around the larva. In the surrounding chamber tissues concentrate a large amount of nutrients: proteins, sugars and biologically active substances [1]. Galls is especially rich in polyphenolic compounds. The outer layer of the galls contains particularly high concentrations of tannins - up to 90% [2]. None of the *Quercus robur* organs contains tanning material at such a high concentration. The largest amount of tanning material is observed in the “mirror” cortex of the Oak reaches 16 and even 20%, but on average 12%; Ordinary oak bark contains 4-10% of tannin [3].

A high concentration of tanning material helps prevent gall-fly damage and eating galls with herbivorous animals.

Tannin is one of the most widely known representatives of gallic tanning material. It turns out to be esters of D-glucose and gallic acid. There are mono-, di-, tri-, tetra-, penta- and polygaloyl ethers. The content of tannins varies depending on the type of gall and is 3,4-89,1% for dry material [2]. At later stages of development, galls acquire stiffness due to the formation of lignin. Lignin, like tannins, also belongs to the group of polyphenolic compounds. In oak galls phenolic compounds, flavonoids, saponins, alkaloids [4]. Of particular interest are saponins and steroid compounds. In methanol extracts, oak galls were identified: γ-Sitosterol; 8-en-11-one, 3-hydroxy-spirostan, Ethyl iso-allocholate, Bisnorhopane [5]. Among the alkaloid compounds, an indole alkaloid of the strychnan group, akuammicine, and pterin-6-carboxylic acid have been identified. Akuammicine alkaloids are a family of monoterpene indole alkaloids with new medicinal properties. At present, the synthesis of these organic compounds remains impossible and the only source are plants. These bases act as ligands for a heterogeneous group of molecular targets and, therefore, exhibit a wide range of pharmacological actions. For example, pseudoakuammicine exhibits opioid activity in vivo, it is believed that echitamine has promising cytotoxic activity, and corymine behaves as a glycine receptor antagonist [6]. Pterins are referred to the pigments of the wings and eyes of insects, they are also referred to as vitamins of the folic acid group. Lipophilic compounds of oak galls are represented by fatty acids, waxes, resins. The Chinese Pharmacopoeia of the Russian Federation indicates the content of palmitic, lauric, myristic acid, dipropyl ester of myropic acid, pentacosan. Elementary and physicochemical analysis showed the presence of important minerals such as calcium (Ca), magnesium (Mg), phosphorus (P), oxygen (O2), potassium (K), aluminum (AI), carbon (C), zinc (Zn), iron (Fe), manganese (Mn), nickel (Ni) and silica (Si) [7].

## Materials and Methods

### Plant collection

We have studied the galls collected in 4 forest areas in the Kiev region in September-October 2017. Three forest tracts, located in the Irpen forestry between Nikolaevka and Khmelnaya villages of the Kiev-Svyatoshinsky district, the fourth - in the Claudian larches near the village of. Dibrova.

Quantitative content of tannins was determined by the Leventhal method [8]. For comparative analysis of the tannin content, the medicinal plant material from the collection of the pharmacognosy department: *Rhuis coriáriae folia*, *Theae sinénsis folia*, *Quercus cortex* was used.

Quantitative content of free organic acids was carried out by the indicator titration method [8].

Quantitative content of ascorbic acid was determined by typing with sodium 2,6-dichlorophenolindophenolate [8].

The content of the total protein was determined by the biuret method. The method is based on the formation of a biuret complex (having a violet color) of peptide bonds of proteins with divalent copper ions. The method uses the so-called. Biuret reagent consisting of KOH, CuSO4 and sodium citrate. In the complex formed, copper is bound to 4 nitrogen coordination bonds, and with 2 oxygen - electrostatic. A complete complex is formed only with peptides consisting of more than 4 residues. The optical density of the solution was determined on a photodetector calibrator Apel AP-101, manufactured in Japan at 540-560 nm. Reagents from the company "Filsit". The advantages of the method include the low sensitivity to foreign matter, low error. To prepare the extraction, the analytical sample of the raw material was ground to 2 mm. 2.5 g of crushed raw material was triturated in a mortar with glass and poured into 20 ml of water. The mixture was kept for 2 hours with periodic stirring, then the extract was filtered, 1 ml of filtrate was taken for analysis.

The content of free glucose was determined by glucose oxidase method. The essence of the method is that, when the reaction of sugar with oxygen in the air, the reagent is oxidized. Hydrogen peroxide is released into the solution. This substance reacts with orthotoluidine to form a colored compound. The behavior of this reaction requires the presence of special enzymes. In the oxidation reaction, glucose oxidase should be present, and when percolation of the liquid is peroxidase. The intensity of the solution color will depend on the glucose content and be more intense at high content. The intensity of the coloring of solutions was determined on a biochemical semi-automatic analyzer "BA-88", manufactured by Germany. Reagents from the company "Filsit". To prepare the extraction, the analytical sample of the raw material was ground to 2 mm. 2.5 g of crushed raw material was placed in a 50 ml flask with reflux, poured 20 ml of water and kept for 2 hours in a boiling water bath, then the extract was filtered. The tannins were precipitated by adding 2 ml of a 2% solution of papaverine hydrochloride to 2 ml of extractor, filtered and analyzed.

For the comparative content of protein, glucose, organic acids and ascorbic acid, medicinal herbal raw materials was used from the collections of the department: *Rosae fructus, Viburni fructus, Sorbi fructus*.

## Results and Discussion

### Comparative content of tannins in *Quercus roburis galls* and official types of plant raw materials

For medicinal purposes in Ukraine as a source of tannin, raw materials *Cotini coggigria folia* and *Rhois coriariae folia* are used, on its basis preparations "Galaskorbin", "Novikova Fluid", which are used as astringent and antiseptic agents, are obtained, respectively. Leaves of these plants contain up to 30% tannins [9]. In our studies, Sumach leaves were used to compare the content of tannins in wild oak galls collected in the Kiev region. It was found that the tannin in the sumac leaves is contained in an amount of 26.73 + 1.12%, which is consistent with previous data. On the other hand, traditional (and popular) medicines, which are used as astringent and aseptic, are preparations (galenic and newgalenic) based on Quercus cortex, such as: "Stomatophyte", "Polyhemostat", "Polifitol-1", etc. Many literary sources confirmed that the bark of an oak tree is rich in tannins [9,10]. This was also confirmed by the present study, which demonstrates that the tannin content in the *Oak bark* was about 12%. We also confirmed that *Theae folia* contains 30% of tannins. A very popular source of tannin are *Gallae Turcicae* and *Gallae Chinensis*, which are traditionally used in India, China and countries of Asia Minor [11]. Wild-growing Quercus roburis galls can be an alternative to regions where traditional Chinese and Turkish galls are not used [12]. Since our research has established that galls of wild oaks contain between 70 and 80% tannins (Table 1). Although this value is lower in comparison with Chinese or Turkish galls [13], the absolute values of tannin content in wild galls of the Kiev region are still quite high, but there are no statistically significant differences in the content of tannin in galls from 4 plots of the forest. They can be considered as raw materials rich in tannins for factory processing, for the purpose of obtaining tannin, as well as pharmaceutical preparations based on them.

| Raw materials | The content of tannins in %, in terms of absolutely dry raw materials | The content of tannins in %, in terms of absolutely dry raw materials (according to lithium sources) |
|---|---|---|
| *Quercus roburis gallae* (Forest 4) | 73,17 ± 0,82 | 50-70% (up to 89%) [13] |
| *Quercus roburis gallae* (Forest 1) | 76,34 ± 1,17 | |
| *Quercus roburis gallae* (Forest 2) | 73,58 ± 1,25 | |
| *Quercus roburis gallae* (Forest 3) | 72,5 ± 2,22 | |
| *Rhois coriariae folia* | 26,73 ± 1,12 | 25-30% [9] |
| *Quercus cortex* | 12,42 ± 0,77 | Up to 15% [14] |
| *Theae folia* | 29,26 ± 1,34 | 8-30% [15] |

**Table 1:** Tannins contents in *Quercus roburis gallae, Rhois coriariae folia, Quercus cortex and Theae folia.*

It should be borne in mind that the composition of polyphenols of different types of gall is different, which can also manifest itself on the pharmacological effect of antimicrobial activity against different species of bacteria, however, the general pharmacological properties inherent in tannins will be manifested. Further pharmacological studies and studies of forms of tannins present in Quercus roburis galls using solvents of different polarity will add clarity to this question. In general, taking into account the high content of tannin in wild oak galls and the supposedly diverse composition of polyphenols, it can be considered that the wild-growing Quercus roburis galls harvested on the territory of the Kiev region are suitable as an alternative source of tannin for the production of medicines.

### Comparative content of organic acids and ascorbic acid in *Quercus roburis gallae* and fruits of official plant species

Tannins contained in galls can be attributed to the main active substances, but the therapeutic value of medicinal plant raw materials is determined by the incoming biologically active substances in their composition, to which all substances capable of influencing the biological processes occurring in organism. Such, so-called concomitant substances include vitamins, organic acids, minerals, sugars, etc. It should be noted that there are groups of medicinal plant raw materials that are used due to the content of vitamins (*Sorbi aucupariae fructus, Viburni fructus*) or even standardized by content ascorbic acid (*Rosae fructus*).Vitamins in plant raw materials are contained in a form that is easier to digest than synthetically produced analogs. The use of galenic preparations from plant raw materials, gives an additional source of vitamins, as their water-soluble part goes into infusions and decoctions [17,18]. The second group of hydrophilic compounds of plant raw materials are organic acids. They participate in the metabolic processes of the organism, promote a higher tone, have a broad spectrum of pharmacological action [10]. However, extracts with acid reaction of the medium are contraindicated in patients with gastritis and peptic ulcer of the gastrointestinal tract, which should be taken into account when prescribing medicinal products [19,20]. Therefore, information on the content of organic acids can be valuable for the standardization of medicinal plant material *Quercus roburis gallae.* Taking into account that in the organoleptic analysis *Quercus roburis gallae* was determined along with the astringent and sweet and sour taste, as well as the fact that the extraction of tannins is carried out with water, we studied the content of organic acids and ascorbic acid content in galls. For comparison, we investigated *Sorbi aucupariae fructus, Viburni fructus* and *Rosae fructus* (Table 2)*.* It was found that the greatest amount of organic acids was contained in the *Quercus roburis gallae* collected in the Claudian forestry near the village of. Dibrova (array 4). Their amount significantly differed from other types of raw materials, the absolute value of ascorbic acid content was also higher, but there were no significant differences in the content of ascorbic acid in three types of gall. The amount of ascorbic acid in the investigated galls is comparable to their amount of *Rosae fructus* taken from the collection of the department and higher than in *Sorbi aucupariae fructus* and *Viburni fructus*. It should be noted that in the fruits of mountain ash, taken for analysis, contained less organic acids, and ascorbic acid than indicated in the literature (Table 3).Organic acids are an important component of plants, they play a role in many metabolic reactions that promote plant growth and can accumulate in large quantities as dissolved free anions [21-24]. The level of organic acids in plants varies depending on the composition of the soil, the amount of moisture, lighting and other factors. Organic acids can bind metal elements, thus reducing their toxic effects on plants [21]. Higher concentrations of organic acids have been found in plants tolerant to heavy metals than in sensitive plants [21,22,25].

The high content of organic acids in galls can also be considered on the one hand as a response of plant tissues to stress. These mechanisms allow to neutralize the effect of insect metabolism products on plants. On the other hand, organic acids and ascorbic acid are necessary for the larva *Cynips quercustolii* for normal development, which can be attributed to the biochemical features of the pathologically altered tissues of the host plant. A unique ratio of a high amount of tannins and ascorbic acid can allow wild-growing *Quercus roburis gallae* take a worthy place among the sources of phytopreparations in Europe.

| Raw materials | The content of organic acids in %, in terms of malic acid in absolutely dry raw materials | The content of organic acids in %, in terms of malic acid in absolutely dry raw materials (according to lithium sources) |
|---|---|---|
| *Quercus roburis gallae* (Forest 4) | 3,5530 ± 0,2532 | - |
| *Quercus roburis gallae* (Forest 1) | 2,1498 ± 0,3341 | |
| *Quercus roburis gallae* (Forest 2) | 1,9998 ± 0,1752 | |
| *Quercus roburis gallae* (Forest 3) | 2,5263 ± 0,3877 | |
| *Rosae fructus* | 2,6300 ± 0,3782 | 2,87 [19] |
| *Sorbi aucupariae fructus* | 2,1189 ± 0,2612 | 3,78[ 19] |
| *Viburni fructus* | 2,7193 ± 0,3354 | 1,22-3,68 [20] |

**Table 2:** Organic acids contents in *Quercus roburis gallae, Rosae fructus, Sorbi aucupariae fructus* и *Viburni fructus*.

| Raw materials | The content of ascorbic acid, in terms of absolutely dry raw materials in % | The content of ascorbic acid, in terms of absolutely dry raw materials in % (according to lithium sources) |
|---|---|---|
| *Quercus roburis gallae* (Forest 4) | 0,2600 ± 0,0418 | - |
| *Quercus roburis gallae* (Forest 1) | 0,1766 ± 0,0274 | |
| *Quercus roburis gallae* (Forest 2) | 0,1403 ± 0,0221 | |
| *Quercus roburis gallae* (Forest 3) | 0,1683 ± 0,0319 | |
| *Rosae fructus, fructus* | 0,2100 ± 0,0396 | 0,20 ± 0,01% [19]; 0,32 [20] |
| *Sorbi aucupariae* | 0,0508 ± 0,008 | 0,23 ± 0,01% [19]; 0,12 [20] |
| *Viburni fructus* | 0,0476 ± 0,004 | 0,06 [20] |

**Table 3:** Ascorbic acid contents in *Quercus roburis gallae, Rosae fructus, Sorbi aucupariae fructus* и *Viburni fructus*.

| Raw materials | Protein content, in terms of absolutely dry raw materials in % | Protein content, calculated on the basis of absolutely dry raw materials in % (according to literary sources) |
|---|---|---|
| *Quercus roburis gallae* (Forest 4) | 3,4900 ± 0,2517 | - |
| *Quercus roburis gallae* (Forest 1) | 4,6550 ± 0,2833 | |
| *Quercus roburis gallae* (Forest 2) | 4,9590 ± 0,2415 | |
| *Quercus roburis gallae* (Forest 3) | 5,0300 ± 0,3274 | |
| *Rosae fructus* | 1,3940 ± 0,2127 | 1,6 |
| *Sorbi aucupariae fructus* | 1,3825 ± 0,1920 | 1,5 |
| *Viburni fructus* | 2,1385 ± 0,0199 | 2,0-4,2 [26] |

**Table 4:** Protein contents in *Quercus roburis gallae, Rosae fructus, Sorbi aucupariae fructus* и *Viburni fructus*.

## Comparative protein and glucose content in *Quercus roburis gallae* and officinal plant species

Proteins and sugars are attributed to the products of primary plant synthesis. The quantity and composition of proteins and sugars in different organs of plants is different, in addition, their content depends on the time of day and seasonal changes. Moreover, if the high content of proteins and sugars in the fruits and seeds of plants is a common phenomenon, the increase in the content of sugar and protein in vegetative organs is due either to their metamorphosis, or is a consequence of the reaction to external influences. As a result of the conducted research, it was established that in the *Sorbi aucupariae fructus*, protein was contained more than in the other two officinal forms of medicinal plant material (Table 4). The protein content in four variants of galls was significantly higher than the protein content of the hips, rowan and grass fruits, and ranged from 3 to 5%, based on the absolutely dry raw material. The amount of protein found in us in the officinal species of plants raw materials corresponds to that specified in the literature [26].

The amount of glucose (Table 5) was the highest in the fruits of mountain ash and guelder rose. In *Quercus roburis gall*s, the amount of glucose was lower, however, it turned out to be comparable to the amount of glucose in hips, which was about 0.8%, based on absolutely dry raw materials.

High content of protein in pathologically altered *Quercus robur* molding tissues can be explained by the plant's reciprocal stress, since the response of all living organisms to stress influences is the synthesis of proteins whose activity is aimed at protecting cells and supporting homeostasis. Such a reaction is observed under the influence of extreme temperatures, ultraviolet and radioactive irradiation, toxic substances, changes in the aqueous regime, as well as pharmacologically active molecules [7,9,27]. Sugars, in turn, serves as a substrate for the synthesis of stressful proteins. It is probable that the accumulation of nutrients (proteins and sugars) is similar to their accumulation in the fruits of plants and is associated with the vital activity of the parasitic phytophagus.

## Conclusion

In *Quercus robius gallae*, the following groups of biologically active substances have been identified: ascorbic acid, glucose, proteins, tannins, catechins, saponins and alkaloids. It is established that the amount of tannins is 70-75%. The amount of ascorbic acid

| Raw materials | The content of glucose, in terms of absolutely dry raw materials in % | The content of glucose, in terms of absolutely dry raw materials in % (according to literary sources) |
|---|---|---|
| *Quercus roburis gallae* (Forest 4) | 0,8330 ± 0,0491 | - |
| *Quercus roburis gallae* (Forest 1) | 0,8470 ± 0,0475 | |
| *Quercus roburis gallae* (Forest 2) | 0,8330 ± 0,0443 | |
| *Quercus roburis gallae* (Forest 3) | 0,7730 ± 0,0411 | |
| *Rosae fructus* | 0,9637 ± 0,0386 | 0,9-8,1 [31]<br>9,6 [30] |
| *Sorbi aucupariae fructus* | 2,8980 ± 0,2174 | 2,8 [29] |
| *Viburni fructus* | 2,5720 ± 0,1552 | 3,5% [28] |

**Table 5:** Glucose contents in *Quercus roburis gallae, Rosae fructus, Sorbi aucupariae fructus* и *Viburni fructus*.

is comparable to that in *Rosae fructus* and is 0.2%. The present study shows the prospects of using wild galls in Europe as alternatives to the import of *Gallae Turcicae*, *Gallae Chinensis* and *Gallae Pistaceae* and the development of new drugs on their basis. For the first time we studied the content of ascorbic acid in this object, a significant amount of which opens new opportunities in the development of galenic preparations of *Quercus roburis gallae* of vitamin, antimicrobial and polypharmacological action.

## Conflict of Interest Statement

We declare that we have no conflict of interest.

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