Pharmaceutical Biology
2005, Vol. 43, No. 2, pp. 129–134

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Antimicrobial Activity of Essential Oils Obtained from Aromatic Plants of Argentina

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Abstract

The aim of this work was to evaluate the antibacterial and antifungal activity of essential oils obtained from medicinal plants of the Argentine Republic. The antimicrobial activity of the essential oils of 14 plants collected from different zones was analyzed. The microorganisms used were *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus cereus*, *Micrococcus luteus*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella* sp., *Proteus mirabilis*, *Pseudomonas aeruginosa*, and the yeast *Candida albicans*. The disk diffusion method was performed to test antimicrobial activity. *B. cereus* and *S. aureus* were inhibited by most of the essential oils. *Aloysia triphylla*, *Psila spartoides*, and *Anemia tomentosa* were the most effective compounds against *B. cereus*, while *A. triphylla* and *Baccharis flabellata* were effective against *S. aureus*. None of the oils inhibited *P. aeruginosa*. *B. flabellata* and *Pectis odorata* were active only against Gram-positive bacteria. *A. triphylla* and *P. spartoides* inhibited all tested microorganism, and the remaining essential oils showed variable activity. The minimum inhibitory concentration (MIC) of *A. triphylla* and *P. spartoides* essential oils were determined using the disk diffusion method. The lowest MICs were against *S. aureus* (1/16), *B. cereus* (1/16), *S. epidermidis* (1/8), and *C. albicans* (1/32) for *A. triphylla*. The lowest MICs were against *S. aureus* (1/32), *B. cereus* (1/32), *P. mirabilis* (1/32), and *C. albicans* (1/64) with *P. spartoides*. The results showed that *B. cereus* and *S. aureus* were the most sensitive microorganisms, and *P. aeruginosa* was the most resistant microorganism. This study may contribute to improve ethnobotanical knowledge and would help to discover substances with potential therapeutical uses, as food preservants or as food-borne pathogen inhibitors.

Keywords: Antibacterial activity, antifungal activity, essential oils, natural substances.

Introduction

The Argentine country includes large regions areas of tropical, moderate, and cold climatic zones where many varieties of native vegetable species live. The growth characteristics of these plants depend on soil and weather ecological factors. However, despite the fact they can develop under varied environmental conditions, the composition and yielding of the variety of active compunds that they produce could be modified because of this situation (Zygadlo et al., 1996; Zygadlo & Juliani, 2001). Ethnobotanics has recognized various species: tinctoreal, aromatic, ornamental, pollen and nectar producers, foragers, combustibles, medicinals and nutritious species (Nuñez & Cantero, 2000). Indigenous tribes currently use many of these species to treat several illnesses, contributing with traditional and popular knowledge that could become useful to the pharmaceutical industry (Gutkind et al., 1981; DiMayuga & Keer-Garcia, 1991; Rojas et al., 1992). The majority of medicinal plants have the ability to synthesize aromatic compounds such as essential oils, which are obtained by hydrodistillation or by a pressing process. Essential oils are constituted mainly of a complex mixture of organic compounds including monoterpenes, diterpenes, carbonilated products, and poliennes. There are many studies that suggest the antibacterial and antifungal activity of these compounds (Cobos et al., 2000; Mwangi et al., 2001; Primo et al., 2001; Juliani et al., 2002). Moreover, it has been probed that essential oils have beneficial properties for

Accepted: October 25, 2004

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DOI: 10.1080/13880200590919438

human and animal health as antitumor factors (Crowell et al., 1996; Crowell, 1999), citogastric protector (Tambe et al., 1996; Singh & Majumdar, 1999), antioxidant (Zygadlo et al., 1995; Tiziana-Baratta et al., 1998; Youdim & Deans, 2000), and antimicrobial (Barel et al., 1991; Zygadlo & Grosso, 1995; El-Sahkawy et al., 1998; Dolara et al., 2000).

The importance of the study of the antimicrobial properties of the essential oils of medicinal plants is recognized worldwide, as this may improve ethnobotanical knowledge. The study of the antimicrobial properties that these plants have may help to discover substances with potential therapeutical uses, as food preservants or as food-borne pathogen inhibitors (Deans et al., 1995; Smith-Palmer et al., 1998). The purpose of this work was to evaluate the antibacterial and antifungal activity of essential oils coming from the autochthonous flora of the Argentine Republic.

Materials and Methods

Obtaining the essential oils

The aereal parts of the medicinal plants used were collected from different regions of Argentina. Specimens are kept in the Herbarium of the Museo Botánico of the Facultad de Ciencias Exactas Fisicas y Naturales of the Universidad Nacional of Cordoba (Argentina). The essential oils (EO) were obtained from the vegetable material, which was hydrodistilled in a Clevenger-like apparatus. The oil obtained was kept in a partition "ampolla" and stored in a freezer (−80°C) until analysis.

Microorganisms

The activity of the oils was tested against the following microorganisms: *Bacillus cereus*, *Staphylococcus aureus* ATCC 25212, *Staphylococcus epidermidis*, *Micrococcus luteus* ATCC 9341, *Enterococcus faecalis* ATCC 29212, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella* sp., and *Pseudomonas aeruginosa*. The yeast *Candida albicans* was used in order to probe antifungal activity.

Tubes containing Triptein soy broth (TSB) inoculated with the microorganisms were incubated during 18 h, at 37°C. From these tubes, 10-fold dilutions were made, until an OD ≅ 0.04 (10^6cfu/ml) was reached. The antifungal activity was determined with the same methodology but using that dilution with an OD ≅ 0.4 (10^6 cfu/ml).

Determination of the antimicrobial activity of the essential oils

Analysis of the antibacterial activity

The antimicrobial studies were carried out according to De Pooter et al. (1995). Each experiment was performed in duplicate.

Disk diffusion method: 200 µl of each inoculum were spread over plates containing Mueller-Hinton agar (MHA); paper filter disks (6-mm diameter), impregnated with 10 µl of each essential oil, were placed on the surface of the media. The plates were left 30 min at room temperature to allow the oil diffusion, and then they were incubated at 37°C during 24 h. After this time, the inhibition zone around the disk was measured with a caliper. Disks with gentamicin (10 µg) were used as positive control.

Analysis of the antifungal activity

Antifungal experiments were performed in the same way as those with bacteria using Sabouraud agar (SA) for the plates. Disks with amphotericin B (2 µg/ml) were used as positive controls.

Minimum inhibitory concentration assay

The minimum inhibitory concentration (MIC) was performed according to the method previously described by De Feo et al. (1998), determined only with microorganisms that showed inhibitory zones larger than 10 mm. It was determined by twofold dilutions of essential oils in dimethyl sulfoxide (DMSO), placing 10 µl of each dilution on a filter paper disk. The disks were placed on the surface of a TSA plate, previously inoculated with 200 µl of each inoculum, and left at room temperature to allow the diffusion of the oil. Then, they were incubated at 37°C during 24 h. After this time, the inhibition zone around the disk was measured with a caliper. MIC was defined as the lowest concentration that inhibited visible growth. The MIC with fungus was determined in the same way as with bacteria using Sabouraud agar in the plates.

The negative control consisted of a paper disk impregnated with 10 µl of DMSO. The positive control was a disk impregnated with the antibiotic gentamicine (10 µg) for bacteria. For *C. albicans*, amphotericin B (2 µg/ml) was used.

Results

Essential oil

Table 1 summarizes the 14 medicinal plant species. They were collected from different regions of Argentina: Córdoba (center region), Salta (north region), Túcuman (northwest region), and Río Negro (Patagonia region).

Antimicrobial activity

The results of the antimicrobial activity of the essential oils against Gram-positive and Gram-negative bacteria and the yeast *C. albicans* are shown in Table 2. The essential oils of *A. tryphilla* and *P. spartoides* inhibited

Table 1. Medicinal plants of the Argentine Repúblic.

Family	Species	Common name	Collection place
Asteraceae	*Achyrocline satureioides*	Marcela hembra	Potrero de Garay (Córdoba)
	Artemisia annua L.	Artemisa	Río Segundo Córdoba)
	Baccharis flabellata Hooker et Arnott var. *Flabellata*	Clavillo, Romerillo, Chilquilla o Carqueja	Dpto Colón (Córdoba)
	Ophryosporus charrua (Grisebach)	Charrua	Sierras chicas (Córdoba)
	Pectis odorata Grisebach	---	La Calera (Córdoba)
	Psila spartoides (H. et A) J. Rimy	Carqueja	Villa Regina (Río Negro)
Chenopodiaceae	*Chenopodium ambrosoides* L.	Paico	Saldán (Córdoba)
Lamiaceae	*Hyptis mutabilis* (Rich.) Briq.	Salvia azul	Sierra de Guasapampa. (NO de Córdoba)
Laureaceae	*Phoebe porphyria* (Griseb)	Laurel de la falda	Provincia fitogeográ-fica de las Yungas (Salta)
Myrtaceae	*Blepharocalyx tweeidiei*	Anacahuita	Yerba buena (Tucumán)
Schizaceae	*Anemia tomentosa*	Doradilla	---
Verbenaceae	*Aloysia triphylla* L'Herit.) Briton	Cedrón	Río Primero (Córdoba)
	Aloysia polistachia	Poleo real, te de burro	Sierras de Chicas (Córdoba)
	Lippia turbinata Grisebx	Poleo	Mina Clavero (Córdoba)

all tested microorganisms, except *P. aeurginosa*. *B. flabellata* and *P. odorata* inhibited all Gram-positive bacteria but had no effect against Gram-negative ones. The essential oils of *A. satureioides* and *H. mutabilis* were active against *S. aureus*, *B. cereus*, *M. luteus*, *E. faecalis*, and *P. mirabilis*. *A. tomentosa* inhibited only *B. cereus*, whereas *A. polistachia* inhibited *S. aureus, B. cereus*, and *P. mirabilis*. *A. annua* was active against *S. aureus*, *B. cereus*, *Klebsiella* sp., and *P. mirabilis*. The essential oil of *B. tweeidiei* inhibited only *E. coli* and *P. mirabilis*, and *Ch. ambrosoides* was active against *S. aureus*, *S. epidermidis*, *B. cereus*, *E. coli*, *Klebsiella* sp., and *P. mirabilis*. *L. turbinata* inhibited *S. aureus*, *B. cereus*, *E. faecalis*, and *P. mirabilis*. *P. porphyria* was active against *S. aureus*, *B. cereus*, *M. luteus*, and *E. faecalis*.

B. cereus and *S. aureus* were the most susceptible Gram-positive bacteria to the essential oils action. The inhibition zone produced against *B. cereus* by *A. triphylla* was 36 mm, by *P. spartoides* was 25 mm, by *A. annua* was 22 mm, by *P. porphyria* was 19 mm, and by *A. tomentosa* was 18 mm. *S. aureus* showed inhibition zones of 36 mm with *A. triphylla*, 14 mm with *B. flabellata*, 13 mm with *P. porphyria*, and 11.5 mm with *P. odorata* and *P. spartoides* (Table 2).

Between all tested essential oils, the inhibitory activity of *A. triphylla* and *P. spartoides* is remarkable, showing

Table 2. Antibacterial and antifungal activity of essential oils (inhibition zone in mm).

Microorganisms / Plants species	*S. aureus*	*S. epidermidis*	*B. cereus*	*M. luteus*	*E. faecalis*	*E. coli*	*Klebsiella* sp.	*P. mirabilis*	*P. aeruginosa*	*C. albicans*
A. satureioides	7	NI	10	8	8.5	NI	NI	10	NI	NI
A. tomentosa	NI	NI	18	NI	NI	NI	NI	NI	NI	NI
A. polystachia	8	NI	8	NI	NI	NI	NI	7	NI	NI
A. triphylla	**36**	**10.5**	**36**	**17**	**11**	**8**	**8**	**11**	**NI**	**16**
A. annua	7	NI	22	ND	ND	NI	7	7	NI	NI
B. flabelata	14	8	15	17	0.9	NI	NI	NI	NI	ND
B. tweeidiei	NI	NI	NI	NI	NI	7	NI	12	NI	NI
Ch. ambrosoides	9	7.5	8	NI	NI	9.5	9	9	NI	ND
H. mutabilis	7	NI	9	7	9	NI	NI	8	NI	NI
L. turbinata	8	NI	9.5	NI	8	NI	NI	10	NI	NI
O. charrua	NI	NI	10	8.5	NI	NI	NI	NI	NI	NI
P. odorata	11.5	8	10	10	8.5	NI	NI	NI	NI	ND
P. porphyria	13	NI	19	14.5	9	NI	NI	NI	NI	NI
P. spartoides	**11.5**	**10.5**	**25**	**12.5**	**7.5**	**10.5**	**8**	**14**	**NI**	**39**

NI, no inhibition; ND, not determined.

Table 3. Minimum inhibitory concentration of the essential oil of *Aloysia triphylla*.

Concentration (μg/disk) / Microorganisms	*Aloysia triphylla* (Inhibition zone in mm)					
	900	450	225	112.5	56.25	28.12
S. aureus ATCC	36	20.5	16	10.5	11	NI
S. epidermidis	11	11	10	**9**	NI	NI
B. cereus	36	2	15	11	**1**	NI
M. luteus	17	**11**	NI	NI	NI	NI
E. faecalis	11	8.5	**8**	NI	NI	NI
P. mirabilis	11	9	**8**	NI	NI	NI
C. albicans	30	20	15	13	13	**8**

NI, no inhibition.

activity against all Gram-positive and Gram-negative microorganisms tested but not against *P. aeruginosa*. Furthermore, these compounds were active against the yeast *C. albicans*, showing inhibitory zones of 16 mm for *A. triphylla* and 15 mm for *P. spartoides*. *P. mirabilis* was inhibited by seven of the tested essential oils [*P. spartoides* (14 mm), *B. tweeidiei* (12 mm), and *A. triphylla* (11 mm)]. *P. aeruginosa* was not inhibited by any of the oils tested.

Minimum inhibitory concentration

Due to the size of the inhibition zone and the inhibitory spectrum of *A. triphylla* and *P. spartoides*, the minimum inhibitory concentration was performed for them. With the essential oils of *A. triphylla*, the MIC obtained for *C. albicans* was 28.12 μg/disk, and for bacteria the MICs values were 56.25 μg/disk for *S. aureus* and *B. cereus*, followed by *S. epidermidis* 112.5 μg/disk, *E. faecalis* and *P. mirabilis* 225 μg/disk. The MIC values of *E. coli* and *Kebsiella* sp. were the same as the concentration of the pure essential oils (900 μg/disk) (Table 3).

The MIC value for the essential oil of *P. spartoides* with *C. albicans* was 14.06 μg/disk. For *S. aureus*, *B. cereus*, and *P. mirabilis*, the MIC values were 28.12 μg/disk. Only the pure oil inhibited the other microorganisms (900 μg/disk) (Table 4).

Discussion

The variability of antimicrobial activity of the essential oils toward the microorganism investigated can be attributed to qualitative and quantitative differences in the constituents of individual oils (De Pooter et al., 1995). It has been observed that the composition of the essential oils varies according to local climatic and environmental conditions; as a consequence, they have different bioactivities. Some essential oils and their constituents are very active against bacteria although not against fungi and *vice versa*, whereas some essential oils stimulate the growth of some microorganisms (Zygadlo & Juliani, 2000). Between the species studied in this work, it has been reported that *A. satureioides* and *B. flabelatta* essential oils have hydrocarbon-rich pure compounds, whereas the rest of plant species have essential oils rich in oxygenated compounds (Zygadlo & Juliani, 2001).

A. triphylla is a vegetable included in the Verbenaceae that is distributed all along South America and is commonly named *cedrón*. Its medicinal properties are as digestive, carminative, and tonic. The essential oil that can be obtained from this vegetable is rich in oxygenated compounds. Some authors have included geranial, limonene, meral, nerolidol, and mircenona among its main pure components (Zygadlo & Juliani, 2001). The antimicrobial activity of pure components such as limonene and 1,8-cineol has been described (Deans et al., 1995;

Table 4. Minimum inhibitory concentration of the essential oil of *Psila spartoides*.

Concentration (μg/disk) / Microorganisms	*Psila spartoides* (Inhibition zone in mm)							
	900	450	225	112.5	56.25	28.12	14.06	7.03
S. aureus	11.5	9	9	8	8	**8**	NI	NI
B. cereus	25	13.5	10.5	10.5	9	**8.5**	NI	NI
P. mirabilis	14	11.5	10.5	10.5	9	**8.5**	NI	NI
C. albicans	39	25	24	15	13	12	**9.5**	NI

NI, no inhibition.

Chinou et al., 1997; Demo et al., 2001), attributing the antimicrobial activity to these components (Demetzoos et al., 1997; El-Sahkawy et al., 1998; Demo et al., 2001).

P. spartoides is distributed from the Patagonia to the north of Argentina. The plant has shown variability in the essential oil composition, according to the collection zone. The majority of the essential oils obtained from plants of the central region of Argentina contains oxygenated monoterpenes in their composition, whereas the species collected from the Patagonia are formed by oxygenated sesquiterpenes (Zygadlo & Juliani, 2001). The essential oil of *P. spartoides* described in this work were from Rio Negro province, located in the Patagonia region, and showed antimicrobial activity against all tested microorganisms. At the same time, the essential oil of *P. spartoides* collected in La Rioja province (northwest region) demonstrated a smaller antimicrobial spectrum and smaller inhibition zones (data not shown). The differences in the antimicrobial activity could be attributed to variations in the essential oil composition of plants collected in different regions.

Various publications have documented the antimicrobial activity of essential oils and plant extracts against Gram-positive and Gram-negative bacteria and yeast. Several researchers have found that essential oils have more activity against Gram-positive bacteria than Gram-negative ones. The most sensitive species observed were *B. cereus* and *S. aureus* (Gutkind et al., 1981; DiMayuga & Keer-Garcia, 1991; Chinou et al., 1997; Demetzoos et al., 1997; De Feo et al., 1998; Hammer et al., 1999; Primo et al., 2001). These data are in agreement with our results. Gram-negative species exhibited different sensitivity to the essential oils, *P. aeruginosa* being the most resistant microorganism (Deans et al., 1995; Bağci & Diğra, 1996; De Feo et al., 1998; Vataru Nakamura et al., 1999; Primo et al., 2000). Natural resistance of Gram-negative bacteria to some hydrocarbon compounds is due to the chemical composition of the outer membrane, which surrounds the peptidoglycan layer. The outer membrane functions as a molecular sieve through which molecules with a molecular mass greater than 600 to 1000 Da cannot penetrate. Despite the presence of porins with low specificity, the outer membrane shows very low permeability toward hydrophobic compounds, which has been ascribed to the presence of the lipophilic lipopolysaccharide (LPS). Besides, some Gram-negative species, like *Pseudomonas* sp., can develop some adaptations that allow them to grow normally, even in toxic compound presence (Sikkema et al., 1995).

In summary, *A. triphylla* and *P. spartoides* inhibited all tested microorganisms while the other essential oils were active against some of them. *B. cereus* and *S. aureus* were the most sensitive microorganisms. This study confirms that many essential oils possess *in vitro* antibacterial and antifungal activity. However, if plant oils and extracts are to be used for food preservation or medicinal purposes, issues of safety and toxicity will need to be addressed (Hammer et al., 1999).

Acknowledgments

The authors would like to thank Agencia Cordoba Ciencias S.E., SECYT-UNC, and SECYT-Universidad Nacional de Río Cuarto for providing the funding necessary to perform this work. Fellowholders: Maria de las Mercedes Oliva (Agencia Córdoba Ciencia) and Maria L. Lopez and Maria P. Zunino (CONICET).

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