

LABORATORY SCREENING TESTS WITH BISQUINOLIZIDINE ALKALOIDS FOR PROTECTION AGAINST MICROFUNGI

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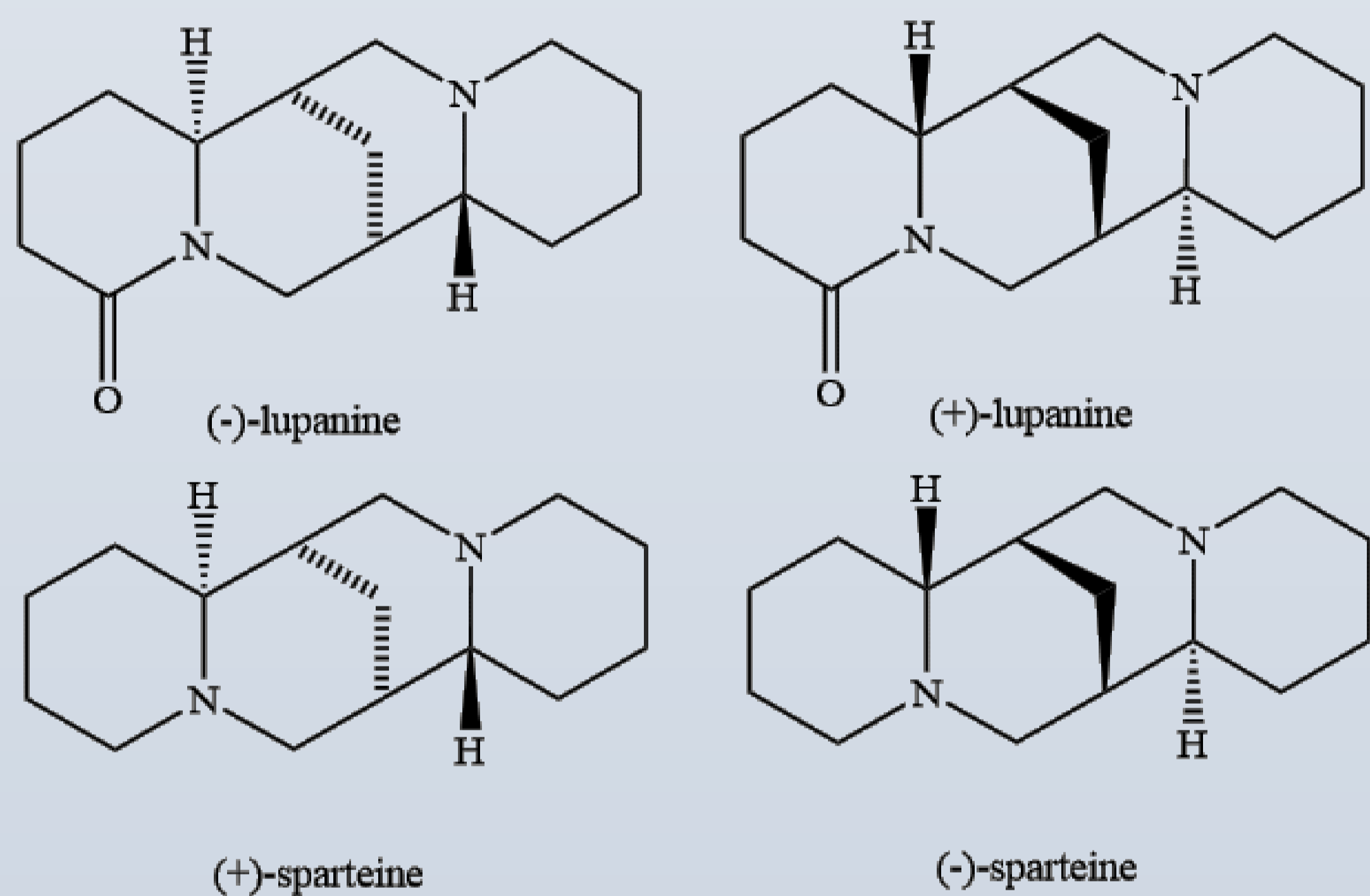
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ABSTRACT

The bisquinolizidine alkaloids sometimes called “lupin alkaloids” have been for the great interest because of their biological and biochemical properties. The pharmacological features include oxytocic, hypoglycemic, hypotensive and antiarrhythmic activities. This group of alkaloids, metabolized by plants of the *Fabaceae* sp. family, are essential for defense against predators such as herbivores, viruses and other microorganisms.¹

The aim of this study was determination of fungistatic properties of the quinolizidine alkaloids: sparteine (1), lupanine (2) and their derivatives by using TLC-bioautography method against microfungi. This method belongs to microbiological screening tests commonly used for identification of the compounds that inhibit the growth of moulds.² The bioassay was tested in determination of effective alkaloid concentrations for 100% growth reduction (LD₁₀₀). The microfungal strains used in the microbioassay were *Aspergillus niger* van Tieghem, *Penicillium cyclopium* Westing. The sparteine and (±)-lupanine derivatives include enantiomers: (-)-sparteine, (+)-sparteine, (+)-lupanine and (-)-lupanine were prepared and characterized using spectroscopy methods according to literature data.³ All analyzed compounds revealed activity against microfungi.



INTRODUCTION

Alkaloids, heterocyclic nitrogen compounds, are considered to be the most important secondary metabolites in plants and are known to have antifungal activity.³ Quinolizidine alkaloids are a fairly large group of alkaloids with very different properties, which is why more than a hundred years are the subject of intensive research. Derivatives of quinolizidine alkaloids also known as lupin alkaloids. They occur mainly in angiosperms in the family Fabaceae (*Leguminosae*) and Legumes (*Papilionaceae*).

The physiological role of alkaloids has still not been fully elucidated. Like many other secondary metabolites they aren't end products of metabolism and on the basis of many studies it was found that they perform a variety of biological functions. Scientists assume that the quinolizidine alkaloids play the role "of chemical weapons" against pathogens and insects. Their concentration is usually higher in essential for the survival and reproductive organs of plants such as flowers, fruits or seeds.⁴ Plants provide an excellent source of biologically active natural products. Over the centuries, many plants were used as a source of biocides, but now traditional pesticides of natural origin play a minor role. However, plant natural products still have great potential.



Lupin (*Lupinus polyphyllus*)
fot. Anna K. Przybył

EXPERIMENTAL BIOLOGICAL SECTION

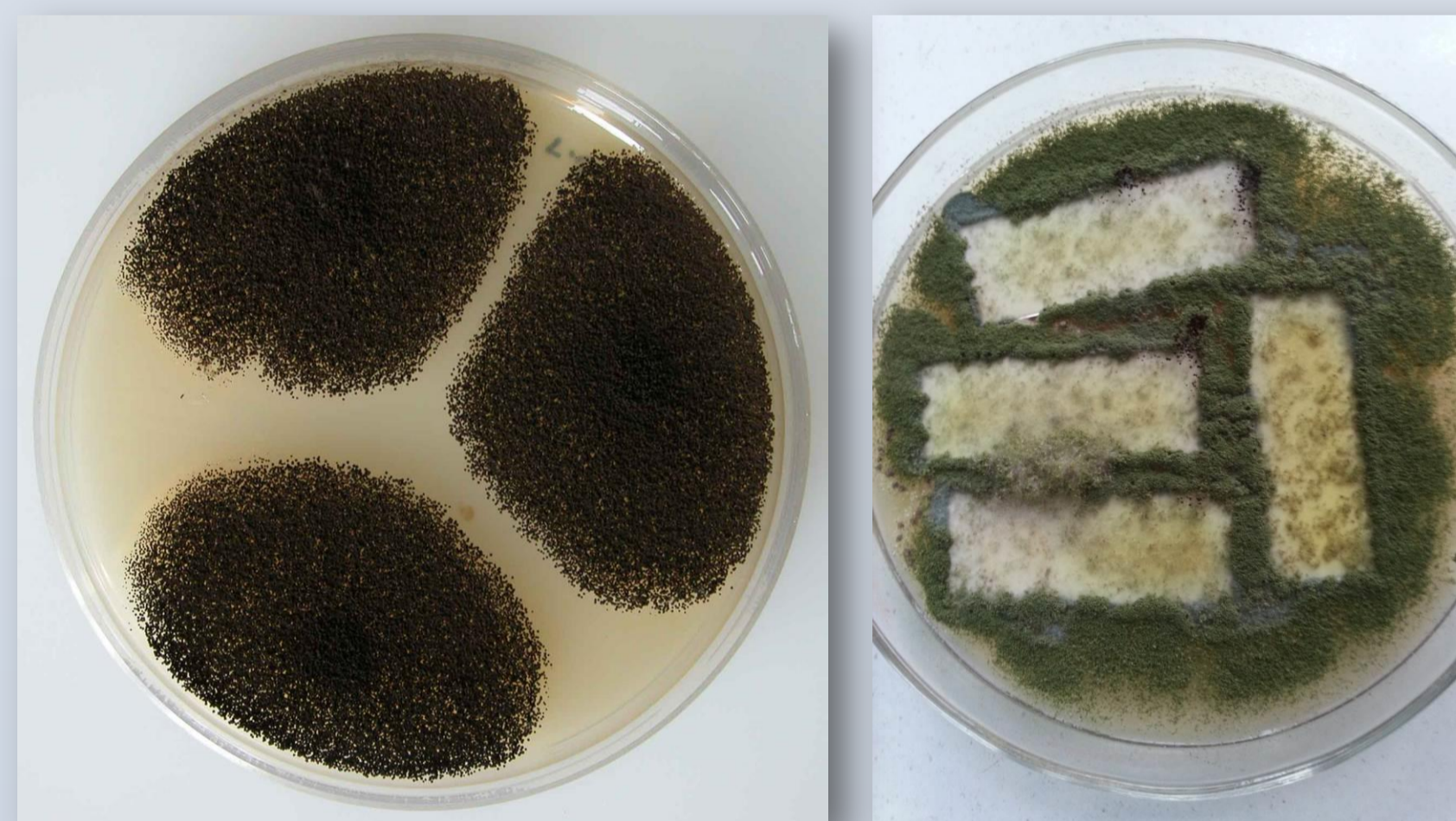
MATERIALS

In the study of activity against micro-fungi were selected following alkaloids: (-)-sparteine, (+)-sparteine, (+)-lupanine and (-)-lupanine.

Sparteine is one of the basic and probably the most important system tetracyclic with a core quinolizidine. It can be modified by the introduction of additional functional groups causes a large collection of compounds. Lupanine belongs to them, acting as sparteine in all sorts of different concentrations of lupine. Tetracyclic quinolizidine alkaloids as sparteine and lupanine can be isolated from almost all species of lupine.

Fungal infections pose a continuous and serious threat to human health. These fungal infections in humans can be classified into allergic reactions to fungal proteins, toxic reactions to toxins present in certain fungi and infections like mycoses. The following microfungi species were used in the test of antifungal activity of bisquinolizidine alkaloids: *Aspergillus niger* van Tieghem and *Penicillium cyclopium* Westling. agar slants were two weeks and come to terms with the collection of the Institute of Wood Chemical Technology. *A. niger* causes a disease called black mold on certain fruits and vegetables such as grapes, onions and is a common contaminant of food. It occurs in the soil and indoor environments. *Aspergillus* is virtually ubiquitous.

P. cyclopium form a green coating (mold) and can occur indoors even if the relative humidity is low.



Aspergillus niger *Penicillium cyclopium*

METHODS

To rapid identification of compounds with antifungal properties used bioautography method combined with thin-layer chromatography (TLC-bioautography). This method ensured the substance resistance to non-enzymatic reactions and oxidation and can be used to quickly verification of antifungal properties of practically every compound. The bisquinolizidine alkaloids dissolved in methanol were spotted on TLC plates in determination of effective alkaloid concentrations for 100% growth reduction of fungi. After drying the plates are placed on the inoculated agar surface to allow diffusion. Agar medium were prepared with Czapeks Dox, agar and malt extract. The plates were incubated on agar medium in Petri dish at 28°C for 1 week and the air humidity was 95%. Each experiment was repeated three times with triplicates of each compounds. The antifungal activity of the alkaloids tested was compared with commercial fungicides IPBC (3-iodo-2-propynyl butylcarbamate).

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RESULTS AND DISCUSSION

Screening studies were performed to identify the chemical substances showing or not any antifungal activity against moulds and to select the lead compound whose derivatives would be tested to generate a new biocide.

The quinolizidine alkaloids used in assay inhibits the hyphal of tested microfungi to 2 days after inoculation of the agar medium.

The results of bioassay tests are show below in table 1.

Table 1. Bioassay test on *Aspergillus niger* and *Penicillium cyclopium*
0 – no growth, 1 - growth of hyphae without spores;
2 – sporulation mycelium

comp.	24h	48h	72h
(-)-sparteine,	0	1	1
(+)-sparteine	0	1	1
(+)-lupanine	0	1	1
(-)-lupanine	0	1	1
IPBC	0	0	0
Ref.	1	2	2



ACKNOWLEDGEMENTS:

The study was supported by Norway Grants and the National Centre for Research and Development of Poland (NCBiR) as a part of Polish-Norwegian Research Programme: *Superior bio-friendly systems for enhanced wood durability*. No. Pol-Nor/203119/32; DURAWOOD).