

Communication

Antimicrobial Activity of Three *Baccharis* Species Used in the Traditional Medicine of Northern Chile

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Abstract: The antimicrobial activities of aqueous ethanol and chloroform extracts of three *Baccharis* species currently used in Northern Chile folk medicine for the treatment of several infectious and inflammatory disorders were tested against Gram-positive and negative bacteria and fungal spp. using the agar-disc diffusion assay. The results indicated that the activity was more pronounced against Gram-positive than against Gram-negative bacteria and yeast. No significant differences on the antibacterial activity were observed in the aqueous ethanol versus chloroform extracts. None of the plant extracts evaluated exhibited any activity against ten fungi tested.

Keywords: Antimicrobial activity; *Baccharis microphylla*; *Baccharis petiolata*; *Baccharis santelicensis*.

Introduction

In Northern Chile, as well as in other developing countries, medicinal plants still represent the main therapeutic tool in traditional medicine [1]. The genus *Baccharis*, one of the most important genera of *Asteraceae*, is widespread throughout South America and is represented in Chile by 48

species [2]. Several of these are used in traditional medicine. The species selected for the present study – *Baccharis microphylla*, *Baccharis petiolata* and *Baccharis santelicensis* – are popularly known as “chilca” and they are commonly used to protect stomach and liver, restore blood circulation, reduce inflammatory processes and cure ulcers, burns and skin wounds [3-8]. The *Baccharis* species under study grow at 3,200-3,800 meters above sea level in the Puna de Atacama, which is characterized by areas of arid climate, low atmospheric pressure, high solar radiation and extremely broad seasonal and diurnal temperature ranges. No chemical studies on *B. microphylla* have been carried out. *B. petiolata* [9] and *B. santelicensis* [10] were previously reported to contain labdane and clerodane diterpenoids and flavonoids (flavones and flavanones); other terpenoids (C-15 and C-30) and mixed biogenesis metabolites have also been isolated from these species [10]. The main objective of this study was to identify Northern Chilean medicinal plants with antimicrobial activity which might serve as good candidates for the development of new antimicrobial agents and/or standardized phytomedicines.

Results and Discussion

The results of the antibacterial and antifungal screening of aqueous ethanol and chloroform extracts from the three studied *Baccharis* species against seven bacterial species and ten fungal strains are summarized in Table 1 (inhibition zones in the agar disc diffusion assay at 25 mg extract /disc) and Table 2 (MIC values). The following tested microorganisms are omitted from Tables 1 and 2 because no extract inhibited their growth: *E. coli*, *P. aeruginosa*, *C. albicans*, *C. tropicalis*, *C. neoformans*, *S. cerevisiae*, *A. fumigatus*, *A. flavus*, *A. niger*, *M. gypseum*, *T. tubrum* and *T. mentagrophytes*. The extracts exhibited a broad spectrum of activity. Gram-positive bacteria are more sensitive to the extracts than Gram-negative ones [11]. None of the plant extracts evaluated inhibited the growth of the Gram-negative *E. coli* and *P. aeruginosa*, nor did they exhibit any activity against the ten fungi tested, including *C. albicans*. The most susceptible bacterium was *B. subtilis*.

Table 1. Antimicrobial activity of aqueous ethanol and chloroform extracts from three *Baccharis* species from Northern Chile (diamater of inhibition zone in mm)*

| Plant species | <i>B. microphylla</i> | | <i>B. petiolata</i> | | <i>B. santelicensis</i> | |
|--------------------------------|-----------------------|------------|---------------------|------------|-------------------------|------------|
| | aq. ethanol | chloroform | aq. ethanol | chloroform | aq-ethanol | chloroform |
| <i>Staphylococcus aureus</i> | 8.2 ± 1 | 13.8 ± 2 | 15.8 ± 2 | 3.8 ± 2 | 23.5 ± 3 | 8.3 ± 0.9 |
| <i>Enterococcus faecalis</i> | 13.2 ± 2 | 24.5 ± 4 | 5.5 ± 0.9 | 4.6 ± 1 | 7.5 ± 3 | 26.4 ± 0.8 |
| <i>Bacillus subtilis</i> | >30 | 28.2 ± 3 | 15.8 ± 2 | 23.5 ± 3 | 28.4 ± 3 | 23.5 ± 2 |
| <i>Acinetobacter baumannii</i> | 5.8 ± 1 | 4.6 ± 1 | 5.1 ± 0.8 | 6.3 ± 1 | — | 8.3 ± 0.9 |
| <i>Salmonella typhi</i> | 8.4 ± 0.9 | 13.8 ± 2 | — | — | — | — |

* The values are the mean of three experiments ± S.E. of the mean. – No inhibition zone.

If extracts displayed an MIC less than 100 µg/mL, the antimicrobial activity was considered as good; from 100 µg/mL to 500 µg/mL the antimicrobial activity was moderate; from 500 µg/mL to 1000 µg/mL the antimicrobial activity was weak; over 1000 µg/mL the extract was considered inactive. Aqueous ethanol and chloroform extracts of *B. microphylla*, *B. petiolata* and *B. santelicensis* presented a good activity against *Bacillus subtilis*, with MIC values of 7.8, 15.6, 62.5, 31.2, 15.6 and

31.2 µg/mL, respectively. The aqueous ethanol extract of *B. santelicens* showed good activity against *S. aureus* (31.2 µg/mL), but was inactive against *E. faecalis* (> 1000 µg/mL), while the chloroform extract of this plant that displayed good activity against *E. faecalis* (15.6 µg/mL) and was inactive against *S. aureus* (> 1000 µg/mL).

Table 2. Minimum Inhibitory Concentration (MIC, µg/mL) values of aqueous ethanol and chloroform extracts from three *Baccharis* species from Northern Chile *

| Plant species | <i>B. microphylla</i> | | <i>B. petiolata</i> | | <i>B. santelicens</i> | |
|--------------------------------|-----------------------|------------|---------------------|------------|-----------------------|------------|
| | aq. ethanol | chloroform | aq. ethanol | chloroform | aq-ethanol | chloroform |
| <i>Staphylococcus aureus</i> | >1000 | 500 | 500 | >1000 | 31.2 | >1000 |
| <i>Enterococcus faecalis</i> | 500 | 125 | >1000 | >1000 | >1000 | 15.6 |
| <i>Bacillus subtilis</i> | 7.8 | 15.6 | 62.5 | 31.2 | 15.6 | 31.2 |
| <i>Acinetobacter baumannii</i> | >1000 | >1000 | >1000 | >1000 | NA | >1000 |
| <i>Salmonella typhi</i> | >1000 | 500 | NA | NA | NA | NA |

* The values are the mean of three experiments. NA: no activity

Conclusions

Although this study investigated the *in vitro* antimicrobial activity, the results showed that the extracts from *Baccharis microphylla*, *Baccharis petiolata* and *Baccharis santelicens* possessed good antibacterial activity, confirming the great potential of these plants used in Northern Chilean ethnomedicine for the production of bioactive compounds and are useful for rationalizing the use of these medicinal plants in primary health care. *In vivo* data may be helpful in determining the real potential usefulness of these plants for the treatment of infectious diseases.

Experimental

Plant materials

Aerial parts of *Baccharis microphylla*, *Baccharis petiolata* and *Baccharis santelicens* were collected near Socaire, in Northern Chile (23° 36'40" S; 67° 50'33" W) at 3,500 m above sea level. The plant's classifications were confirmed by Professor Clodomiro Marticorena, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Chile. A sample of each plant was deposited at the Herbario de la Universidad de Concepción.

Preparation of extracts and dry residues

The aqueous ethanol extract were prepared by adding EtOH-H₂O (1:1, 50 mL) to ir-dried and coarsely powdered plant (5.0 g), and heating the mixture at reflux condenser for 10 min with continuous magnetic stirring. Afterwards, the extract was covered with a watch glass, left standng for 30 min to cool, centrifuged and filtered through sterilized cellulose nitrate. The dry residue from the extracts was obtained by vacuum-evaporation followed by lyophilization at 5 mm Hg pressure and

-50°C in a Freezone lyophilizer (Labconco). For chloroform extraction, samples (5.0 g) were ground to a fine power in a mill and mixed with chloroform (50 mL). The obtained extracts were filtered and the solvent evaporated in a rotary evaporator at 50°C to afford the corresponding dry extracts. Extracts were tested at a concentration of 100 mg/mL in water-DMSO (9:1).

Microorganisms tested

The following bacteria strains were employed in the screening: Gram-positive: *S. aureus* (ATCC 25923), *E. faecalis* (ATCC 29212) and *B. subtilis* (ATCC 6633). Gram-negative: *A. baumannii* (ATCC 19606), *S. typhi* (ATCC 3492), *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853). In the antifungal screening the following fungi were tested: *C. albicans* (ATCC 90028), *C. tropicalis*, *C. neoformans* (ATCC 32264), *S. cerevisiae* (ATCC 9763), *A. fumigatus* (ATCC 26934), *A. flavus* (ATCC 9170), *A. niger* (ATCC 9092), *M. gypseum* (C115), *T. tubrum* (C113) and *T. mentagrophytes* (ATCC 9972).

Antibacterial testing

Antimicrobial activities of the aqueous ethanol and chloroform extracts were determined using the agar-disc diffusion method [12]. The media used were Mueller-Hinton agar for the bacteria and Sabouraud dextrose agar for the fungi. After 24 h, the culture of selected bacteria/yeast was mixed with physiological saline solution and the turbidity was corrected by adding sterile physiological saline to a MacFarland turbidity standard of 0.5 (10^8 CFU/mL). Afterwards, a top layer of Mueller-Hinton agar inoculated with 0.2% microbial suspension was poured over the Petri dishes. Sterile filter discs 6 mm in diameter were impregnated with 250 μ L of solution of extract (100 mg/mL, equivalent to 25 mg of extract) per disc and then placed onto the inoculated plates. The plates were then incubated at $37 \pm 0.1^\circ\text{C}$ for 24 h. The test was carried out in triplicate. Antimicrobial activity was measured as the diameter (mm) of clear zone of growth inhibition. Ampicillin (10 μ g/disc), streptomycin (25 μ g/disc) and chloramfenicol (30 μ g/disc) were used as positive control antibiotics in all plates and their inhibition zones against *E. coli* were 17, 9 and 18 mm, respectively. Nystatin (100 μ g/disc) and Amphoterecin B (200 μ g/disc) were used as positive control for the fungi in all plates and their inhibition zones against *C. albicans* were 26 and 17 mm, respectively. DMSO, ethanol and methanol were included in every experiment as negative controls. The Minimum Inhibitory Concentration (MIC) values of extract from *Baccharis* were determined in triplicate by using the microdilution broth method in 96-well microplates [13]. The extracts were dissolved in DMSO (1 mg/mL), and then diluted in tryptone soya broth to achieve concentrations ranging from 1000 to 7.8 μ g/mL. The final concentration of DMSO was 10% (v/v). The inoculum was adjusted to each microorganism to yield a cell concentration of 10^8 CFU/mL. Standard antibiotics were used as positive controls and their concentrations ranged from 5.9 to 0.01 μ g/mL. The microplates were incubated at 37°C for 24 h (bacteria) or at 30°C for 48 h (yeast), and aqueous 2,3,5-triphenyltetrazolium chloride solution (0.5%, 20 μ L) was added to indicate the viability of bacteria and yeast. MIC values were determined as the lowest concentration of the extract capable of inhibiting visible microorganism growth.

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Sample Availability: Available from the corresponding author.