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Anti-Bacterial Activity of the Vallus Tissue Ballota Nigral L in Vitro

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ABSTRACT

The antibacterial activity of *Ballota nigra* L. callus tissue obtained in vitro against Escherichia coli and Staphylococcus aureus was studied. The sterilization mode of plant explants was optimized when introduced into an in vitro culture. The most effective nutrient media for the growth of *B. nigra* cell cultures were selected. A comparative analysis of the antibacterial activity of plant extracts obtained from callus tissues and intact plants was carried out. It was found that plant extracts from callus tissue *B. nigra* and all its dilutions had greater antimicrobial activity against *E. coli* than extracts from parts of an intact plant (flowers and leaves) and 100% extracts from callus tissues and their dilution 1:10 and 1: 100 exhibited a practically similar antibacterial effect to *S. aureus*, as did an intact plant.

Keywords: Antibacterial activity, callus tissue, *Ballota nigra* L., plant extracts, cell cultures, in vitro, *Escherichia coli*, *Staphylococcus aureus*.

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INTRODUCTION

The modern flora provides great opportunities for the use of plant objects in such industries as pharmaceutical, food, cosmetic, agricultural. However, plant stocks are declining, and their special traditional cultivation requires considerable effort. Therefore, the use of new biotechnologies, which make it possible to obtain valuable plant components in the laboratory, is currently relevant. One of the promising tasks of biotechnology is to obtain drugs based on plant tissue culture. Such in vitro cultivation technologies reduce financial and temporary labor costs and eliminate the seasonality of work and allow us to get an environmentally friendly, biosafe plant component, regardless of where the plant naturally grows [1-5].

Therefore, an alternative method for the synthesis of cost-effective biologically active substances is the in vitro culture of plant cells and tissues [6]. With the right cultivation technology, the productivity of callus crops and the quality of biomass of plant raw materials are many times higher than in intact plants. On the basis of this promising method, drugs such as reserpine, raunatin, and ajmaline from the cells of *Rauvolfia serpentina*, which gives 5 times more ajmaline alkaloid than the intact diosgenin plant, from the cells of *Dioscorea*, bio-ginseng, produced from callus tissue of *Panax*, shikonin synthesized from the cell culture of *Lithospermum erythrorhizon* were produced [1, 7].

One of the promising plants is the black horehound (*Ballota nigra* L.) - a perennial herbaceous plant of the Lamiaceae family. The composition of *B. nigra* grass is represented by terpene, phenolic compounds [8-10]. The plant is also rich in organic acids, flavonoids, alkaloids, diterpenes, in particular marubin and ballotinone, phytosterols, up to 13% tannins, choline, tannins, bitterness, and pectins. Glycosides and polyphenols possess neurosedative properties. Flavonoids can have decongestant and hemolytic effects. Diterpenes have an antispasmodic effect on smooth muscles. Phenolic acids have antioxidant, anti-inflammatory and antimicrobial properties. In addition, it was found that plant extracts of *B. nigra* had antifungal and antiprotozoal activity [11-13]. It is this factor that makes this species an interesting and relevant object for further research.

The aim of the work is to study the antibacterial activity of extracts from calus tissue of *B. nigra* obtained in vitro and in the aerial part of an intact plant.

MATERIAL AND METHODS

The materials and objects of the study were callus tissue obtained in vitro and intact *B. nigra* plants (flowers and leaves) growing in Belgorod Region, as well as test objects, gram-negative bacteria of the species *Escherichia coli* (strain VKPM-M17) and gram-positive - *Staphylococcus aureus* (strain MDC 5233).

All work and experiments with plant explants, callus tissues and microorganisms were carried out in the Lamsystems class II and class III protection laminar boxes, type A2-A3 (manufactured by Russia, ZAO Laminar Systems) in compliance with aseptic rules [14]. Obtaining callus tissues was carried out by introducing plant explants of *B. nigra*, collected in the Belgorod region, into in vitro culture. Plant explants were subjected to step sterilization. First, plant material, washed in a soap solution, was placed in 70% ethanol for 1 minute, and then in a sterilizing solution of a certain concentration for a certain time and was washed three times in sterile distilled water. As sterilizers we used: lysoformin 3000 (5%), biocide (5%), sodium hypochlorite (5%, 2.5%), chloramine B (5%), mercuric chloride (0.1%). The exposure time for each disinfectant was 3 and 5 minutes. Then, sterile plant explants were wounded in order to induce callusogenesis and cultivated on modified nutrient media Murashige and Skoog of various compositions with the addition of various concentrations of phytohormones IAA, BAP and sucrose (20, 30 g/l) [15]. Callus tissues were cultured in a thermostat at a temperature of 23.5 ° C and passaged every 3-4 weeks for fresh nutrient in order to propagate and support cultures.

Plant extracts of callus tissue and intact *B. nigra* plants were obtained using the method of preparation of alcoholic extracts [16]. After obtaining a 100% plant extract, the procedure for obtaining serial dilutions was carried out according to the standard procedure [17]. For

the preparation of plant extracts from callus tissue, cell cultures of passage 2 were used.

The study of antimicrobial activity was carried out using the disk diffusion method. Diurnal cultures of *E. coli* (strain VKPM-M17) and *S.aureus* (strain MDC 5233) were preliminarily obtained on slant agar using the timing medium; microorganism suspensions were prepared according to standard methods [18]. Filter disks with a diameter of 14 mm were impregnated with the studied solutions (extracts). For the control, a 40% solution of ethanol (included in the extracts) and antibiotic solutions were used. The control antibiotic gentamicin was used for *E. coli*, and ceftriaxone for *S. aureus*. The diameters of the zones of growth inhibition of microorganisms were evaluated according to standard indicators [18]. Data processing was performed on the statistical analysis by Microsoft Excel software package. The following statistical characteristics were used: arithmetic mean (x), mean error (Sx). To assess the significance of differences between the control and experimental groups, the Fisher test was used [19].

RESULTS AND DISCUSSION

Obtaining callus tissues of *B. nigra* was carried out by introducing plant explants (leaf blades) into in vitro culture. To determine the most effective sterilizing agent plant explants were sterilized with five disinfecting solutions: lysoformin 3000 with a concentration of 5%, biocide with a concentration of 5%, sodium hypochlorite with a concentration of 5% and 2.5%, chloramine B 5%, a prominent concentration of 0.1%, with an exposure time of 3 and 5 minutes. The results made it possible to indicate (Table 1., Fig. 1.) that the most effective sterilizing solution for leaf blades of the *B. nigra* species is sodium hypochlorite at a concentration of 5% when exposed for 5 minutes.

With this sterilization regime, the maximum number of viable plant explants was obtained (93.3% sterile and 66.6% viable explants), which were capable of inducing callus tissue. Reducing the exposure time of this sterilizer to 3 minutes leads to a decrease in the number of viable explants (40%) and sterile seeds (80%). It is also possible to use a mercuric chloride 0.1% for 3-5 minutes but reducing the sterilization time to 3 minutes also leads to a decrease in the production of viable and sterile *B. nigra* explants. The remaining sterilization regimes using 2.5% sodium hypochlorite, 5% chloramine B, 5% biocide and 5% lysoformin did not produce viable explants, the explants were covered by infection and did not lead to callusogenesis, so their use is not advisable.

Table 1. The effect of sterilizing solutions on plant explants *B. nigra*

Sterilizing solution and its concentration	Sterilization duration (minutes)	Sterile explants number (%)	Viable explants number (%)
Sodium hypochlorite (5%)	3	80±3,09	40±1,54
Sodium hypochlorite (5%)	5	93,3±3,61	66,6±2,57
Sodium hypochlorite (2,5%)	3	53,3±2,06	0±0
Sodium hypochlorite (2,5%)	5	60±2,3	0±0
Sulema (0,1%)	3	66,6±2,5	6,66±0,25
Sulema (0,1%)	5	86,6±3,35	13,33±0.51
Chloramine B (5%)	3	33,3±1,28	0±0
Chloramine B (5%)	5	46,6±1,79	0±0
Biocide (5%)	3	13,3±0,51	0±0
Biocide (5%)	5	26,6±1,03	0±0
Lysoformin 3000 (5%)	3	0±0	0±0
Lysoformin 3000 (5%)	5	6,66±0,25	0±0
Control (distil. water)	30	0±0	0±0

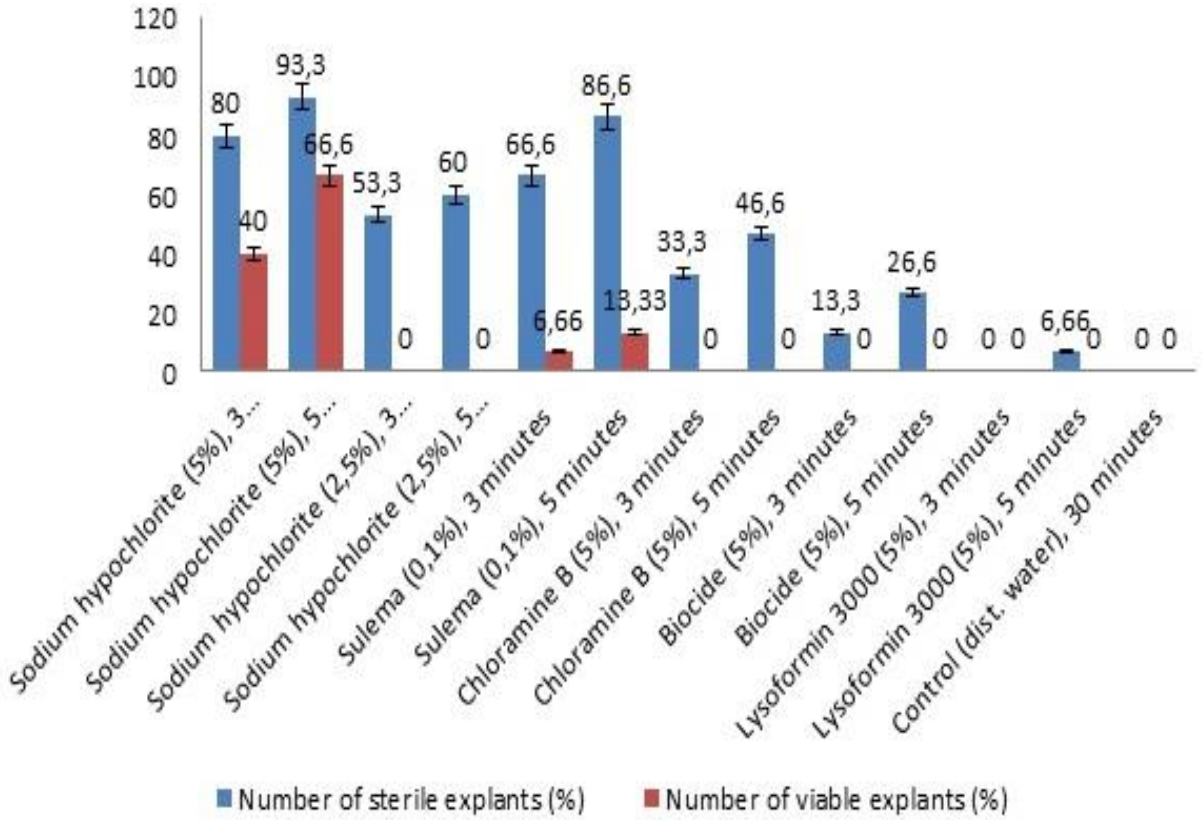

Fig 1. The effect of sterilizing solutions on the ratio of sterile and viable explants of the *B. nigra* species

Using the Fisher criterion, it was found that at a significance level of P> 0.05, all values of viable and sterile *B. nigra* explants, presented in Table 1. are statistically significant. Using the Fisher criterion, it was found that at a significance level of P> 0.05, all values of viable and sterile *B. nigra* explants, presented in Table 1. are statistically significant. Viable isolated cultures, obtained by sterilization, were grown on various modified nutrient media in order to obtain callus tissues.

To conduct a comparative analysis of the antimicrobial properties, alcoholic plant extracts were obtained from callus tissues cultured in vitro in the laboratory and from the flowers and leaves of intact plants, collected in Belgorod Region. The results of the antimicrobial action of the extract from the flowers, leaves of an intact plant and callus tissue of B. nigra on microorganisms of the species E. coli and S. aureus are presented in Table 2 and diagrams (Fig. 2, 3).

Table 2. The number of sterile and viable plant explants *E. purpurea* the effect of plant extracts from flowers, leaves of an intact plant and callus tissue of *B. nigra* on microorganisms *E. coli* and *S. aureus*

Extract concentration	Diameters of the zones of growth inhibition of microorganisms, mm					
	Intact plant				Callus tissue	
	Flowers		Leaves			
Microorganisms	*E. coli*	*S. aureus*	*E. coli*	*S. aureus*	*E. coli*	*S. aureus*
100%	6,68±2,22	9,5±3,1	5,98±1,9	10,1±3,3	8,8±2,9	9,18±3,06
1:10	5,39±1,79	6,51±2,17	3,75±1,25	8,04±2,68	7,69±2,56	6,94±2,31
1:100	2,64±0,88	1,16±0,38	3,65±1,2	5,75±1,9	6,18±2,06	6,08±2,02
1:1000	2,97±0,99	1,07±0,35	3,2±1,06	1,61±0,53	5,69±1,89	2,64±0,88
1:10000	1,19±0,39	1,08±0,36	1,07±0,35	0,97±0,32	4,73±1,57	2,56±0,85
control	1,21±0,4	4,18±1,3	3,66±1,2	3,96±1,32	1,92±0,6	0,23±0,07
antibiotic	10,46±3,48	9,96±3,32	8,6±2,8	9,2±3,06	9,9±3,3	9,98±3,32

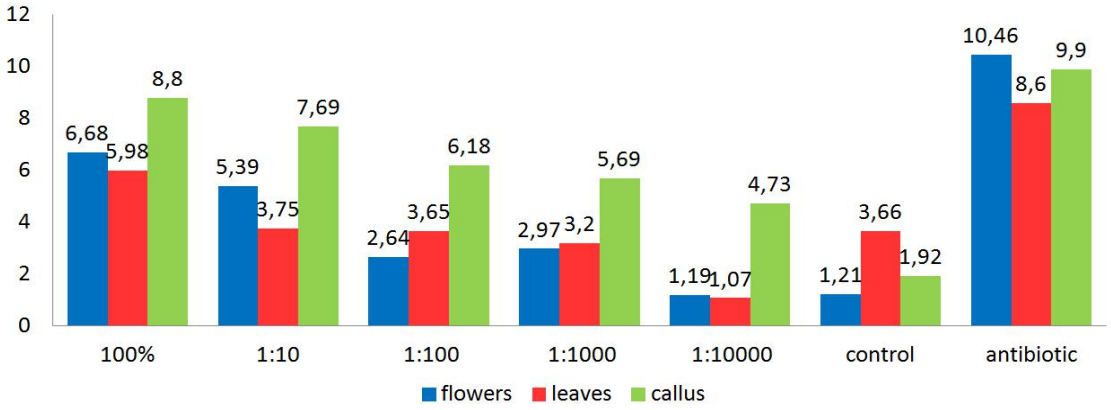

Fig 2. Comparative analysis of the antimicrobial activity of extracts of *B. nigra* from flowers, leaves and callus tissue in relation to *E. coli.*

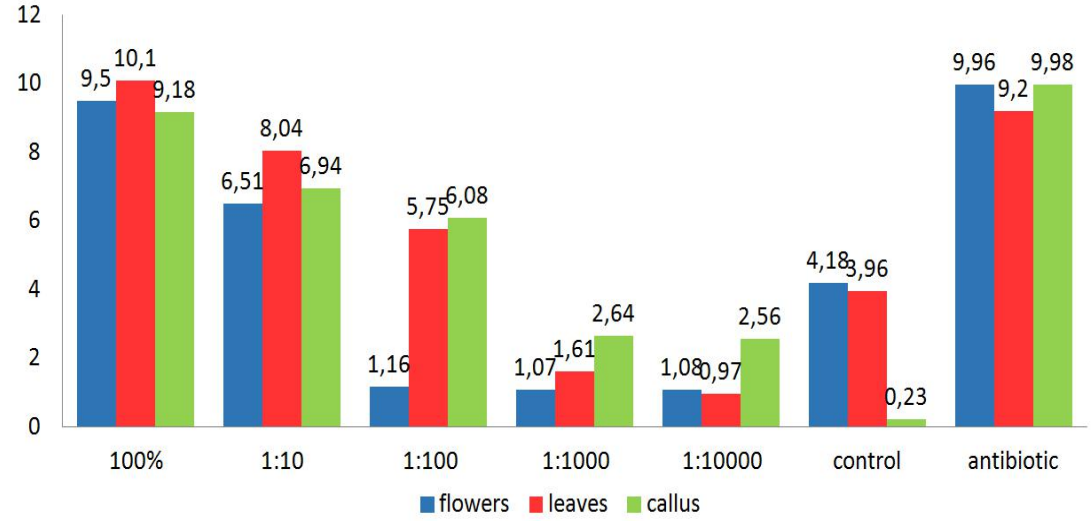

Fig 3. Comparative analysis of the antimicrobial activity of extracts of *B. nigra* from flowers, leaves and callus tissue in relation to *S. aureus.*

As it can be seen from table 2 and Fig. 2, 3, 100% extract from the flowers of the intact *B. nigra* plant and its dilution 1:10, 1: 100, 1: 1000 and 1: 10000 exhibit weak antibacterial activity against *E. coli.* In relation to *S. aureus*, 100% extract and its 1:10 dilution also have a weak antimicrobial effect. A 100% plant extract from the leaves of an intact *B. nigra* plant and its dilution 1:10 exhibit a weak antimicrobial effect against *E. coli.* Dilutions of 1:10 and 1: 100 of leaf extract in relation to *S. aureus* also have a weak antibacterial effect, and 100% of the extract exhibits moderate antimicrobial activity.

As shown in table 2 and figure 2 and 3, 100% extract from callus tissue *B. nigra*, as well as all its dilutions have a weak antimicrobial effect against *E. coli,* and 100% extract from callus tissue *B. nigra,* and its dilutions of 1:10 and 1: 100 exhibit weak antimicrobial activity against *S. aureus.* The indicators of all other dilutions were below the control value, therefore, did not have antibacterial properties.

Based on the above and comparative diagrams in Fig. 2, 3, 100% extract from callus tissue *B. nigra* and all its dilutions have greater antimicrobial activity against *E. coli* than extracts from flowers and leaves of an intact plant. But 100% extracts from callus tissues and their dilutions of 1:10 and 1: 100 exhibit a practically similar antibacterial effect to *S. aureus.*

CONCLUSION

Therefore, the study of the callus culture of B. nigra has further prospects and can be used to obtain callus tissue in bulk to synthesize substances with the antibacterial action, as well as to obtain a cell culture at different passages of cultivation and to test it on a wider range of microorganisms.

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