ORIGINAL PAPER

# ANTIBACTERIAL ACTIVITY OF SPRUCE BARK (*PICEA ABIES* L.) EXTRACT AGAINST *ESCHERICHIA COLI*

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**Abstract:** The increase of antibiotic resistant bacteria in lasts years resulted in limited options for treatment of bacterial diseases. *Escherichia coli* is one of the most common Gram-negative bacterial pathogen and a cause of both community and hospital acquired infections. Medicinal plants are alternative rich sources of useful antibacterial agents. The antimicrobial activities of the spruce (*Picea abies* L.) bark polyphenolic extracts were examined for their capacity to inhibit the growth of *Escherichia coli*. Spruce bark extract was obtained by conventional aqueous extraction and with ultrasounds. The minimum inhibitory concentration was determined by microdilution method. The antibacterial effect of both extracts was strong against *Escherichia coli*. The antimicrobial effect of polyphenolic extracts on *Escherichia coli* was expressed at a concentration of 15 mg/ml. Antimicrobial activity of spruce bark compounds suggest a possible use of spruce bark in pharmaceutical preparations.

**Keywords:** antimicrobial agents, *Escherichia coli*, polyphenols, spruce bark, *Picea abies* L.

## 1. Introduction

The drug resistance in human pathogenic bacteria and many adverse effects of antibiotics has led to a search for new antimicrobial agents with plant origin. It is known that plants produce antimicrobial compounds. Thus, crude plant extracts have been used for a various purposes for a very long time (Moradi et al., 2016; Sharif et al., 2016). The antimicrobial activity of crude plant extracts was the basis of various applications, such as food preservation, pharmacy, natural therapies and medicine (Lis-Balchin and Deans 1997; Dorman and Deans, 2000; Sathasivampillai et al., 2017).

According to the classification system from "Vascular plants from Romania - Field Illustrative Determinator" (Sârbu et al., 2013), *Picea abies* (L.) H. Karst has the following systematic classification: Vegetable Kingdom, Pinophyta Division, Pinatae Class, Pinales Order, Pinaceae Family, *Picea* Genus, *abies* species. Norway spruce is very widespread in the Romanian forests. Spruce present great economic importance, being used as construction wood, paper manufacturing, and in phytotherapy.

The literature data show many studies on volatile oil from spruce, with different antimicrobial activity and intensity depending on tested strains. The inhibitory effect was noticed against the Gram-positive and fungal strains (Radulescu et al., 2011; Chauhan and Dahiya, 2016). In the spruce bark alcoholic extract, was identified gallic acid, catechin and vanillic acid in high concentration (Ignat et al., 2013). The results of Ignat et al. (2013) showed that the spruce bark ethanol extracts exerted antibacterial activity against *Staphylococcus aureus* and for *Escherichia coli* and *Pseudomonas aeruginosa* being less susceptible.

The aim of this study was to evaluate the antimicrobial activity of *Picea abies* L. bark aqueous crude extracts against *E. coli*.

## 2. Materials and Methods

### 2.1 Plant/bacteria materials

Spruce (*Picea abies* L.) bark it is a waste product from a wood processing company (Vatra Dornei, Romania). The spruce bark was air-dried at room temperature and milled in a GRINDOMIX GM 2000 mill (0.5 mm diameter). The biomass was used without any pre-treatments.

To determine the antibacterial activity on *Escherichia coli,* ATCC 25922 strain was selected from the collection of Laboratory of Microbiology, Virology and Parasitology (Faculty of Medicine - University of Medicine and Pharmacy, Tîrgu Mureș).

### 2.2 Aqueous extraction

For extraction was used 20 g of ground spruce bark over which added 125 mL distilled water. The mixture was incubated for 45 min in a water bath at 85-90°C (Tanase et al., 2018a). Ultrasound assisted extraction was performed under ultrasounds action in accord with method previously described (Tanase et al., 2018a).

### 2.3 Minimum inhibitory concentration of *E. coli* (MIC)

To determine the MIC of the obtained extract against *E. coli*, the microdilution method was used, as previously described (Tanase et al., 2018a). Shortly, from fresh bacterial culture, a standard inoculum was prepared in liquid culture medium. One-hundred microliters of the tested extracts were mixed in the first well of the microplate with 100 µl of bacterial inoculum. We evaluated the MIC of the tested extracts in the first well with no bacterial growth. The MICs were calculated in mg/ml, by adjusting the obtained concentrations with the dilution factor and processed volumes.

*E. coli growth rate*

For *E. coli* growth rate determination, the method described in a previously paper was used (Tanase et al., 2018a). To determine if the tested solutions affect the *E. coli* growth rate, stock solutions of the both extracts was prepared. The concentration of the stock solution was adjusted to correspond to the MIC that was assessed by the microplate method. The total number of colony forming units/ml (CFU/ml) at time 0 (H0), 3 hours (H1), 6 hours (H2) was determined inoculating 50 µl from 1/100 diluted working solution and from 1/100 diluted control on Mueller Hinton agar plates. After 24 hours of incubation, the number of the colonies was counted using the colony counter "IUL Flash & Grow" and mathematically adjusted in order to be expressed as CFU/ml.

### 2.4 Statistical analysis

Using the calculated CFU/ml numbers from each time point, absolute growth curves were plotted. The following formula was used in order to assess the growth rate (*r*) for the tested sample and control, after 6 hours of incubation. The statistical significance was assessed by GraphPad InStat 3 software, at a significance threshold value of $p<0.05$.

$$r = \frac{LN(CFU/ml \text{ for } H2 - CFU/ml \text{ for } H0}{\text{no. hours for } H2}$$

## 3. Results and discussions

### 3.1 Extract characterization

The previous results summarized that spruce bark extracts (EAM and USM) contain considerable quantities of bioactive aromatic compounds (EAM - 0.135 mg GAE/mL and USM 0.114 mg GAE/mL). The compounds identified in samples by HPLC were vanillic acid and taxifolin in small amounts (Tanase et al., 2018b).

### 3.2 Minimum inhibitory concentration of *E. coli* (MIC)

The results indicate that the two aqueous extracts (EAM and USM) of different concentrations have antibacterial activity against *E. coli*. The minimum inhibitory concentrations (MIC) of polyphenolic extracts required for growth inhibition of *E. coli* was 15 mg/mL both for EAM and for USM.

*E. coli growth rate*

As shown in **Table 1**, when the growth medium was enriched with both tested solutions (EAM and USM), the bacterial growth was significantly inhibited, compared with control. After three hours of incubation, the growth of *E. coli* was significantly reduced (**Table 1**, **Fig. 1** and **Fig. 2**). At H2 time, EAM inhibited the growth of *E. coli* **(Fig. 1**), while USM presented bactericidal effect (**Fig. 2**). As a research direction, it would be interesting to investigate which component of the USM extract induced the bactericidal effect on *E. coli*.

The biological activity of *P. abies* and other species of the Pinaceae family was previously tested on other strains. For the aqueous extract of *P. abies* bark, *Staphylococcus aureus*, methicillin-resistant *S. aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were used (Tanase et al., 2018b). For the spruce bark ethanol extracts, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* were tested (Ignat et al., 2013). The ethereal extracts from *Pinus nigra*, *Pinus halepensis*, *Abies equi-trojani*, *Abies bornmulleriana*, *Abies cilicica*, *Abies nordmanniana*, *Cedrus libani* and *Picea orientalis* were examined against *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Candida albicans* (Eryilmaz et al., 2015). The authors concluded that all the tested extracts, except *Abies bornmulleriana*, *Cedrus libani* and *Pinus halepensis* showed weak antibacterial activity against the various tested bacteria comparing with the standards.

From previous studies, it is known that spruce bark contains vanillic acid and taxifolin (Tanase et al., 2018). Moon et al., (2006) concluded that vanillic acid accelerated the death of *E. coli*. It was also found that taxifolin have antibacterial action against human pathogens inclusive *E. coli* (Asmi et al., 2017). Thus, the vanillic acid and taxifolin identified in the spruce bark crude extract can have a role in antibacterial activity against *E. coli*. The results of the present investigation suggest that the spruce bark crude extracts can be used as potential leads to discover new antibacterial agents to control *E. coli* bacterial infections.

**Table 1.** Data presenting the growth rate of *E. coli* in presence of EAM and USM ($p<0.0001$)

| Experimental variants | CFU/ml | | | Growth rate ($h^{-1}$) | Generation time (min) |
|---|---|---|---|---|---|
| | **H0** | **H1** | **H2** | **r** | **g** |
| EAM | $1.4 \times 10^4$ | $2.8 \times 10^4$ | $3.1 \times 10^7$ | 1.28 | 32.42 |
| Control | $5.6 \times 10^4$ | $5.3 \times 10^6$ | $3.6 \times 10^8$ | 1.46 | 28.47 |
| USM | $2.8 \times 10^4$ | $2.1 \times 10^5$ | 0 | N/A | N/A |
| Control | $1.4 \times 10^4$ | $2.1 \times 10^6$ | $3.3 \times 10^8$ | 1.68 | 24.76 |

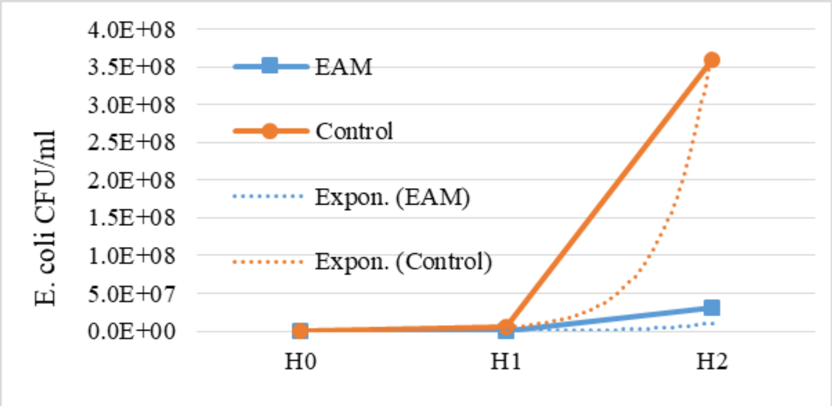


**Fig. 1.** The graphics representation of the growth rate for *Escherichia coli* in the presence of EAM comparing to the Control

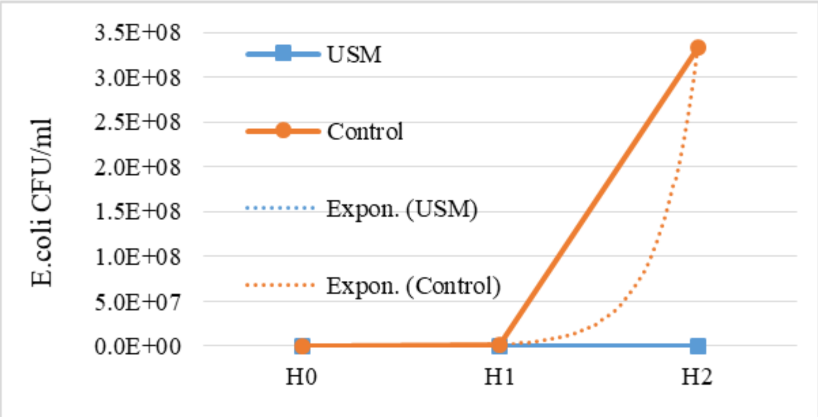


**Fig. 2.** The graphics representation of the growth rate for *Escherichia coli* in the presence of USM comparing to the Control

## Conclusions

The results of this study reveal that the spruce bark extract (obtained by hot water with or without ultrasounds) can be effective against Gram-negative bacteria such as *Escherichia coli*. Antimicrobial activity of this spruce bark extract, may suggest its use in pharmaceutical preparations. By demonstrating antimicrobial capacity of polyphenolic extracts, we can follow a new direction of research, namely reducing the pharmacological resistance of microorganisms to antibiotics, by using polyphenolic extracts.

## Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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