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PLANT SCIENCE

Antibacterial activity of organic extracts from *Zinnia peruviana* (L.) against gram-positive and gram-negative bacteria

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Abstract

Latin American countries have a long tradition in the use of plants that can produce varied therapeutic effects. *Zinnia peruviana* (L.) (Asteraceae) is a native plant used in folk medicine for the treatment of malaria, for stomach pain, as hepatoprotective and antiparasitic, antifungal and antibacterial agents. The antibacterial activity *in vitro* of extracts of *Z. peruviana* was evaluated against methicillin-resistant *Staphylococcus aureus* ATCC 43300, *Pseudomonas aeruginosa* ATCC 27853, *Listeria monocytogenes* CLIP 74910, *Escherichia coli* and *Bacillus cereus*. Different extracts were prepared using ethyl acetate and mixtures of n-hexane and ethyl acetate of increasing polarity on flash chromatography. *Z. peruviana* extracts showed antibacterial effects against all gram-positive and gram-negative pathogenic bacteria tested, with significant antibacterial activity against methicillin-resistant *S. aureus*, *L. monocytogenes* and *B. cereus*. The results open a path for future studies in the search for new molecules of natural origin with antibiotic activity.

Key words: Antibacterial activity, Organic extracts, *Zinnia peruviana*

Introduction

The production of drugs for the pharmacological treatment of diseases began with the use of herbs. Latin American countries have a long tradition in the use of plants which contain compounds with biological activity that can produce therapeutic effects varied (Goleniowski et al., 2006). Microbial resistance to different antibiotics used today, has caused a trend to search for drugs of natural origin.

Z*innia peruviana* (L.) (Asteraceae) is a native plant known by the vernacular names "Chinita del Campo". This specie is found in the center and north of Argentina (Barrie et al., 2011; Cantero et al., 2000). It is an erect herbaceous annual, 70-100 cm in height, with lance linear to broadly ovate or elliptic leaves, and 4-5 cm wide capitula with ray florets in a single whorl. Ligules are either yellow or scarlet red (Stimart et al., 2007). Nowadays, there is not much information of the bioactivity of different organic extracts of this plant. However, this specie is widely used in folk medicine for the treatment of malaria (Carrizo et al., 2002; Del Vitto et al., 1997; Goleniowski et al., 2006), for stomach pain, as hepatoprotective and antiparasitic (Salgado, 2007), antifungal and antibacterial agents (Barboza et al., 2009). The purpose of the study presented here, was to evaluate *in vitro* the antibacterial activity of organic extracts of *Z. peruviana*.

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Materials and methods

Plant material

Z*innia peruviana* (L.) (Asteraceae) aerial parts were collected in Rio Grande, San Luis, Argentina in February 2008 (Figure 1). Voucher specimen was identified by Ing Luis Del Vitto *et al*. and lodged in the University of San Luis (Argentina) herbarium.

Preparation of extracts

Previously dried aerial parts (500g) at room temperature and finely powdered were macerated with acetone at room temperature for 48h. Acetone extract was separated by filtration. Extraction was replicated three times. Extraction fluids were concentrated under reduced pressure yielding 330 g of dark syrup, then; it was dissolved with acetone and absorbed on silica gel column (700g). Each acetone extract was partitioned by chromatography

"Flash" using as elution solvents ethyl acetate (AcOEt) and mixtures of n-hexane and AcOEt of increasing polarity. The progress of separation was monitored by thin layer chromatography (TLC) using as mobile phase benzene: dioxane: acetic acid (120:20:4) and as revealing a mixture of H_2SO: AcOH: H_2O (2:20:1) followed by heating at 120°C. The extracts of *Z. peruviana* tested in this study were: 100% ethyl acetate, 30% ethyl acetate/n-hexane and 40% ethyl acetate/n-hexane.

Microorganism

A total of five bacteria were selected for this study. Methicillin-resistant *Staphylococcus aureus* ATCC 43300, *Pseudomonas aeruginosa* ATCC 27853, *Listeria monocytogenes* CLIP 74902 (Collection Listeria Institute Pasteur), *Escherichia coli* and *Bacillus cereus* isolated in UNSL Laboratory.

Determination of Minimal Inhibitory Concentration (MIC)

The antibacterial activity was assayed *in vitro* using microplate method (*microwell dilution)* according to the CLSI method (Wilkinson, 2007) in tripticase soya broth (Britania, Argentina) pH7.2 supplemented with 0,01% (w/v) of 2,3,5-triphenyltetrazolium chloride (TTC) used as visual indicator of bacterial growth.

The inoculum of each strain was prepared from 24h broth culture and adjusted to concentration of 10^6CFU/ml. Organic extracts were dissolved in dimethylsulfoxide and tested in a concentration ranging from 8 to 0.1 mg/ml.

The 96-well plates were prepared by dispensing into each well 95µl of nutrient broth and 5 µl of the inoculum (final concentration of 10^4 CFU/ml). One hundred microlitre aliquot from the serial dilutions of extracts was transferred into consecutive wells. The final volume in each well was 200 µl. Controls of nutrient broth, strains and extracts were included. After 24- hour incubation at 37ºC, the antibacterial activity of the extracts (MIC) was defined as the lowest concentration of the extract in the medium in which there no visible grown. The experiments were replicated at least twice.

Determination of minimal bactericidal concentration (MBC)

Extracts that showed inhibitory activity in the preliminary broth assay were submitted to a subculture on the surface of the tripticase soya agar plates, in order to evaluate bacterial growth. MBC was defined as the lowest concentration that showed no bacterial growth in the subcultures after 24 h of aerobic incubation at 37°C.

Results and Discussion

Argentina is a country with both rich floral biodiversity and cultural diversity. Traditional herbal medicines are important in the health care of most people, and rely heavily on the use of indigenous plants (Landa et al., 2007).

The increasing prevalence of multidrug resistant bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the specter of untreatable bacterial infections and adds urgency to the search for new infection-fighting strategies (Sieradski et al., 1999). In the present study, the extracts of *Z. peruviana* showed antibacterial effects against all gram-positive and gram-negative pathogenic bacteria tested (Table1). Although, Gram positive bacteria showed the highest sensitivity against the tested extracts. *Z. peruviana* 30% and 40% ethyl acetate/n-hexane extracts showed a similar behavior, with significant antibacterial activity against methicillin-resistant *S. aureus, L. monocytogenes* and *B. cereus* CIM of 0.2 mg/ml. An interesting finding was that all the tested extracts showed activity against methicillin-resistant *S. aureus*. This organism is highly infectious and often is resistant to multiple drugs which making it very difficult the therapeutic options (Drew, 2007). Furthermore, we observed that *P. aeruginosa*, nosocomial opportunistic microorganism which is characterized by presenting multiple mechanisms of antimicrobial resistance and to produce serious infections in hosts with altered defenses, was sensitive to all the extracts tested at a concentration of 4 mg/ ml. *E. coli* showed MIC of 8 mg/ml for *Z. peruviana* 30% and 40% ethyl acetate/n-hexane extracts (Figure 2). Moreover, *B. cereus* and *L. monocytogenes* pathogenic bacteria that cause foodborne infections serious were strongly inhibited by those extracts (MIC 0.2 mg/ml). *Z. peruviana* 100% ethyl acetate extract, showed significant activities against all tested strains at doses of 4 mg/ml. The MBC values were identical to the MIC values or two fold higher than the corresponding MIC (Table 1).

There are few reports on the antimicrobial activity of this plant. Some authors demostrated that aqueous and alcoholic extracts from *Z. peruviana* showed different degrees of antibacterial activity against gram-positive and gram-negative microorganisms such as *Enterococcus faecalis, E.coli, Klebsiella pneumoniae, P. aeuruginosa, Serratia marcescens, S. aureus, Streptococcus agalactiae, Streptococcus pyogenes* (Amani et al., 1998).

Table 1. Antibacterial activity of *Z.peruviana* extracts.

Bacterial strains	Extracts MIC/MBC (mg/ml)		
	100% ethyl acetate	30% ethyl acetate/n-hexane	40% ethyl acetate/n-hexane
S. aureus ATCC 43300	4/4	0.2/0.4	0.2/0.4
P. aeruginosa	4/10	4/8	4/8
L. monocytogenes CLIP 74902	4/8	0.2/0.4	0.2/0.2
E. coli	4/8	8/8	8/8
B. cereus	4/8	0.2/0.4	0.2/0.4

MIC: Minimal Inhibitory Concentration (mg/ml); MBC: Minimal Bactericidal Concentration (mg/ml).

Figure 1. *Zinnia peruviana* (L.) (Asteraceae) collected in Rio Grande, San Luis (Argentina). February 2008.

To our knowledge, there are no reports available in the literature on the phytochemical screening of *Z. peruviana*. Some authors detected compounds such as saponins, steroid, flavonoids and glycosides from *Zinnia elegans* (Ehsanulhaq, 2001; Hafizaet et al., 2002). Several authors have demonstrated that these compounds obtained of different medicinal plants inhibited the growth of gram positive and gram negative bacteria (Aslam et al., 2009; Gurinder et al., 2009; Sarojini et al., 2011). So, if some of these phytoconstituents are present in *Z. peruviana*, they could be responsible, partly or completely, of antimicrobial activity observed here from organic extracts of this plant.

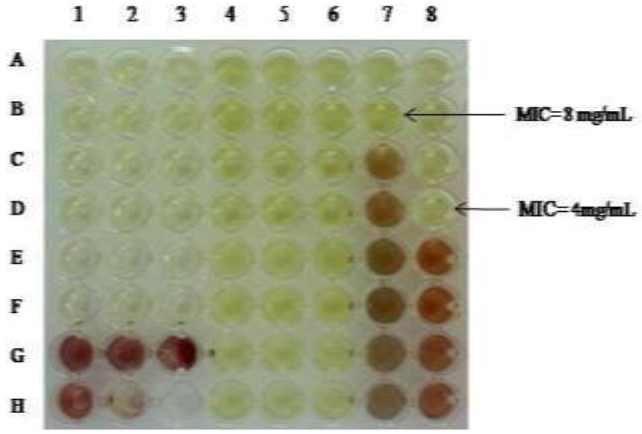

Figure 2. Microdilution plate used for broth microdilution method with *Z. peruviana* 30% ethyl acetate/n-hexane extract against *Staphylococcus aureus* ATCC 43300 (4), *Listeria monocytogenes* CLIP 74902 (5), *Bacillus* cereus (6), *Escherichia coli* (7) and *Pseudomonas aeruginosa* ATCC 27853 (8).
File 1: A-F: broth controls, G and G: controls of strains. Files 2 and 3, A-F extract controls. G and H: controls of strains.

Conclusions

This study contributes to knowledge of the antibacterial properties of regional plant extracts and opens a path for future studies in the search for new molecules of natural origin with antibiotic activity.

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