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**«ҚАЗАҚСТАН РЕСПУБЛИКАСЫ
ҰЛТТЫҚ ҒЫЛЫМ АКАДЕМИЯСЫ» РҚБ**

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PHYTOCHEMICAL STUDY OF MEDICINAL PLANT - HUMULUS LUPULUS L., FOR THE PURPOSE OF OBTAINING BIOLOGICALLY ACTIVE SUBSTANCES

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Abstract. Relevance. A phytochemical study was carried out on Humulus lupulus L., a promising species from the Cannabaceae family. Qualitative and quantitative analyses revealed that, regardless of environmental conditions, the Kazakh variety contains alkaloids, anthraquinones, proteins, hydrolysable compounds, and other bioactive components. This study aimed to investigate the phytochemical composition, identify key active substances, and evaluate the potential of various plant organs as raw material for medical and pharmaceutical use. Differences in extract coloration with analytical reagents suggest variability in the quantity of each compound group. Analysis showed that hop stems contain significant levels of alkaloids, anthraquinones, polysaccharides, and phenolic and hydroxycinnamic acids. Leaves accumulate more monosaccharides and flavonoids. Hop cones are a rich source of proteins, tannins, coumarins, xanthones, triterpenoids, phospholipids, and essential oils. In total, 31 substances were identified in the Kazakh variety for the first time: 6 anthraquinones, 5 tannins, 4 xanthones, 7 coumarins, 4 triterpenoids, and 5 carbohydrates. The composition and content of bioactive substances varied depending on the plant part analyzed. Practical value. These findings confirm the potential of Kazakh Humulus lupulus L. as a local source of pharmacologically valuable compounds and provide a scientific basis for further studies and the development of plant-based pharmaceuticals and nutraceuticals.

Keywords: Humulus lupulus, analysis of chemical composition, quantitative analysis of compounds, bioactive compounds

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ДӘРІЛІК HUMULUS LUPULUS L. ӨСІМДІГІНЕН БИОЛОГИЯЛЫҚ БЕЛСЕНДІ ЗАТТАРДЫ АЛУ МАҚСАТЫНДАҒЫ ФИТОХИМИЯЛЫҚ ЗЕРТТЕУ

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Аннотация. Өзектілігі. Фитохимиялық зерттеу Cannabaceae тұқымдасына жататын келешегі зор өсімдік түрі Humulus lupulus L. өсімдігіне жүргізілді. Бұл зерттеу аясында сапалық және сандық талдаулар жүргізіліп, қоршаған орта жағдайларына тәуелсіз түрде Қазақстан аумағында өсетін осы өсімдіктің құрамында алкалоидтар, антрахинондар, белоктар, гидролизденетін қосылыстар және басқа да биологиялық белсенді компоненттердің бар екендігі анықталды. Зерттеудің негізгі мақсаты — өсімдіктің фитохимиялық құрамын терең зерттеу, оның құрамындағы негізгі белсенді заттарды анықтау, сондай-ақ әртүрлі өсімдік бөліктерінің (сабақ, жапырақ, шишка) медициналық және фармацевтикалық мақсатта шикізат ретінде қолданылу әлеуетін бағалау болып табылады. Аналитикалық реагенттермен алынған сығындылардың түсінің әртүрлілігі фитохимиялық топтардың сандық құрамында өзгеріс бар екенін көрсетеді. Өсімдіктің әртүрлі бөліктеріне жүргізілген талдау нәтижесінде хмельдің сабағында алкалоидтар, антрахинондар, полисахаридтер, сондай-ақ фенолдық және гидроксикоричный қышқылдарының жоғары мөлшерде шоғырланғаны анықталды. Жапырақтары, негізінен, моносахаридтер мен флавоноидтарға бай екені белгілі болды. Ал құлмақтың шишкалары (гүлдері) ақуыздар, таниндер, кумариндер, ксантондар, тритерпеноидтар, фосфолипидтер және эфир майлары сияқты фармакологиялық тұрғыдан маңызды заттардың бай көзі болып табылады. Жалпы алғанда, Қазақстанда өсетін Humulus lupulus L. түрінің құрамынан алғаш

рет 31 әртүрлі химиялық қосылыс анықталды. Атап айтқанда, 6 антрахинон, 5 танин, 4 ксантон, 7 кумарин, 4 тритерпеноид және 5 көмірсу түрі тіркелді. Бұл заттардың құрамы мен мөлшері өсімдіктің қай бөлігінен алынғанына қарай өзгеріп отырды. Практикалық құндылығы. Аталған ғылыми нәтижелер Қазақстан аумағында өсетін Humulus lupulus L. өсімдігінің фармакологиялық тұрғыдан құнды табиғи қосылыстардың жергілікті көзі ретінде үлкен әлеуетке ие екенін дәлелдейді. Сонымен қатар, бұл мәліметтер өсімдік негізіндегі фармацевтикалық және нутрицевтикалық өнімдерді әзірлеуге арналған ғылыми негіз қалыптастырады әрі болашақтағы зерттеулерге жол ашады.

Түйін сөздер: Humulus lupulus L., химиялық құрамды талдау, қосылыстардың сандық талдауы, биоактивті қосылыстар

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ФИТОХИМИЧЕСКОЕ ИССЛЕДОВАНИЕ ЛЕКАРСТВЕННОГО РАСТЕНИЯ – HUMULUS LUPULUS L. С ЦЕЛЬЮ ПОЛУЧЕНИЯ БИОЛОГИЧЕСКИ АКТИВНЫХ ВЕЩЕСТВ

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Аннотация. Актуальность. Фитохимическое исследование перспективного вида из семейства Cannabaceae – Humulus lupulus L., произрастающего на территории Казахстана, было проведено с целью всестороннего анализа его химического состава. В рамках данного исследования были проведены как качественные, так и количественные фитохимические анализы, которые показали, что независимо от условий произрастания казахстанская разновидность хмеля содержит ряд биологически активных веществ. К ним относятся алкалоиды, антрахиноны, белки, гидролизуемые соединения и другие важные метаболиты, обладающие потенциальной фармакологической активностью. Основной задачей исследования являлось выявление состава фитохимических компонентов,

определение ключевых активных веществ, а также оценка потенциала различных органов растения (стебли, листья, шишки) в качестве сырья для медицинского и фармацевтического применения. Разнообразие окраски экстрактов при использовании аналитических реагентов указывает на количественную вариативность групп соединений, что говорит о сложной и неоднородной химической природе растения. В результате анализа было установлено, что стебли хмеля содержат значительные концентрации алкалоидов, антрахинонов, полисахаридов, а также фенольных и гидроксикоричных кислот. Листья, в свою очередь, характеризуются более высоким содержанием моносахаридов и флавоноидов. Наибольшее богатство биологически активных веществ было выявлено в шишках хмеля были обнаружены белки, дубильные вещества, кумарины, ксантоновые соединения, тритерпеноиды, фосфолипиды, а также эфирные масла, играющие важную роль в фармакологии. Всего в казахстанской популяции хмеля впервые было идентифицировано 31 химическое соединение, в том числе 6 антрахинонов, 5 танинов, 4 ксантона, 7 кумаринов, 4 тритерпеноидных соединения и 5 различных углеводов. Установлено, что состав и концентрация этих веществ варьируются в зависимости от исследуемой части растения. Практическое значение. Полученные научные данные подтверждают высокий потенциал Humulus lupulus L. казахстанского происхождения как ценного местного источника биологически активных веществ с фармакологическим значением. Исследование служит научной основой для проведения дальнейших работ, направленных на разработку лекарственных средств и функциональных добавок растительного происхождения, а также расширяет представление о применимости данной культуры в фармацевтической и нутрицевтической промышленности.

Ключевые слова: Humulus lupulus, анализ химического состава, количественный анализ соединений, биоактивные соединения

Introduction. The growing interest in plant-derived bioactive substances has led to a notable increase in the use of natural products in modern medicine. Wild flora remains a fundamental source of therapeutic raw materials. However, due to increasing demand, there is an urgent need to improve collection practices and implement sustainable resource management within natural ecosystems. Medicinal plants are recognized for their broad pharmacological spectrum and mild therapeutic action, particularly in the treatment of chronic diseases. Nevertheless, anthropogenic pressure continues to diminish the reserves of such valuable biological resources. This reinforces the importance of researching the chemical profiles and pharmacological effects of medicinal plant species. Among the botanicals with promising pharmacological potential is Humulus lupulus L. (common hop), a species known for its anti-inflammatory, sedative, and anxiolytic properties. It is traditionally applied to manage nervous tension, sleep disturbances, and neuralgic pain. Despite its wide distribution, the natural populations of Humulus lupulus L. remain relatively scarce. This plant is widely used in both official and traditional phytotherapy and finds roles

in various industries (Callemien, et al., 2005).

This study aims to conduct a detailed analysis of the chemical constituents and biological features of Humulus lupulus L., assess its potential for use in medicine and traditional healing practices, and explore its broader applications, with attention to sustainable utilization and conservation strategies. Humulus lupulus L. is a herbaceous perennial climbing vine that reproduces via dioecious flowers. The perennial base includes underground organs such as rhizomes, roots, and dormant buds, while the aerial shoots develop annually from spring to autumn. The plant exhibits a climbing habit, with stems reaching lengths of 3–5 meters or more. These stems have ribbed surfaces and are covered in fine hooked spines arranged along the ribs. The foliage consists of large, rough-textured leaves with serrated margins, typically heart-shaped at the base and sharply pointed at the tips. Petioles are either equal to or shorter than the leaf blade. The stipules are lanceolate, often with bifurcated tips.

Male flowers form terminal panicles with stamens that contain long anthers. Female flowers are grouped in dense inflorescences, enclosed by bracts that later develop into characteristic cone-like structures. These cones, measuring 2.5–3.5 cm in length, are composed of enlarged bracts with glandular trichomes that produce biologically active substances. The plant is strictly dioecious, bearing either male or female inflorescences. The fruit is an ovoid achene enclosed in the persistent calyx. In herbal medicine, the female inflorescences (cones) are the principal part used (De Keukeleire, et al., 2003). The plant naturally occurs in riparian zones, forest clearings, and shrub thickets, and it is also cultivated in home gardens. In Kazakhstan, Humulus lupulus L. is found in river valleys and foothill zones. Its global range includes various parts of Europe, North America, the Caucasus, and Asia Minor (Yedilova, et al., 2025). Chemical investigations have identified a wide array of compounds in this species, including cyclitols (up to 0.03%) (Yedilova, t al., 2020), various mono- and diterpenes (e.g., α-pinene, myrcene, linalool, camphor), as well as numerous sesquiterpenes, steroids, and alicyclic compounds. These include humulon, lupulon, and several derivatives such as cohumulon and adhumulon. Phenolic components such as hydroxybenzaldehyde and phloroglucinol glycosides have also been identified, alongside stilbenes (e.g., resveratrol), flavonoids, chalcones, and xanthohumols (Silva, et al., 2023; Yu, et al., 2014; Zhoua, et al., 2018). Additionally, the plant contains important vitamins (B1, B2, C), phenolic acids (e.g., caffeic, gallic), and organic acids (e.g., citric, succinic) (Tanaka, et al., 2014).

Pharmacologically, compounds such as xanthohumol and 6-isopentenylnaringenin exhibit antifungal activity. Aqueous infusions and decoctions are applied in clinical phytotherapy to reduce inflammation, support gastrointestinal function, stimulate appetite, and aid in healing chronic skin lesions. These herbal preparations also find use in treating insomnia, menopausal syndromes, and functional nervous disorders. In gynecology, decoctions are used to treat amenorrhea, dysmenorrhea, and lactation issues. Clinical studies suggest that hop preparations improve sleep and emotional well-being and are applied in balneotherapy. Moreover, compounds like humulon and lupulon have demonstrated antimicrobial properties, while derivatives such as

cohumulon and adluplon exhibit antibacterial action (Sangiovanni, et al., 2019; Alonso-Esteban, et al., 2019). Scientific data also confirm that hop extracts have adaptogenic effects under chronic stress, protecting internal organs from degenerative changes and helping to stabilize neurotransmitter systems. Furthermore, the essential oil has insect-repelling properties. The pharmacological versatility of Humulus lupulus L. includes analgesic, antioxidant, sedative, and antimicrobial actions (Schiller, et al., 2006; Luzak, et al., 2016; DeGrandi-Hoffman, et al., 2012).

Given its phytochemical richness and pharmacological profile, studying the Kazakhstani variety of Humulus lupulus L. is highly relevant. This research seeks to address the limited data on this local ecotype and contribute to the development of phytopreparations based on Kazakhstan's endemic plant resources.

Methods and materials.

Sample collection

The plant (Humulus lupulus L.) was selected and then collected in the month of July 2021 from the Alakol district of Almaty Region during the fruiting period (Figure 1).

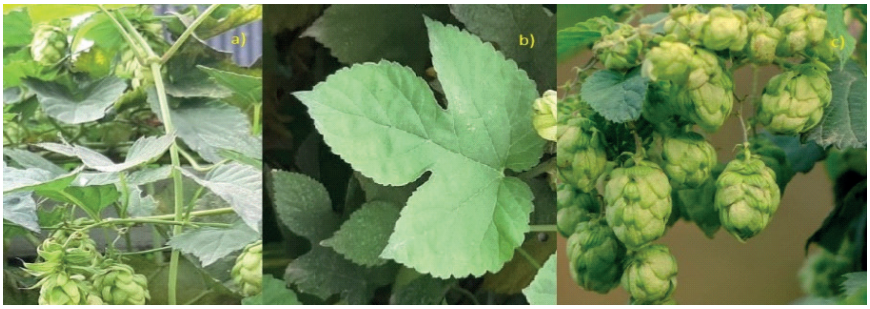


Figure 1. a) stems b) leaves c) cones.

Phytochemical analysis

The study was carried out using standard techniques widely applied in the analysis of medicinal plant materials.

Estimation qualitative analysis of alkaloids:

To detect alkaloids, 2 ml of 50% aqueous-alcoholic extract was mixed with 2 ml of 1% phosphomolybdic acid solution. The emergence of a yellow precipitate, which turned blue within 20 minutes, indicated a positive reaction.

Estimation quantitative analysis of alkaloids:

The amount of alkaloids was measured using a modified analytical technique, with the results expressed in percentage relative to berberine bisulfate content.

Estimation qualitative analysis of anthraquinones:

A 2 ml sample of the hydroalcoholic extract was combined with 2 ml of 1% zirconium nitrate in acidic medium and heated in a water bath for 5 minutes. The formation of a red-purple precipitate confirmed the presence of anthraquinones.

Estimation quantitative analysis of anthraquinones:

After hydrolyzing glycosidic forms using 5% sulfuric acid, anthraquinone content was determined using a refined procedure and calculated in terms of chrysophanic acid.

Estimation qualitative analysis of proteins:

For protein detection, 2 ml of aqueous extract was reacted with Millon's reagent. The mixture was heated in a boiling water bath for 10 minutes. A white precipitate appeared, turning brick-red with continued heating.

Estimation quantitative determination of protein content:

Protein concentration was established using the biuret reaction according to standard protocols.

Estimation qualitative analysis of tannins:

To determine the presence of tannins, 2 ml of aqueous extract was treated with 2 ml of 5% nitrosomethylurea and heated for 10 minutes. The resulting precipitate was filtered, and the filtrate was tested with 1% ammonium iron alum and a sodium acetate crystal. The purple coloration signified a positive result.

Estimation quantitative determination of tannins:

Tannin content was quantified using methods approved by the pharmacopoeia.

Estimation qualitative analysis of xanthones:

2 ml of hydroalcoholic extract was mixed with 2 ml of 5% aluminum chloride solution. A green-blue coloration indicated the presence of xanthones.

Estimation quantitative determination of xanthones:

The amount of xanthones was determined by applying thin-layer chromatography.

Estimation qualitative analysis of coumarins:

To 2 ml of hydroalcoholic extract, 3 ml of pyridine was added, followed by the gradual addition of 2 ml of 0.1 N sodium hydroxide. A color shift from yellow to green and then to blue was observed upon addition of 3 drops of bromothymol blue alcohol solution.

Estimation quantitative determination of coumarins:

The coumarin content was evaluated using an optimized method described in Bland, et al, (2015).

Estimation qualitative assessment of polysaccharides:

To detect polysaccharides, 5 ml of aqueous extract was mixed with four volumes of 95% ethanol. The formation of a visible precipitate confirmed their presence.

Estimation quantitative evaluation of polysaccharides:

Quantification was based on dry weight and conducted using an optimized analytical approach (Bland, et al., 2015).

Estimation qualitative analysis of triterpenoids:

The Kedde reaction was applied: 2 ml of chloroform extract was treated with 2 ml of 1% alcoholic m-dinitrobenzoic acid in an alkaline medium. A bright green coloration indicated a positive result.

Estimation quantitative evaluation of triterpenoids:

An improved method was used to calculate triterpenoid content, expressed as a percentage of absolutely dry raw material in terms of daziantozide.

Estimation qualitative analysis of phenolic acids:

To the acetone extract, an equal volume of reagent mixture (1% potassium ferricyanide and 2% ammonium iron alum) was added. A dark blue coloration was observed, indicating the presence of phenolic acids.

Estimation of phenolic acids (quantitative):

The content was determined using a refined spectrophotometric technique.

Estimation qualitative analysis of flavonoids:

The Martini-Bettolo reaction was employed: a saturated solution of antimony pentachloride in carbon tetrachloride was added to the hydroalcoholic extract, leading to the appearance of an orange hue.

Estimation quantitative identification of flavonoids:

Flavonoid content was measured based on the concentration of quercetin, the main reference component in the plant material.

Estimation qualitative and quantitative assessment of phospholipids:

Both qualitative and quantitative analysis of phospholipids were performed using a gravimetric (mass-based) approach. The total phospholipid content was calculated relative to absolutely dry plant mass.

Estimation qualitative analysis of essential oils:

To identify essential oils, the Sabettai reaction was applied. Equal volumes of 1% bromine in chloroform and chloroform extract were mixed, resulting in a blue color (Bland, et al., 2015).

Estimation quantitative determination of essential oils:

The technique described by Itavo, et al. (2015) was used: the ground raw material was weighed, placed into a 300 ml round-bottom flask with 100 ml of hot water, and subjected to steam distillation for 2–4 hours. Essential oils were collected in a calibrated tube and the volume measured after cooling, then expressed as a percentage of the initial material mass.

Results and Discussion. To evaluate the quality indicators of the medicinal plant material, standardized techniques outlined in the State Pharmacopoeia were applied (Itavo, et al., 2015). As part of the commodity analysis, moisture content was assessed by determining the percentage of weight loss upon drying.

In addition, the total ash content was measured, including both the portion insoluble in a 10% hydrochloric acid solution and the fraction containing sulfates. All values were expressed as percentages and are illustrated in (Figure 2).

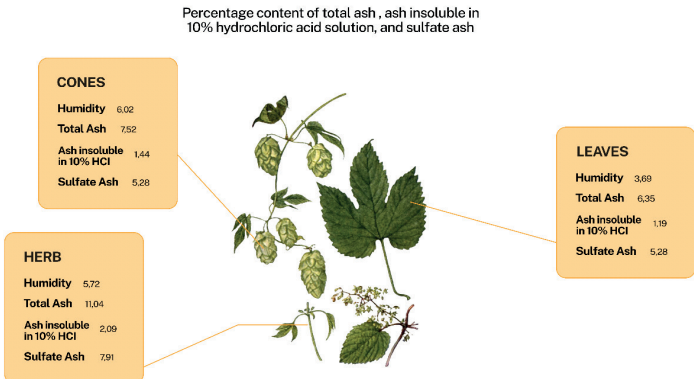
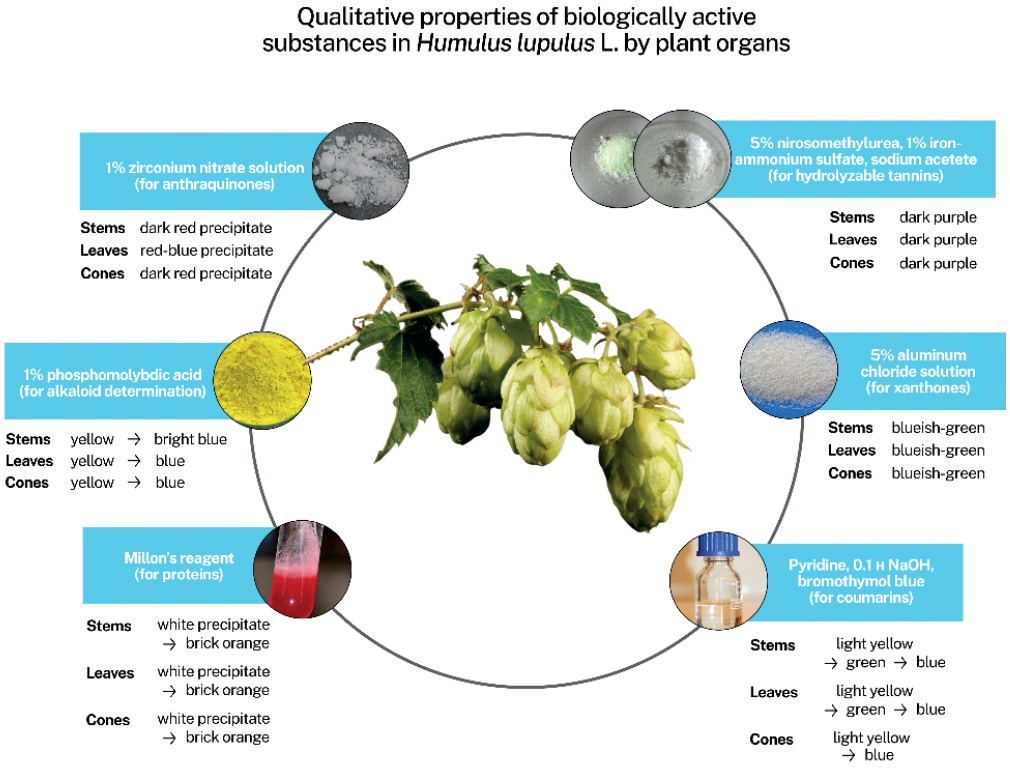


Figure 2. Percentage amount of overall ash, ash not soluble in 10% hydrogen chloride solution, and ash containing sulfate

The evaluation of the obtained data confirmed that the analyzed medicinal plant material met the quality requirements established by the Pharmacopoeia of the Republic of Kazakhstan.

To perform a qualitative assessment of the biologically active compounds present in *Humulus lupulus* L., a variety of solvents with different polarities were utilized. These included aqueous acetone solutions at concentrations of 10%, 30%, 50%, and 70%, as well as aqueous ethanol solutions at the same respective concentrations. Non-polar solvents such as hexane, benzene, and chloroform were also incorporated into the extraction process. For the selective isolation of mono- and polycarboxylic acids, phenolic acids, siliceous compounds, and proteins, a 10% ethanol-in-water solution was applied. Extraction of anthraquinones, alkaloids, flavonoids, tannins, and coumarins was carried out using 50% and 70% hydroalcoholic solvents. Chloroform was employed specifically to extract triterpenoids and phospholipids.

All resulting extracts from *Humulus lupulus* L. were subjected to qualitative phytochemical screening via one-dimensional and two-dimensional paper chromatography using various solvent systems. Visualization of compound groups was performed with specific detection reagents. The findings of this chromatographic analysis are illustrated in Figure 3.



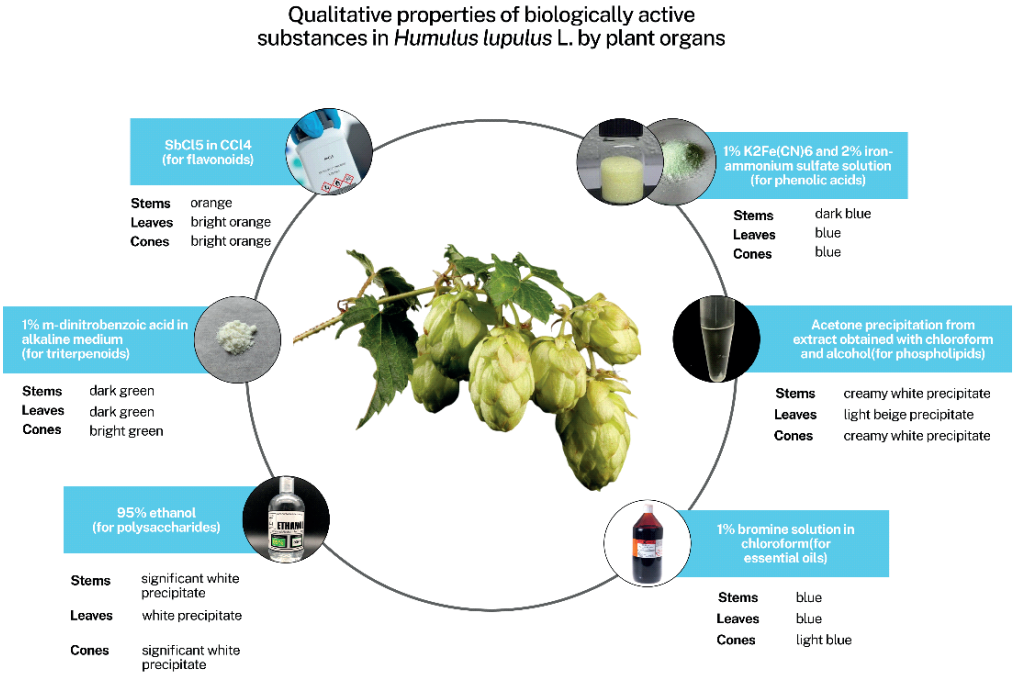


Figure 3. Qualitative properties of biologically active substances in Humulus lupulus L. by plant organs.

The chromatographic profiles presented in Figure 3 confirm that *Humulus lupulus* L. specimens from different ecological regions of Kazakhstan consistently contain alkaloids, anthraquinones, and other classes of biologically active compounds. Variations in the intensity of color reactions with diagnostic reagents reflect differences in the relative concentration of each structural group of metabolites. To perform a detailed phytochemical investigation of these compound classes, the powdered air-dried aerial parts of *Humulus lupulus* L. were subjected to a 2-hour thermal extraction using solvents selected based on polarity and compatibility with the chemical nature of the targeted metabolite group. This selective extraction approach was guided by the principle of structural affinity between solvents and specific natural compounds. Quantitative determination of the detected bioactive constituents was carried out in accordance with pharmacopoeial guidelines and internationally recognized protocols. The chemical profiling of anthraquinones, tannins, xanthones, coumarins, and carbohydrate derivatives was performed using high-performance liquid chromatography (HPLC) with the use of certified reference standards. The analytical results are presented in Figure 4

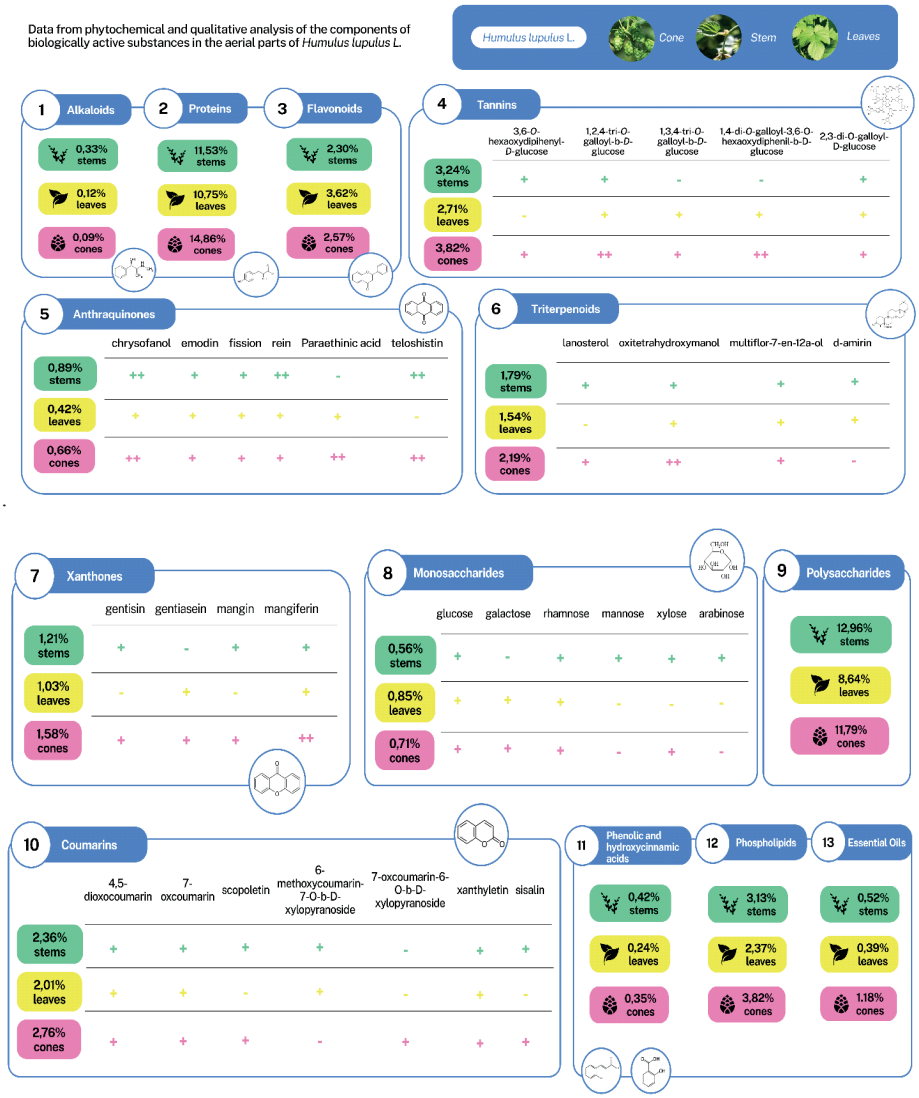


Figure 4. Data from phytochemical and qualitative assessment of the components of bioactive substances in the aerial parts of *Humulus lupulus* L.

An examination of the data summarized in Table 3 revealed distinct distribution patterns of phytochemicals across the aerial organs of *Humulus lupulus* L. The stem tissue demonstrated higher concentrations of alkaloids, anthraquinone derivatives, polysaccharides, as well as phenolic and hydroxycinnamic acids. Leaf samples showed predominant accumulation of flavonoids and monosaccharides. The inflorescences, or cones, were identified as a rich source of proteins, hydrolyzable tannins, xanthones, coumarins, triterpenoids, phospholipids, and essential oil components, positioning them as valuable raw material for phytopharmaceutical development.

Conclusion. The present study provided the first comprehensive chemical characterization of the aerial parts of the Kazakh variety of *Humulus lupulus* L., focusing on the diversity and concentration of key categories of biologically active constituents. Phytochemical screening confirmed the presence of a broad spectrum of bioactive substances within this species.

Notably, 31 compounds were identified for the first time in this Kazakh ecotype of *Humulus lupulus* L., including 6 anthraquinones, 5 hydrolyzable tannins, 4 xanthones, 7 coumarins, 4 triterpenoids, and 5 carbohydrate-type metabolites. The study also revealed significant organ-specific differences in both the qualitative profiles and the quantitative levels of these phytochemicals.

These findings underscore the chemical uniqueness and pharmacological promise of the Kazakh *Humulus lupulus* L., laying a scientific foundation for its further exploration. The data highlight its potential as a raw material for the development of novel herbal medicines and offer a valuable contribution to Kazakhstan's phytochemical resource base. Continued research on this species may facilitate the creation of targeted phytopreparations and expand its application in pharmaceutical and nutraceutical fields.

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