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Research article

Antimicrobial Activity of Aqueous Extracts of Leaves and Silage from *Paulownia elongata*

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Abstract

Studies to determine the *in vitro* antimicrobial activity of fresh leaf of *Paulownia elongata* and silage derived from it were performed in order to assess the capabilities for decontamination after the entry of pathogens, with regard to the epizootiological safety of such feed. Eighteen pathogenic bacterial strains were used, belonging to the species *Salmonella enterica, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes, Paenibacillus alvei* and *Candida albicans.* The experiments were conducted using the classical agar-gell diffusion method of Bauer-Kirby and the method for determining of the minimum inhibitory concentrations (MICs). The average value of the MIC_{50} of the aqueous extract of the leaves of *Paulownia elongata* was 80.6 ± 19.5 μg/mL, while of the aqueous extract of the silage – 58.3 ± 13.9 μg/mL. It was found that the leaves of the plant and silage of them exhibit inhibitory activity *in vitro*, as the effect being more pronounced against the Gram-negative bacteria.

Keywords: *Paulownia elongata*, silage, antimicrobial activity, minimum inhibitory concentrations.

1. Introduction

Paulownia elongata is a fast developing plant that in recent years began to be cultivated in Bulgaria. At an early age the *Paulownia* trees show intensive growth combined with excellent characteristics for use in wood, fodder, ornamental and medicinal purposes. They thrive even under more severe weather conditions, such a semi arid regions of northern Mexico at an altitude of over 1900 m and temperatures ranging from -18 to 35° C [1]. All species of the genus are fast growing trees, which is why some of them like *Paulownia elongata, Paulownia tomentosa, Paulownia fortunei* and others are used for industrial timber, feed, receiving of ethanol, paper and others. Historically China's the largest manufacturer of *Paulownia* spp. These species are cultivated also as park trees. As they blossom abundantly, are also used as a honey plants. Their colors are rich in nectar and the honey derived from them is with extremely high quality [2].

Foliage of *Paulownia spp*. in its composition is particularly suitable for preparing of forage for herbivores. It has a high protein content (approximately 20%) and its qualities are similar to those of alfalfa. Large yields provide low cost food, which is one of the most important parameters in the selection of feed in industrial livestock [2]. Very good results in feeding the goats with such feed reported [3] in the U.S. The leaves are very tasty to the animals. According [4] nutritional value of the fallen leaves surpasses that of the straw from wheat or rice. In China, it is widely used as a cheap source of feed fed to pigs and ruminants. Fodder by *P. elongata* is of high quality and favorable levels of energy and protein [5]. In our country also has tendency to use it.

Fallen leaves, however, may be carriers of microorganisms, including pathogenic for animals, which easily fall from the soil and fertilizers, as well as by dissemination by rodents and other animals. Therefore, this study aims to investigate the leaves and silage *P. elongata* for antimicrobial effect on pathogens in order to assess the potential for decontamination and the epidemiological safety of their providing as food for animals. To assess the effects of leaves and silage in this respect is essential the availability of data for the minimum inhibitory concentrations for microorganisms of different groups. Since such data are not found in the available literature, the purpose of these studies is to determine the smallest concentrations that inhibit the multiplication of Gram-positive and Gram-negative microorganisms.

2. Materials and Methods

2.1. Extracts.

The effect of leaves and leaf silage from *Paulownia elongata* was tested. Extracts (10%) were prepared by boiling for 5 min in distilled water, followed by filtration through gauze and fractionated sterilization with water vapor. In determining the antimicrobial action of the substances, as a control was used the antibiotic thiamphenicol with broad spectrum activity.

2.2. Microorganisms.

Eighteen strains by different types of Gram-negative and Gram-positive microorganisms were used, respectively, by three strains of *Salmonella enterica, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes, Paenibacillus alvei* and *Candida albicans.* They were isolated from animals with various chronic infections, and *P. alvei* - from honeycombs with European foulbrood.

2.3. Antimicrobial tests.

The classical agar-gel diffusion method of [6] was used. Bacterial suspensions were inoculated at a dose of 2.10^6 cells/mL on Mueller-Hinton's agar (Antisel - Sharlau Chemie S. A., Spain) with pH 7.2 – 7.4 in Petri dishes with diameter 9 cm. The extracts and control were applied by dropping by 0.1 mL (30 μg of antibiotic) in 9-mm wells in agar of Mueller-Hinton with a thin agar layer at the bottom. Results were recorded by measuring the diameters of inhibitory zones in mm, including the hole diameter, after incubation during 24 h at 37°C. According to the tree-degree system of Bauer-Kirby, inhibitory effect was established at zones > 12 mm for the extracts and >17mm for the antibiotic.

The determination of the *minimum inhibitory concentrations* (MICs) was performed by the method of twofold serial dilutions on Mueller-Hinton's agar (Antisel - Sharlau Chemie S. A., Spain) with pH 7.2-7.4, as per [7]. Bacterial suspensions were inflicted at a dose of 10^6 cells/ml. After incubation at 35-37°C for 18 to 24 hours the number of developed colonies was determined. The extracts were applied in double increasing concentrations from 64 to 2048 μg/mL agar. MIC_{50} and MIC_{90} were calculated mathematically depending on the number of the colonies of the agar medium with the respective extract or antibiotic dilution compared to the control medium colonies without drugs.

2.4. Statistical analysis.

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett post-hoc test.

3. Results and Discussion

3.1. In the agar-gel diffusion method.

In the research conducted by the diffusion method of [6] was found that both extracts show antimicrobial effect. The results are presented in Table 1.

The data indicate high sensitivity of Gram-negative bacteria and *C. albicans* and lower - of staphylococci and streptococci. The studied bacilli also not showed high sensitivity to the extract of silage. Differences between types of microorganisms are not significant, except those between *S. pyogenes* and *S. enterica* ($P<0,05$).

In the silage was reported pH 4.0, in the aqueous extract of silage – 4.5, and in the extract of the leaves – 6.5.

3.2. Determination of the minimum inhibiting concentrations.

The results from the studies to determine the minimum inhibiting concentrations of aqueous extract of silage from *Paulownia elongata*, conducted for 18 strains of 6 different types of Gram-positive and Gram-negative pathogens, are presented in Table 2.

As can be seen from the summary data, the MICs of the silage for most organisms tested were not high (50.0±0.0 μg/mL). Slightly higher concentrations were needed to suppress the growth of a sustainable to chemical effects *Pseudomonas aeruginosa* – 66.7±28.8 μg/mL, and of *Streptococcus pyogenes* – 83.3±28.8 μg/mL, but differences with the other types were not significant (P>0.05).

From Table 3 one can see the results of the studies conducted to determine the minimum inhibiting concentrations of leaves from *P. elongata*, conducted for 18 strains of 6 different types of pathogens.

The data shows that the most sensitive to the inhibitory action of the extract of the leaves were *Paenibacillus alvei* and *Salmonella enterica* (MIC_{50} respectively 50.0±0.0 and 66.7±28.8 μg/mL). *Streptococcus pyogenes* and *Candida albicans* were inhibited by higher concentrations ($МПК_{50}$ – 100.0±0.0 μg/mL). The differences between the averages for the various types of microorganisms studied, however not statistically significant (P>0.05).

Obviously, aqueous extracts of leaves and silage from *P. elongata* exhibit antimicrobial effect on all tested microorganisms. Sensitive to their effects are bacteria (positive and Gram negative) and oval fungi *Candida albicans*. The studied microorganisms exhibit higher sensitivity to the extract of silage. This is probably due to its lower pH. It is worth noting the high sensitivity of *Paenibacillus alvei*, which indicates that this plant extract could possibly be used to prevent European foulbrood in bee larvae. *Streptococcus pyogenes* and *Candida albicans* show lowest sensitivity and for inhibiting their development are required the highest concentrations of leaf extract.

The data presented in the tables show that the extract of silage has a higher antimicrobial effect than that of leaves. The reported average MIC_{50} values were respectively 58.3±13.9 and 80.6±19.5 μg/mL, although P>0.05. The differences between the two extracts are more indicative and statistically significant (P<0.05) at comparing the MIC_{90}, the average values in which are respectively 161.1±38.9 and 233.3±42.2 μg/mL.

It is important that the high nutritional value of the leaves and silage from *Paulownia elongata* is complemented by their antimicrobial activity against several common pathogens, established by us, which increases the value of their usage in livestock. Application of such feed would have and preventive role against development of some common conditionally pathogenic microorganisms in livestock.

Interestingly, the leaves are valuable not only in agriculture but also from a pharmaceutical point of view. They contain substances that benefited the liver and kidneys, and also work well at pulmonary diseases, with which may additionally be useful for animal health. In China some of such its properties are known long ago and even there, the

pharmaceutical industry is concerned with industrial manufacture of drugs obtained from leaves of *Paulownia* spp. [2]. Our findings of the antimicrobial activity further enhances value of the leaves from *Paulownia elongata* from a medical standpoint.

Conclusion

Water extracts of the leaves of *Paulownia elongata*, and silage of them exhibit antimicrobial activity *in vitro* against *Salmonella enterica, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes, Paenibacillus alvei* and *Candida albicans.* The inhibitory effect is better expressed against Gram-negative bacteria. After ensiling inhibitory effect of leaves increases, probably due to the lowering of pH and supplementing it with the effect of the resulting lactic acid.

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Tables

Table 1: Inhibitory effect of 10% aqueous extracts of silage and leaves of *Paulownia elongata* on pathogenic microorganisms in the agar diffusion method.

Microorganisms	N of strains tested	Inhibition zones in mm		
		Silage	Leaves	Thiamphenicol
S. pyogenes	3	**13,5**±0,3	**13,3**±0,7	**29,7±0,6**
S. aureus	3	**15,8**±2,5	**16,5**±2,2	**24,0±6,0**
P. alvei	3	**13,4**±3,2	**12,7**±2,7	**13,0±2,0**
P. aeruginosa	3	**14,9**±2,3	**13,3**±1,8	**22,3±5,5**
S. enterica	3	**17,3**±2,1	**16,5**±2,2	**19,7±6,6**
Candida spp.	3	**16,3**±0,7	**15,0**±0,5	**26,3±4,6**
Total	18	**15,5**±2,0	**14,6**±1,7	**22,5±5,8**

Table 2: Minimum inhibiting concentrations (MICs) of aqueous extract of silage from *Paulownia elongata* for pathogenic microorganisms.

Microbial species	N of strains tested	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)
Salmonella enterica	3	**50,0**±0,0	**100,0**±0,0
Pseudomonas aeruginosa	3	**66,7**±28,8	**166,7**±57,7
Staphylococcus aureus	3	**50,0**±0,0	**200,0**±0,0
Streptococcus pyogenes	3	**83,3**±28,8	**200,0**±0,0
Paenibacillus alvei	3	**50,0**±0,0	**133,3**±57,7
Candida albicans	3	**50,0**±0,0	**166,7**±57,7
Total	18	**58,3**±13,9	**161,1**±38,9

Table 3. Minimum inhibiting concentrations (MICs) of aqueous extract of leaves from *Paulownia elongata* for pathogenic microorganisms.

Microbial species	Number of strains tested	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)
Salmonella enterica	3	**66,7**±28,8	**266,7**±57,7
Pseudomonas aeruginosa	3	**83,3**±28,8	**300,0**±0,0
Staphylococcus aureus	3	**83,3**±28,8	**200,0**±0,0
Streptococcus pyogenes	3	**100,0**±0,0	**200,0**±0,0
Paenibacillus alvei	3	**50,0**±0,0	**233,3**±57,7
Candida albicans	3	**100,0**±0,0	**200,0**±0,0
Total	18	**80,6**±19,5	**233,3**±42,2