ANTIMICROBIAL ACTIVITY OF *PSORALEA GLANDULOSA* L.

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ABSTRACT

Antimicrobial activity of leaf extracts of Psoralea glandulosa *L. (Papilionaceae) is reported. This study was carried out on the extracts and on the plant's most abundant metabolite, bakuchiol. Antimicrobial activity against Gram positive bacteria was observed. Bioautographic assays showed that bakuchiol was the compound responsible for this activity.*

INTRODUCTION

Psoralea glandulosa L. (Papilionaceae) is a plant commonly known in Chile as culén, culé, cuelén, kulén, or trapil-l-awen. It is a native shrub, and the genus is represented by two species, namely *P. glandulosa* and *P. pubescens* Poir. (Marticorena and Quezada, 1991). *P. glandulosa* is cultivated in neighboring countries (Rodriguez *et al.*, 1982). Its leaves and flowers have been used in folk medicine as febrifuge and in indigestions characterized by diarrhoea; they are also used as a vermifuge and a vulnerary (Hoffmann *et al.*, 1992).

In the present research we describe an antimicrobial study of the extracts of *P. glandulosa*. The extract with the highest activity (petroleum ether) was used to isolate the compound responsible for this activity, by means of a bioassay guided of fractionation, through a bioautographic agar overlay assay. In addition, fractionation was followed by the brine shrimp (*Artemia salina*) bioassay.

Keywords: Antimicrobial activity, bakuchiol, *Psoralea glandulosa.*

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MATERIALS AND METHODS

Plant Material

Leaves of *P. glandulosa* were collected from the Metropolitan region of Santiago, Chile in January 1992. The identity of the plant was determined by Prof. R.C. Peña. A voucher specimen (S. Erazo *s.n.*) was deposited at the Herbarium of the School of Chemistry and Pharmacy, N°19564 SQF of the University of Chile. The sample was dried at room temperature and finely powdered.

Extraction, Isolation and Identification of the Compound

Dried plant material (2,250 g) was successively extracted with petroleum ether, dichloromethane and methanol. The solvents were evaporated *in vacuo* leaving residues of 120, 60 and 300 g, respectively. The petroleum ether extract was fractionated by MPLC with silica gel; fractions were eluted from the column with a mixture of petroleum ether and dichloromethane (8:2). The most abundant compound was isolated and identified by spectroscopic analysis: ^{1}H NMR and ^{13}C NMR. The phenolic monoterpene, bakuchiol was identified (Erazo *et al.*, 1990).

Antimicrobial Activity

The antimicrobial activity of the extracts was determined against Gram negative species (*Escherichia coli, Pseudomonas aeruginosa*), Gram positive species (*Staphylococcus aureus, Micrococcus flavus, Bacillus pumilus, Staphylococcus epidermidis*) and the yeast *Saccharomyces cerevisiae*.

The extracts were dissolved in DMSO. Appropriate dilutions were added to a fixed volume of culture medium: Plate Count Agar (PCA) for bacteria and

Potato Dextrose Agar for the yeast. Plates were poured with media and dried for 24 h. They were then surfaced inoculated with a single line of an overnight culture of the different microorganisms. Plates were then incubated at 37°C for 24 h for the bacteria and at 28°C for 48 h for the yeast. Results were recorded as growth or growth inhibition at each extract concentration. Controls were carried out using 100, 200 and 400 µL DMSO per mL of culture media, in order to assay toxicity due to the solvent.

Minimum inhibitory concentration (MIC) was then determined in those extracts which showed antibacterial activity in the plate assay. The turbidimetric method was used, using serial dilutions of the extract in 4 mL of the Plate Count Broth or Tryptic Soy Broth. Both media were used to assay the MIC of the extracts against bacterial strains. The tubes were inoculated with 0.25 mL of overnight cultures, at concentrations adjusted to OD_{580} = 0.30. After incubation for 24 h at 37°C tubes were inspected for turbidity. Samples were taken from all tubes at concentrations higher than the MIC. These were inoculated onto fresh media with no extract in order to determine survival of the bacteria. In addition, MIC of DMSO was carried out at a concentration range of 100 to 500 µL/mL. All experiments were carried out in triplicate, and three repeats were done. The same experimental procedure was used for the yeast except that the culture medium was Potato Dextrose broth and the tubes were incubated at 28°C for 48 h.

Bioautographic Agar Overlay Assay

TLC of the petroleum ether extract was carried out on silica gel G60 F_{254} glass backed plates (10 cm × 20 cm) and developed with chloroform as solvent. Chromatograms were air dried to remove the solvent and then they were sterilized by UV radiation for 30 min. Plates were overlayed with 38 mL of molten PCA inoculated with 2 mL of a *Staphylococcus epidermidis* culture grown overnight and adjusted to an OD_{580} = 0.5 prior to inocculating the molten agar. This procedure was carried out in a sterile glass box; the chromatograms were placed on glass rods inside the glass chamber, the bottom of which was covered with sterile water as to create a moist chamber. It was then covered with a glass lid and incubated overnight at 37°C. The bioautograms were sprayed with an aqueous solution (2.5 mg/mL) of thiazolyl blue (methyl thiazolyl tetrazolium MTT). Clear inhibition zones were observed against a purple background (Rahalison *et al.*, 1991).

***Artemia salina* Bioassay**

The samples were prepared by dissolving 50 mg of extract and of the isolated compound in 2 mL DMSO. The bioassay was performed according to Meyer *et al.* (1982), in which ten brine shrimp larvae are exposed to different concentrations of the chemicals. Toxicity is determined by death of the shrimp, which is seen as loss of motility. Three repeats were performed for each extract concentration. Toxicity was calculated as lethal concentration 50 (LC_{50}). Controls to assess toxicity due to DMSO were carried out at a concentration 10 µL DMSO per mL of sea water.

RESULTS AND DISCUSSION

The first experiments in this research were done to assay the effect of the solvent, DMSO, on microbial growth. Results showed that there is an inhibition of microbial growth at DMSO concentrations of 200 µg/mL or higher; DMSO at concentrations of 100 µg/mL of lower have no effect on growth of any of the species tested (data not shown). This result allowed us to assay the plant extracts at concentrations up to 100 µg/mL. The results of this research indicate that both the petroleum ether and dichloromethane extracts are active against Gram positive bacteria and inactive against Gram negative bacteria and yeast. Both methanolic and aqueous extracts were inactive against all of the microorganisms tested. In addition, antibacterial activity of an infusion prepared with 1 g of ground, dried leaves in 250 mL boiling water was tested. This is the dosage recommended in folk medicine. Antimicrobial activity of this infusion was negative. When the amount of leaves was increased three fold, low levels of antimicrobial activity were observed.

The most active extract is that in petroleum ether. MIC of this extract against Gram positive strains was 12.5 µg/mL except for *B. pumillus* in which MIC was 25 µg/mL (Table 1). No growth was observed when fresh culture medium was inoculated with samples of those tubes at concentrations above MIC, indicating a possible bactericidal effect. An exception to this was *B. pumilus* in which growth was observed in all tubes, an effect that can be attributed to spore formation. No inhibitory activity was observed against Gram negative strains nor the yeast at the concentrations assayed.

Antimicrobial activity of this extract could be assigned to two compounds as seen in the bioautography. These compounds differed in their Rf and their concentrations; the most active and abundant

Table 1. Minimum Inhibitory Concentration (MIC) of the petroleum ether extract and Bakuchiol.

Microorganism	MIC of petroleum ether extract (μg/mL)	MIC of Bakuchiol (μg/mL)
P. aeruginosa	> 100	> 100
E. coli	> 100	> 100
S. epidermidis	12.5	10.0
S. aureus	12.5	5.0
M. flavus	12.5	10.0
B. pumilus	25.0	10.0
S. cerevisiae	>100	> 100

Table 2. Lethal Concentration (LD_{50}) of the extracts and Bakuchiol in the *Artemia salina* bioassay.

Sample	Concentration (μg/mL)
Petroleum ether extract	< 10
Dichloromethane extract	16
Methanol extract	> 500
Bakuchiol	< 2

compound was the one with the highest Rf, identified as bakuchiol. This monoterpene has been described in the oil obtained from the seed of *Psoralea corylifolia* L. and it has been shown active against *Staphylococcus aureus* (Mehta *et al.*, 1972)

The antimicrobial plate assay was carried out with the isolated bakuchiol. As seen in Table 1, the Gram positive bacteria were sensitive to bakuchiol at concentrations of 5 and 10 μg/mL. Bakuchiol had no effect on the growth of Gram negative bacteria.

Table 2 shows the results from brine shrimp (*Artemia salina*) bioassay. The concentrations at which the extracts showed any activity ranged from < 10 to > 500 μg/mL, while bakuchiol displayed a toxicity level lower than 2 μg/mL (LC_{50} < 2). After 24 h in the test solutions, the shrimp lost motility and are assumed dead; nevertheless, when these were transferred back to sea water without any added extract, most of the shrimp were able to swim again. This result indicate that even though low concentrations of bakuchiol affect the motility of the shrimp, its effect is not lethal. This assay was performed using hexachlorocyclohexane (lindane) as a positive control. No toxicity due to the solvent, DMSO was observed.

Results presented here indicate that leaf extracts from *P. glandulosa* have antibacterial and biological activity, and that these activities are due mainly to a phenolic monoterpene, bakuchiol.

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