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EXTRACTION OF POLYPHENOLIC COMPOUNDS FROM THE *JUGLANS REGIA* L. PELLICLES OF USING ULTRASOUND

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The attention of Russian and foreign scientists is focused on obtaining secondary plant metabolites, including phenolic compounds, due to their pharmacological activity. The *Juglans regia* L. pellicles are a promising raw source for the production of alkaloids, naphthoquinones (juglone), tannins and organic acids. In modern practice of obtaining biologically active substances from plant raw materials, intensive extraction methods are of particular interest. In this extraction method ultrasonic treatment is used to increase the yield and intensify the process of extracting biologically significant substances. Technological parameters of ultrasonic treatment must be selected individually for each type of plant raw material. The aim of the work was to study the effect of ultrasonic treatment on the yield of polyphenolic substances during extraction from the pellicles of breeding varieties *Juglans regia* L. It was found that the content of tannins in the pellicle biomass of the studied varieties *Juglans regia* L. varies from 5.2 to 7.3 g/100 g, phenolic compounds – from 17 to 18.2 mg/100 g. The highest content of these compounds was recorded in the pellicle biomass of the Valentina's Gift variety. It was found that ultrasonic treatment with a power of 200 W for 10 min contributes to the highest yield of polyphenolic substances in the extract from the pellicle biomass of the Valentina's Gift variety. However, the spectral profiles of hydroalcoholic extracts indicate the opposite: 200 W ultrasound reduces the intensity of the extraction of polyphenolic compounds.

Keywords: Juglans regia L., walnut pellicles, extraction, ultrasound, ultrasonic treatment, polyphenolic substances.

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Introduction

One of the raw sources of pharmacological substances are natural substances of plant origin with a wide range of physiological activity. It is known that the most important biologically active substances include plant metabolites – alkaloids, glycosides, saponins, flavonoids, essential oils, tannins and bitter substances, organic acids and mineral salts [1, 2].

Walnut (*Juglans regia* L.) is a medicinal plant widely used in folk medicine and homeopathy. As a medicinal raw material, the fruits of *Juglans regia* L. are used in the stage of milky-wax maturity, green pericarp, leaves, bark of branches and roots, as well as pellicles, which are recommended to be used in the form of infusions and decoctions in the treatment of purulent and fungal skin lesions, diabetes mellitus, tuberculosis, diseases of the thyroid gland, mucous membranes of the mouth and throat, joints, anemia, beriberi, thyrotoxicosis, colitis [3–5].

There is relatively little information in the scientific literature on the composition of the polyphenolic compounds of *Juglans regia* L. In the studies of Kornsteiner M., Li L., Jurd L., Fukuda T., Cerda B., Colaric M., there are data on the study of some walnut polyphenols [6–11]. Ellagic acid and its derivatives, which contribute significantly to the antioxidant activity of walnut, have been identified [6, 9–11]. Colaric et al. [11] found

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that the nucleus of *Juglans regia* L. contains lilac, ellagic acids, and juglone. It has also been shown that these compounds are most often found in the pellicles of *Juglans regia* L. Such acids as p-coumaric, chlorogenic, ferulic, and sinapinic acids are found in lower concentrations [11]. Among the phenolic compounds of the pellicles of *Juglans regia* L., naphthoquinones and anthracene derivatives are noted [3, 12, 13].

Methods for the extraction of biologically active substances from natural raw materials are of great practical importance, since the correctly selected technological method and the completeness of the extraction of valuable compounds from plant raw materials determine the degree of purity of the final product and form the qualitative characteristics of drugs [14, 15]. Of particular interest are intensive extraction methods that use the additional action of various physical factors to increase the efficiency of extracting valuable components from natural sources [16–18].

In most studies, among various physical methods for increasing the yield and intensifying the process of extracting biologically significant substances, preference is given to ultrasound [19]. A number of factors influence the acceleration of the process of extracting valuable medicinal substances from plant raw materials when exposed to ultrasonic waves: an increase in the phase boundary that occurs due to an increase in the degree of dispersion, partial destruction of plant cells, an increase in the concentration gradient due to mixing and convective diffusion, an increase in the extraction temperature due to the occurrence of thermal effects [20].

The use of ultrasonic technologies has advantages over traditional methods of processing raw materials, the main of which is the short duration of the extraction process [1, 20, 21]. One of the key disadvantages of this extraction method is the need to use special equipment, a high level of aggressiveness of ultrasonic waves in relation to a plant cell, which consists in the risk of destruction of its molecules under the influence of hydrodynamic cavitation shocks [20, 21]. It is important to determine the extraction conditions not only for a specific type of raw material, but also for a group of biologically active compounds, since high frequencies of ultrasound can negatively affect a plant cell.

Popova N.V. and Potoroko I.Yu. [22] indicate that the yield of valuable substances in extracts is directly proportional to the duration and frequency of exposure to ultrasound. It was noted that ultrasonic extraction with a power of 120 W for 5 min makes it possible to increase the yield of biologically active substances from aqueous extracts of white grain oats and to increase the antioxidant activity of the obtained extracts. The paper [23] provides information that ultrasonic extraction increases the yield of carotenoids from the terrestrial parts of Mediterranean plants – fig and olive leaves and increases the antioxidant capacity of their aqueous extracts. It was shown [24] that the yield of caffeic and rosmarinic acids and rutin from a medicinal plant of the *Lamiaceae* family increases in the extract with the optimal ratio of technological parameters of ultrasonic extraction.

The aim of this paper was to study the effect of ultrasonic treatment (US-treatment) on the yield of polyphenolic substances during extraction from the *Juglans regia* L. pellicles.

Materials and Methods

Preliminary preparation and analysis of raw materials. The pellicle biomass of the *Juglans regia* L. breeding varieties – Valentina's Gift, Burlyuk, Alminsky Crimean selection (The Nikitsky Botanical Gardens – National Scientific Center of the RAS) that harvested in 2021 was used as a raw material.

A pooled sample of the pellicle biomass obtained after walnut shelling was collected by sampling 1 kg of inshell nuts. After splitting the nut, the pellicles were freed from the shell using tweezers. The cleaned pellicles were crushed in a laboratory mill LZM-1M (Vilitek, Russia) for 3 min and the crushed sample was sifted through a sieve with a mesh diameter of 0.5 mm.

The determination of the mass fraction of moisture was carried out by drying weighed portions of the pellicle biomass in an oven at a temperature of 105 °C to constant weight [25, 26]. The obtained data were used to recalculate the total content of tannins to the mass fraction of dry residue in the pellicles of the *Juglans regia* L. studied varieties.

To determine the total content of tannins in terms of tannin in the pellicle biomass of the *Juglans regia* L., water extracts were preliminarily prepared. To obtain aqueous extracts, weighed portions of the pellicle biomass were placed in conical flasks with a capacity of 500 ml, filled with 250 ml of water heated to a boil, and heated under reflux on a boiling water bath for 30 min with occasional stirring, then the liquids were settled, cooled to room temperature, and decanted for about 100 ml in 250 ml conical flasks [25, 26].

Preparation of extracts. An aqueous solution of ethanol with 50% alcohol by mass was used as an extractant. The ratio of the pellicle biomass of the *Juglans regia* L. and extractant was 1 : 30 by weight.

The samples were mixed with the extractant and treated with ultrasound (US-treatment) on a SCIENTZ-IID instrument (SCIENTZ, China), while varying its US power in the range from 100 to 300 W and the sonication time in the range from 5 to 15 min (radiator frequency 25 kHz, temperature range 20–25 °C).

After US-treatment, the extracts were kept in a sealed container at room temperature for 24–48 h without access to light, after that the extracts were separated from the meal by decantation.

To assess the effect of US-treatment on the completeness of the extraction of polyphenolic compounds, control samples of extracts obtained without US-treatment were used.

Analysis of extracts. The method [25, 26] was used to determine the content of tannins in the obtained extracts. The total content of phenolic compounds in the studied raw materials and extracts was determined by the method [27].

To study the profile of polyphenolic substances in extracts, a SHIMADZU spectrophotometer (SHIMADZU, Japan) was used. The spectra of extracts were recorded in the UV and visible spectrum.

Statistical analysis. The experimental data were processed using the tools of the Microsoft Office Excel (2010) software.

All experimental results were presented as mean values from five measurements, taking into account the confidence interval at a probability of α=0.95 using Student's t-test.

Results and Discussion

Polyphenolic substances of the Juglans regia L. pellicles. Figure 1 shows the results of studies of the content of tannins and phenolic compounds in the samples of the pellicles of the *Juglans regia* L. studied varieties.

The content of tannins in the pellicle biomass of the *Juglans regia* L. varies from 5.2 to 7.3 g/100 g. The results obtained are in good agreement with the literature data [11], which indicate that most of the tannins accumulate in the pellicles of nuts.

The content of phenolic compounds in the pellicle biomass of the *Juglans regia* L. varies from 17.0 to 18.2 mg/100 g. It is known that the content of phenolic compounds will make it possible to evaluate the antioxidant potential of plant raw materials for its use as a source of natural antioxidants and biologically active substances [8, 9, 11, 28].

The highest content of tannins (7.3 g/100 g) and phenolic compounds (18.2 mg/100 g) was noted in the pellicles of the Valentina's Gift variety.

Effect of US-treatment on the extraction of polyphenolic substances from the Juglans regia L. pellicles. Figure 2 shows the results of a study of the effect of US-treatment on the content of tannins in extracts from the pellicle biomass of the Valentina's Gift variety. It has been established that the content of tannins in the obtained extracts varies from 5.72 to 6.25%, which is approximately 1.2 times higher than in the control sample of the extract not subjected to US-treatment. It is noted that that US-treatment with a power of 300 W leads to a significant heating of the sample, so its use for the extraction of biologically active substances is impractical.

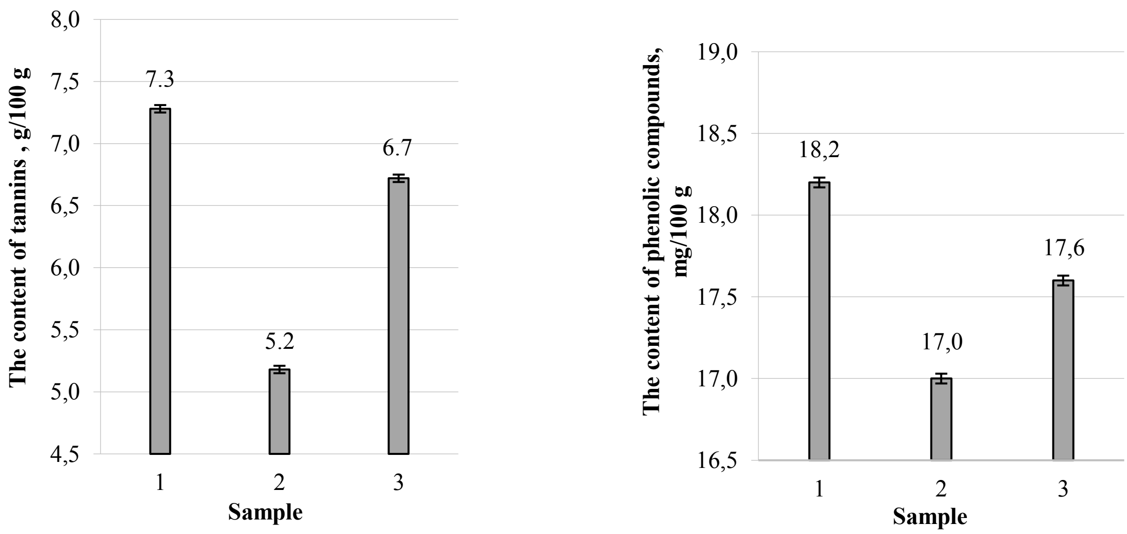


Fig. 1. The content of tannins and phenolic compounds in the pellicle of the *Juglans regia* L. studied varieties: 1 – Valentina's Gift, 2 – Burlyuk, 3 – Alminsky

The dynamics of extraction of tannins from pellicles under US-treatment is shown in Figure 3. It turned out that the duration of exposure to US-treatment for more than 10 min reduces the yield of tannins in extracts. The maximum yield of tannins under the selected extraction conditions is observed during US-treatment with a power of 200 W for 10 min. It should be noted that an increase in the time of extraction of tannins up to 48 h is more effective, since it helps to increase the yield of tannins in extracts.

Figure 4 (a and b) shows the spectral profiles of the obtained extracts from the *Juglans regia* L. pellicles in the UV and visible spectrum.

The absorption spectra in the UV spectrum (a) shows the presence of 3 expressed highs in the range from 340 to 350 nm, which are most likely an additional maximum absorption spectra of phenolic derivatives of the C6-C3 series. It is known that this series includes derivatives of cinnamic acid, which are found in all higher plants. Their characteristic feature is the presence of optical isomerism, as evidenced by the absorption spectra character [29, 30]. It was noted that an increase in the ultrasonic power up to 200 W leads to a decrease in the absorption intensity, which is probably due to destructive changes in the molecules of phenolic compounds.

An expressed absorption maximum in the visible spectrum (b) was recorded in all three samples of extracts in the range from 630 to 700 nm. In the extract obtained by US-treatment with a power of 100 W, an expressed absorption maximum was observed at 670 nm, which is absent in the control sample and in the sample obtained by US-treatment with a power of 200 W. Probably, the use of ultrasound with a power of 200 W leads to the polyphenol oxidation under the influence of cavitation. Therefore, the use of ultrasound with a power of more than 100 W to increase the extractability of polyphenolic substances from *Juglans regia* L. pellicles is undesirable.

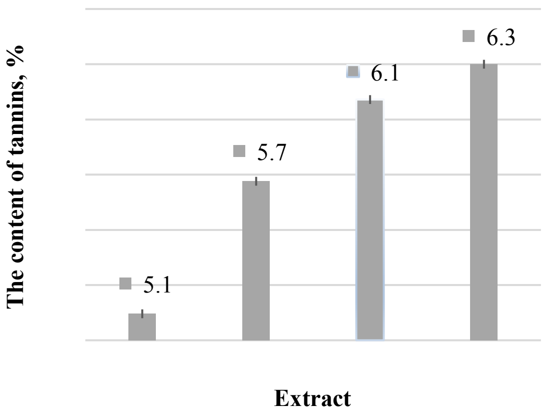
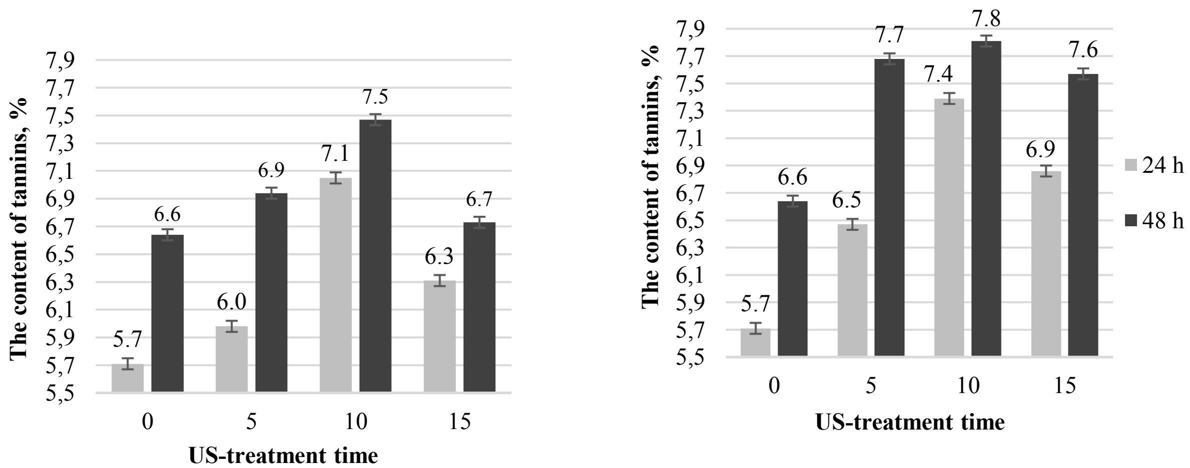
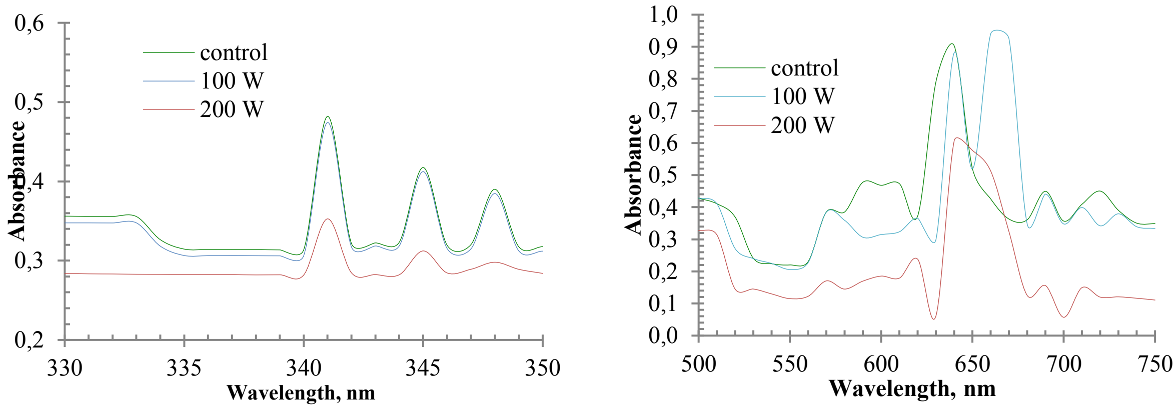


Fig. 2. Influence of US-treatment on the content of tannins in extracts from the *Juglans regia* L. pellicles: 1 – control; 2 – 15 min, 100 W; 3 – 15 min, 200 W; 4 – 15 min, 300 W



a *b*

Fig. 3. Effect of US-treatment on the extraction of tannins from the *Juglans regia* L. pellicles: *a*) 100 W, *b*) 200 W



a *b*

Fig. 4. Spectral profiles of extracts from the *Juglans regia* L. pellicles: *a*) UV spectrum (1 : 50 dilution); *b*) visible spectrum (1 : 6 dilution)

Conclusions

It was noted that the pellicles of the *Juglans regia* L. studied varieties of the Crimean selection are characterized by a high content of polyphenols. Thus, the content of tannins in the pellicle biomass of the Valentina's Gift variety is 7.3 g/100 g, and phenolic compounds – 18.2 mg/100 g. The efficiency of US-treatment in the extraction of polyphenolic substances from the pellicle biomass of *Juglans regia* L. that the most effective extraction of polyphenolic substances is achieved by processing extracts with ultrasonic power of 200 W and a duration of 10 min. However, the spectral profiles of the studied extracts indicate that 200 W US-treatment is less effective than 100 W, as it leads to a decrease in absorption and a change in the spectral profiles of the extracts.

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