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Influence of grinding, shaking and ultrasonic vibrations on tannins extraction from oak bark

Tannins are the class of polyphenolic plant biomolecules, which have an astringent effect. Their biological activity is based on the ability to bind and precipitate proteins or other organic compounds, including alkaloids and amino acids. Tannins are widely distributed in many species of plants, but in this work, oak (*Quercus robur* L.) bark is used as a source of protein precipitating tannins. Plants use tannins for protection against bacteria, fungi, and some insects. Tannins are mainly used by industry for leather processing. Recently, antiviral activity of tannins has been discovered. Tannins showed antiviral activity against HIV and herpes simplex virus 2. In the last case, silver nanoparticles with tannic acid act as microbicide by preventing adsorption of viral particle by the cell [1]. In relation to HIV infection, substances inhibited the replication of HIV-1, since all these tanning substances used in the experiment significantly inhibited the production of the p24 antigen (a structural protein that makes up most of the HIV viral core) [2]. The effect of tannins on protozoa was also revealed. Free-living protozoa species of *Acanthamoeba* genus generally cause significant infections of keratitis and encephalitis. Both pure silver and gold nanoparticles and tannic acid-modified nanoparticles of silver and gold were prepared, and their activities were tested against the clinical strains of *Acanthamoeba* spp. The tannic acid-modified nanoparticles proved more effective and less toxic to eye infection. Moreover, tannic acid-modified silver nanoparticles were well absorbed by the trophozoites eventually inhibit germination of cyst which is a major stage of life cycle of amoeba parasite [3].

The main aim of the work is to evaluate the influence of various factors on the extraction of tannins from oak bark. Tannins were extracted with 50 % water-ethanol solution for 20 min. We have applied the combinations of treatments for extraction efficiency improvement:

- shaking of beakers with extracts on laboratory shaker SHO-10 during extraction;
- sonication of beakers with extracts on laboratory ultrasonic bath UM-4 during extraction;
- grinding of oak bark to the particles of size 0.8 mm by the laboratory grinding machine MLT-2;
- non-ground oak bark samples were used as a control.

The content of protein precipitating tannins was evaluated by radial diffusion assay [1]. In this method, the interaction of tannins with 0.1 % bovine serum albumin in 2 % agar gel is quantified. The insoluble precipitates form rings around a well ($d = 6$ mm) with extract in agar after

24 hours (Fig. 1). The diameter of the rings is proportional to the tannin contents present in bark extracts. The diameters of the rings around the wells were measured three times and averaged. Results of precipitate ring diameters measurements are shown in table 1.

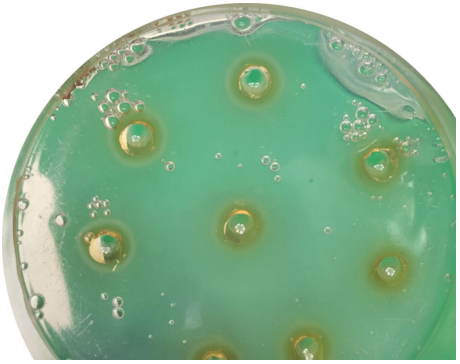

Fig. 1. Results of a radial diffusion test for tannins in extracts of oak bark

Table 1. Diameters of precipitate rings, mm

Treatment	Replications								Mean
	1	2	3	4	5	6	7	8	
Shaking of ground samples	11.33	11.67	11.00	10.67	11.33	12.00	11.67	11.33	11.38
Shaking of non-ground samples	10.33	9.33	10.00	10.33	10.67	10.33	11.00	10.33	10.29
Sonication of ground samples	6.00	7.33	6.67	10.67	9.00	8.67	11.33	9.67	8.67
Sonication of non-ground samples	8.00	7.33	7.33	7.67	8.67	6.67	7.33	6.33	7.42

The performed project had four parts. In each part, the null hypothesis was proposed, that there was no difference between the two means of precipitate ring diameters. The t test was used for testing the hull hypothesis in MS Excel.

In the first part of the project, we compared tannin contents in shaken extracts from ground and non-ground oak bark samples. The value of t obtained was 4.79 and the probability of obtaining this value for a twotailed test was $0{,}28 \cdot 10^{-3}$ (< 0.05), so we are able to reject the null hypothesis and conclude, that there is a significant difference between tannin content in extracts obtained with shaking of ground and non-ground oak bark samples.

In the second part of the project, we compared tannin contents in sonicated extracts from ground and non-ground oak bark samples. The value of t obtained was 1.74 and the probability of obtaining this value for a two-tailed test was 0.1034 (> 0.05), so we are able to accept the null

hypothesis and conclude, that there is no significant difference between tannin content in extracts obtained with sonication of ground and non-ground oak bark samples.

In the third part of the project, we compared tannin contents in shaken and sonicated extracts from ground oak bark. The value of t obtained was 3.95 and the probability of obtaining this value for a twotailed test was 0.0014 (< 0.05), so we are able to reject the null hypothesis and conclude, that there is a significant difference between tannin content in extracts from ground oak bark obtained with shaking and sonication treatment.

In the fourth part of the project, we compared tannin contents in shaken and sonicated extracts from non-ground oak bark. The value of t obtained was 9.28 and the probability of obtaining this value for a twotailed test was $0{,}23 \cdot 10^{-6}$ (< 0.05), so we are able to reject the null hypothesis and conclude, that there is a significant difference between tannin content in extracts from non-ground oak bark obtained with shaking and sonication treatment.

As a result, we propose to use grinding and shaking of oak bark in order to improve tannin extraction efficiency. Experimental results show that ultrasound treatment has no statistically significant effect on tannin extraction from oak bark.

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