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EVALUATION OF THE ANTIBACTERIAL PROPERTIES OF EXTRACTS OBTAINED FROM NATIVE *POROPHYLLUM LANCEOLATUM* IN SAN LUIS, ARGENTINA

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Abstract

Ethereal, dichloromethane, ethyl acetate, and methanolic extracts from *Porophyllum lanceolatum* DC. (Asteraceae/Compositae) were analyzed for *in vitro* antibacterial activity in tripticase soy broth against bacterial organisms like methicillin-resistant *Staphylococcus aureus* ATCC 43300, methicillin-sensitive *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* CLIP 74910, *Escherichia coli* ATCC 35218 and *Pseudomonas aeruginosa* ATCC 27853. The above extracts from *P. lanceolatum* were active against Gram-positive bacteria but in any case inhibited the growth of Gram-negative bacteria. The MIC values were between 2 and 0.5 mg/mL. Ethereal, dichloromethane and ethyl acetate extracts were active against both strains of *S. aureus* at the lowest concentration (0.5 mg/mL). Inhibitory concentrations of the ethereal and ethyl acetate extracts against *S. aureus* were bactericidal (MIC = MBC). MBC of dichloromethane and methanolic extracts for *Staphylococcus* and *Listeria* were 1 or 2 dilutions above the MIC. All Gram-negative bacteria were resistant to the extracts tested in the investigated concentrations. This study showed that extracts of *P. lanceolatum* are promising for future natural therapy against Gram-positive bacteria.

Key words: Antibacterial activity; *Porophyllum lanceolatum*; Gram-positive bacteria; organic extracts

Introduction

The continuous use of certain plants for medicinal purposes over time reflects their therapeutic value. The ethnomedical knowledge associated with plants is one of knowledge that is among the most important developed by man (1). Indeed, international health organizations have recognized its explicitly, while the scientific research and technological advances are increasingly appreciated the importance of ethnomedicine and a return to nature (2, 3). In this context, the emergence of microorganisms resistant to synthetic drugs has stimulated both the search of new treatments and the use of bioactive compounds derivates from plants and other agents from natural origin (4, 5, 6).

The plant genus *Porophyllum* (Asteraceae: Tageteae) comprises about 25 species of annuals or perennials, exclusive of warm and temperate America, most intensely aromatic up to smelly, some of which are used in folk medicine, especially as diaphoretic and antispasmodic (7, 8). In some species have been isolated sulphurate compounds, essential oils, flavonoids, phenylpropanoids and others, whose aerial parts showed anti-inflammatory and insecticidal properties (9, 10, 11, 12, 13). According to the World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to obtain a thorough knowledge about their properties, safety and efficacy (2, 10).

Porophyllum lanceolatum DC. is an annual, glaucous herb, vulgarly known as "hierba del ciervo", "hierba del venado" or "clavelina" (9, 10). The stems are branched towards the upper half, the leaves are linear to lanceolate, and inflorescences (apical capitula) are provided with perfect flowers; the fruits (cypselae) are dark, with pappus fulvous-creamy.

All tender parts shows noteworthy glands, whereby the plant is rich in essential oils. It is native to northern and central Argentina, appearing also in most neighboring countries (Bolivia, Brazil, Uruguay and Paraguay). It grows between 0 and 1,500 m a.m.s.l. on rocky or sandy soils, even in montane grasslands, and sometimes becomes weed of crops and gardens. It is popularly used in west-central Argentina in infusion, primarily as a digestive and antispasmodic, like other species of the genus, as well as diaphoretic and against venereal diseases (9, 10); even the fresh leaves are applied as vulnerary.

The aim of this study was to evaluate the antibacterial properties of four extracts of *P. lanceolatum*, in samples of San Luis, Argentina.

Material and Methods

Plant Material

The aerial parts (leaves, stem, and fruits) of *Porophyllum lanceolatum* was collected in San Luis province (Argentine) in summer 2011 and were authenticated by two of the authors. The voucher specimen (UNSL 537) was deposited in the Herbarium of UNSL.

Preparation of Extracts

A 10 g of air-dried plant powder (leaves and floriferous stems) was soaked in 100 ml of organic solvents, viz., petroleum ether, dichloromethane, ethyl acetate and methanol separately for 72 h in a round bottomed flask at room temperature. Extracts were filtered through the Whatman filter paper No.1. The ethereal (E), dichloromethane (DCM), ethyl acetate (EA) and methanolic (M) extracts were obtained. The extracts were concentrated to dryness under reduced pressure at 37°C using a rotary evaporator. Condensed extracts were weighed and stored in tight containers at 4° C until their use for the antibacterial tests.

Antibacterial activity test

Microorganisms

Antibacterial activity of the extracts was tested individually on Gram-positive and Gram-negative bacterial strains. The Gram-positive bacterial strains used were methicillin-resistant *Staphylococcus*

aureus ATCC 43300 (MRSA), methicillin-sensitive *Staphylococcus aureus* ATCC 25923 (MSSA), *Listeria monocytogenes* CLIP 74910. Gram-negative bacterial strains used were *Escherichia coli* ATCC 35218 and *Pseudomonas aeruginosa* ATCC 27853. Bacterial strains were stored in trypticase soy broth supplemented with 20% glycerol at –80 °C until use.

Determination of Minimal Inhibitory Concentration (MIC)

The minimal inhibitory concentration (MIC) of *Porophyllum* extracts was determined by the microwell dilution assay according to the CLSI method (14), in tripticase soy broth (Britania, Argentina) pH 7.2 supplemented with 0.01% (W/V) of 2,3,5- triphenyltetrazolium chloride as visual indicator of bacterial growth. The inoculum of each strain were prepared from 24 h of culture and the suspension was adjusted to the tube 0.5 of Mc Farland scale (10^8 bacterial cells). Then, they were diluted 10 times. The extracts were dissolved in dimethilsulfoxide (DMSO) to the highest concentration to be tested (8 mg/ml). The final concentration of DMSO in the assay did not exceed 1%. Then, serial twofold dilutions were made in concentration ranges from 8 to 0.5 mg/ml. The 96-well plates were prepared by dispensing into each well 95 µl of nutrient broth and 5 µl of the inoculum.

One hundred microlitre aliquot from the stock solutions of the organics extracts and their serial dilutions initially prepared was transferred into five consecutive wells. The final volume in each well was 200 µl. The plates were covered with sterile plate sealer and then incubated at 37°C for 24 h in aerobic atmosphere and static conditions. MIC was defined as the lowest concentration of the extracts in the medium in which there was no visible growth after incubation (no red colour). In addition, controls of nutrient broth, strains and extracts were also included. The experiments were performed in duplicate and then replicated at least twice.

Determination of Minimal Bactericidal Concentration (MBC)

Extracts that showed inhibitory activity in the broth assay were submitted to a subculture in tripticase soy agar, in order to evaluate bactericidal effect. The plates were incubated under the same conditions as in the MIC experiment. The presence or absence of bacterial growth was determined by visual inspection. The MBC was defined as the lowest concentration that showed no bacterial growth in the subcultures after incubation in the absence of extract.

Results and Discussion

The great concern with the resistance of pathogens to antimicrobials used stimulates research on new compounds that have antimicrobial activity. It is believed that there are many plants containing natural compounds unexplored that could be potential sources to for the reduction or control of bacterial diseases (15).

Our results showed the potential antibacterial properties of organic extracts of *Porophyllum lanceolatum* against Gram-positive strains. As shown in Table 1, the MIC values were between 2 and 0.5 mg/mL for *Staphylococcus* and *Listeria*. DCM, EA and E extracts were active against *S. aureus* at the lowest concentration (0.5 mg/mL). Under the conditions tested, the extracts had no effect on *E. coli* and *P. aeruginosa*, Gram-negative bacteria. It is well known that Gram-positives are susceptible to antibiotics like vancomycin, that do not work or work poorly against Gram-negative. This is a difference related to the ability of antibiotics to be blocked by the pore size of porin proteins in the outer membrane of the later (16).

In this study, the EA, DCM and E extracts of *P. lanceolatum* showed relatively low inhibitory concentration values against methicillin-resistant and methicillin-sensitive *Staphylococcus* (0.5 mg/mL) that indicates its potential as an antibacterial compound. Based on MIC values, extracts can have strong (0.05 to 0.5 mg/ml), moderate (0.6 to 1.5

mg/mL) or weak activity (> 1.5 mg/mL) (17). Using the afore-mentioned criteria, all the extracts evaluated in this study can be considered strong inhibitors for *Staphylococcus* and between moderate and weak inhibitor for *Listeria*. Inhibitory concentrations of the E extract against both strains of *S. aureus* were bactericidal (MIC = MBC). MBC of the EA, DCM and M extracts for methicillin-sensitive *Staphylococcus* and *Listeria* were 1 or 2 dilutions above the MIC. Is known antibacterial activity of many plant species, but there are not reports that evaluate the antibacterial activity in the *P. lanceolatum* specie (12). Takahashi *et al* (18) have demonstrated antioxidant and antileishmanial properties in *P. ruderale*. Also antifungal activity was found in *P. obscurum* (Values of MIC=31.2 -1000 µg/mL (19). In our study, *P. lanceolatum* showed good inhibitory activity against Gram-positive bacteria (MIC=500-1000 µg/mL. These investigations are particularly relevant in developing countries, which harbor the highest biodiversity and simultaneously, the biggest health problems related to diseases of microbial etiology (10).

In resume, the results shown here prove the efficiency of organics extracts of *P. lanceolatum* for to inhibit the growth of Gram-positive bacteria, this is a potential alternative for future natural therapy against Gram-positive bacteria. Further studies for the isolation of active substances for control of pathogens and their mode of action on the microbial cell are underway.

see Table 1.

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Bacterial sp	Organic extracts of *Porophyllum lanceolatum*							
	Ethereal		Dichloro methane		Ethyl acetate		Methanol	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus ATCC 4300	0.5	0.5	0.5	1	0.5	0.5	2	ND
S. aureus ATCC 25923	0.5	0.5	0.5	2	0.5	4	0.5	4
L. monocytogenes CLIP 74910	2	8	1	2	2	4	1	ND
E. coli ATCC 35218	NA	NA	NA	NA	NA	NA	NA	NA
P. aeruginosa ATCC 27853	NA	NA	NA	NA	NA	NA	NA	NA

Table 1. Minimal inhibitory concentration (MIC) and Minimal bactericidal concentration (MBC) obtained for 4 organic extracts from *P. lanceolatum* against Gram-positive and Gram-negative bacteria.

The values of MIC and MBC are expresed in mg/mL.
NA:Extract no active at concentration tested.
ND: Bactericidal concentration no detected at maximun concentration tested (4 mg/ml).