

STUDIES REGARDING THE ANTIBACTERIAL ACTIVITY OF SOME EXTRACTS OF NATIVE PTERIDOPHYTES

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*Au fost demonstrate proprietățile antioxidantă, antimicrobiale, antivirale, antiinflamatorii, antitusive, antitumorale, anti-SIDA și antihelmintice ale unor extracte de ferigă. Scopul acestei lucrări a fost evaluarea acitivității antibacteriale a extractelor obținute din trei specii de ferigă native: *Asplenium scolopendrium*, *Cystopteris fragilis*, *Polypodium vulgare*. Activitatea antibacterială a fost demonstrată pe cinci mostre de bacterii: *Staphylococcus aureus* ATCC 25923, *Streptococcus* sp., *Escherichia coli*, *Enterobacter cloacae* și o bacterie preluată din sol. Cel mai evident efect antibacterial a fost demonstrat pe extractul gametofit de *Cystopteris fragilis*. Mostrele de bacterii cele mai sensibile la activitatea extractului au fost cele de *Escherichia coli*, *Enterobacter cloacae*, și ale bacteriei preluate din sol.*

*The antioxidant, antimicrobial, antiviral, antiinflammatory, antitussive, antitumor, anti-HIV and antihelmintic properties of various fern extracts were demonstrated. The aim of this paper was the evaluation of antibacterial activity of extracts obtained from three native fern species: *Asplenium scolopendrium*, *Cystopteris fragilis*, *Polypodium vulgare*. The antibacterial activity was demonstrated against five bacterial strains: *Staphylococcus aureus* ATCC 25923, *Streptococcus* sp., *Escherichia coli*, *Enterobacter cloacae* and one bacterial strain isolated from soil. The most evident antibacterial effect was demonstrated for *Cystopteris fragilis* gametophytic extract. The most sensitive bacterial strains at extract's activity were *Escherichia coli* and the bacterial strain isolated from soil.*

Keywords: ferns, extract, antimicrobial, leaves, gametophytes

1. Introduction

Ferns and their allies are in a major division of the Plant Kingdom called *Pteridophyta*, and they have been around for millions of years. There are over 250 different genera of ferns and about 12 000 species [1]. It has been observed that

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pteridophytes are not infected by microbial pathogens, which may be one of the important factors for the evolutionary success of pteridophytes and the fact that, they survived for more than 350 million years [2]. Internationally, several researchers showed the therapeutic properties of certain fern species. Thus, the antioxidant, antimicrobial, antiviral, antiinflammatory, antitussive, antitumor, anti-HIV and antihelmintic properties of various fern extracts were demonstrated. The ferns and their therapeutic potential are extremely less exploit in Romania. Among 70 species of fern, in Illustrated Flora of Romania are mentioned just three local ferns used like medicinal herbs: *Huperzia selago* (L.) Schrank et Mart., *Lycopodium clavatum* L. and *Equisetum arvense* L. [3]. The aim of this paper was the evaluation of antibacterial activity of crude extracts obtained from leaves and gametophyte of three native fern species: *Asplenium scolopendrium* L., *Cystopteris fragilis* (L.) Bernh. and *Polypodium vulgare* L.

2. Experimental

Vegetable materials

Fern leaves from *Asplenium scolopendrium* (Aspleniaceae), *Polypodium vulgare* (Polypodiaceae) and fern gametophytes (obtained *in vitro*) from *Polypodium vulgare* and *Cystopteris fragilis* (Woodsiaceae) were used for extract preparation. The specimens of ferns species *Asplenium scolopendrium* and *Polypodium vulgare* were collected from Vâlsan Valley, Argeş County, Romania.

Preparation of plant extracts

5 g of fresh leaves were washed with tap water and distilled water and then surface was sterilized with 90% ethanol. Subsequently, the plant materials were grounded in 50 mL of methanol for alcoholic extracts. The methanolic macerates were kept for 24 h at room temperature. Macerates were squeezed through double-layered muslin cloth and filtered through filter paper. After filtration, aliquot was centrifuged at 6 000 rpm for 20 min at room temperature. The supernatants were filtered through Whatman No.1 filter paper.

Antibacterial assay

The antibacterial activity of crude extracts were tested on *Staphylococcus aureus* (ATCC 25923), *Streptococcus* sp., *Escherichia coli*, *Enterobacter cloacae* and one bacterial strain isolated from soil (from our laboratory collection). Standard antibiotic discs (Piperacillin) and control wells (just with solvent) were used as a positive and negative control. An overnight culture of each bacterium

was prepared by taken one wireloop of the organism from the stock and inoculated into the 2ml sterile nutrient broth, and then incubated at 37°C for 24hr. For testing antibacterial effects were used plates with sterile solid medium (nutrient agar).

Antibacterial activity was performed by agar well diffusion method. Using a sterile Durham tube 7mm diameter, the wells were made according to the number of samples. Each bacterial overnight culture was homogeneous inoculated on the surface of nutrient agar in Petri dishes. The extracts were put into the wells, and positive and negative controls were used, too. The plates were incubated at 37°C for 24 h.

The antimicrobial activity of the fern extracts was appreciated by measuring the diameter of inhibition growth zone (in millimeter), as a clear, distinct zones of inhibition surrounding agar wells [4]. The experiment was carried out three times and the results were the mean of three replicates.

3. Results and Discussions

The growth of the *S. aureus* ATCC 25923 was most inhibited by antibiotic and by extract obtained from *Asplenium scolopendrium* leaves extract and *Cystopteris fragilis* gametophyte extract.

Smaller inhibition zones were observed in the case of *Polypodium vulgare* leaves. The *Polypodium vulgare* extract obtained from gametophyte and sporelings have not antibacterial effects.

Table 1

Antibacterial activity of methanolic extracts obtained from ferns

Extracts	Bacterial strains	Diameters of inhibition zones (mm)				Bacterial strain from soil
		<i>S. aureus</i> ATCC 25923	Str. sp.	<i>E. coli</i>	<i>En. cloacae</i>	
<i>Asplenium scolopendrium</i> (leaves)		9	9	9	-	8
<i>Polypodium vulgare</i> (leaves)		8	9	8	9	12
<i>Polypodium vulgare</i> (G +S)		-	10	11	-	8
<i>Cystopteris fragilis</i> (G)		9	10	10	12	11
Positive control (Piperacillin)		33	22	22	36	27
Negative control (Methanol)		9	-	9	9	8

S. aureus - *Staphylococcus aureus*; Str. sp. - *Streptococcus* sp.; *E. coli* - *Escherichia coli*; *En. cloacae* - *Enterobacter cloacae*; (G +S)-gametophytes+sporelings; (G)- gametophytes.

All crude tested extracts produce growth inhibition zone against *Streptococcus* sp. (Fig. 1); the gametophytes extracts showed the most obviously antibacterial activity.

Similar results have been obtained in the case of *Escherichia coli*; the largest inhibition zones were produced by *Polypodium vulgare* gametophytes and sporelings extract.

The growth of *Enterobacter cloacae* was not inhibited by the *Asplenium scolopendrium* leaves extract and by *Polypodium vulgare* gametophytes and sporelings extract. The diameter of inhibition zones produced by *Cystopteris fragilis* gametophytes extract was 12 mm.



Fig. 1 Inhibition zones against *Streptococcus* sp.

In the case of the bacterial strain isolated from soil, the growth was not inhibited by the *Polypodium vulgare* gametophytes and sporelings extract; *Polypodium vulgare* leaves extract produced the largest inhibition zone.

The best antibacterial effect was demonstrated for *Cystopteris fragilis* gametophytes extract, which inhibited the growth of all tested bacteria. Also, the *Polypodium vulgare* leaves extract inhibited the growth of all tested bacteria.

The antibacterial effect showed by the tested extracts is possibly the result of the ecdysteroids presence. Phytoecdysteroids are plant analogs of insect molting hormones. In some ferns families they are absent, or present in a few species only (e.g., *Aspleniaceae*), whereas in others (e.g., *Polypodiaceae*, *Woodsiaceae*) almost all investigated species contain ecdysteroids. In *P. vulgare*, the highest concentrations of phytoecdysteroids were found in gametophytes [5]. Different researchers have reported antifungal and antibacterial activity of ecdysteroids.

The crude methanolic extracts obtained from leaves of some Indian ferns led to an inhibition zone between 9-11 mm against *Staphylococcus aureus*, similar to Romanian tested ferns. Some extracts such as those obtained from *Athyrium pectinatum* and *Dryopteris cochleata*, have not inhibited the *Staphylococcus aureus* growth [6]. The alcoholic extracts obtained from rhizome and roots of *Athyrium pectinatum* inhibited the *Staphylococcus aureus* growth, while the extract obtained from leaves had no antibacterial activity [7].

4. Conclusions

The best antibacterial effect was demonstrated for *Cystopteris fragilis* gametophytes extract, which inhibited the growth of all tested bacteria. Also, the *Polypodium vulgare* leaves extract inhibited the growth of all tested bacteria. The most sensitive bacterial strains at extract's activity were *Escherichia coli* and the bacterial strain isolated from soil.

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