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Antibacterial activity of essential oil of *foeniculum vulgare* miller against multiresistant gram-negative bacilli from nosocomial infections

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Abstract

The essential oil of *Foeniculum vulgare* Miller was analized by GC and GC/MS. Fourteen components, representing the 99.98% of the oil, were identified; *trans*-anethole (64.08%), α-phellandrene (14.54%), and α-pinene (9.38%) were the most abundant constituents. Antimicrobial tests were carried out against 30 strains of multiresistant Gram-negative bacilli isolated from patients with nosocomial infections. Results show that the oil exhibited a strong inhibitory activity against all strains of the Gram-negative bacilli tested, regardless of bacterial species, type or number of resistance markers. These findings suggest that the essential oil of aerial parts of *Foeniculum vulgare* Miller have inhibitory activity against multiresistant Gram-negative bacilli from patients with nosocomial infections. Thus, the investigated essential oil has potential antibacterial activity, which can be further exploited to develop new drugs to treat infections produced by important multiresistant Gram-negative human pathogens.

Key words: Antibacterial activity; essential oil; *Foeniculum vulgare* Miller; nosocomial infections.

Actividad antibacteriana del aceite esencial de *Foeniculum vulgare* Miller contra bacilos gramnegativos multirresistentes provenientes de infeciones nosocomiales

Resumen

El aceite esencial proveniente de *Foeniculum vulgare* Miller fue analizado mediante GC y GC/MS. Catorce componentes constituyeron el 99,98% del aceite, siendo el *trans*-anetol (64,08%), α-felandreno (14,54%) y el α-pineno (9,38%) los más abundantes. Las pruebas para evaluar la actividad antibacteriana fueron llevadas a cabo utilizando 30 cepas de bacilos gramnegativos multirresistentes aisladas de pacientes con infección nosocomial. Los resultados obtenidos demostraron que el aceite exhibió una fuerte actividad inhibitoria contra todas las cepas de bacilos gramnegativos probadas, independientemente de la especie bacteriana, del tipo y número de marcadores de resistencia. Estos hallazgos sugieren que el aceite esencial prove-

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niente de las partes aéreas de *Foeniculum vulgare* Miller tienen actividad inhibitoria contra bacilos gramnegativos multirresistentes provenientes de pacientes con infección nosocomial. De manera que, el aceite esencial investigado tiene actividad antibacteriana potencial, lo cual podría ser explotado para el desarrollo de nuevas drogas que puedan ser empleadas en el tratamiento de infecciones causadas por patogenos humanos importantes, entre ellos las bacterias gramnegativas multirresistentes.

Palabras clave: Aceite esencial; actividad antibacteriana; *Foeniculum vulgare* Miller; infección nosocomial.

Introduction

The pharmacological industries have produced a number of new antibiotics in the last decade. However, bacterial resistance is growing due to the indiscriminate use these conventional drugs. This has caused severe clinical problems in the treatment of infectious diseases, particulary nosocomial infections that have a high mortality (1-3). It is possible that clinically significant new drugs, with antimicrobial activity and structures widely different from those of conventional use, might be found in products from higher plants (2-4).

Foeniculum vulgare Miller is a well-known aromatic plant belonging to the *Umbellifarae* family, a small genus of annual, biennial or perennial herbs distributed in central Europe and Mediterranean region. It is widely cultivated throughout the temperate and tropical regions of the world for its aromatic fruits, which are used as a culinary spice (5). This aromatic plant has been introduced to Venezuela, where it is known as "hinojo", and it is used as anti-inflammatory, anti-spasmodic, anti-diarreic, diuretic, and to treat menstrual disturbances (6).

The present *in vitro* study was carried out to establish the antibacterial activity and the influence of the inoculum size and volume of essential oil of *Foeniculum vulgare* Miller against multiresistant Gram-negative bacilli isolated from patients with nosocomial infections.

Materials and Methods

Aerial parts of three individuals of *F. vulgare* Miller were collected at El Playón, Mérida, Venezuela at 1700 m of altitude during the flowering stage on February 2005. A voucher specimen (C. Ferranti, 18) is kept at the Herbarium of the Faculty of Pharmacy, of The Andes University, Mérida. Venezuela.

Fresh flowers, leaves and twigs were ground and subjected to hydrodistillation for 3 hours using a Clevenger-type apparatus to yield 0.22% of yellowish oil, which after drying was submitted to GC and GC/MS analysis. A Perkin Elmer AutoSystem gas chromatograph (GC), fitted with an HP-5 (60 m x 0.25 mm x 0.25 μm film) fused silica capillary column was used for GC/FID and Kovats indices determination (7). The temperature program used was the following: initial temperature 60°C followed by heating at 4°C/min to 200°C; final temperature was kept for 20 min. The injection and detector temperatures were 200°C and 250°C respectively. Helium was used as the carrier gas (1.0 mL/min). Mass spectra were obtained on a 5973 Hewlett Packard MSD system operating in the EI mode at 70 Ev, equipped with an HP-5MS capillary column (30 m x 0.25 mm x 0.25 μm film). The oven temperature program was the same used for GC analysis. The sample was diluted with diethyl ether (20 μL in 1.0 mL) and 1.0 μL was injected at 200°C (split ratio 1:100). The carrier gas was He at 0.9 mL/min. The identity of the oil components was established from their GC retention indices (8), comparison of their mass spectra by library search (Wiley 6th ed.), and co-chromatography with authentic compounds.

Thirty Gram-negative bacilli strains (15 *Pseudomonas aeruginosa*, 10 *Acinetobacter* spp and 5 *Alcaligenes faecalis*) resistant to wide-spectrum β-lactam antibiotics and aminoglycosides, were isolated from blood cultures of adult patients with nosocomial infection at the intensive care unit (ICU) of The Andes University Hospital (9). *P. aeruginosa* ATCC 27853, *A. baumannii* ATCC 19606 and *A. faecalis* ATCC 8750 were used as control strains for susceptibility testing.

The inoculum of each isolate, prepared by culture in Mueller-Hinton (Difco) broth, was incubated in air at 36°C for 2 hours. Cultures were further diluted in Ringer ¼ (Oxoid) to obtain different inocula of approximately 10^7, 10^5 and 10^3 cfu/mL. The bacterial suspensions were inoculated in triplicate in Mueller-Hinton agar (40 mL in Petri dishes) in wells with different volume capacities made by means of a sterile cover mold manufactured ad hoc. Five different essential oil volumes (30, 25, 20, 15 and 10 μL) were placed in the wells and sealed with sterile paraffin to avoid oil evaporation during incubation. After aerobic incubation during 16-20 h at 36°C, the agar plates were examined. The relative susceptibility of the organism to the oil was demonstrated by the diameter of the clear zone of growth inhibition around the wells, which was measured using a vernier calipers (Mitutoyo). Fleroxacin (4 μg), a fluoroquinolone (F. Hoffman La Roche Ltd.) was used as a standard antibiotic to control the sensitivity of the tested microorganism.

Results and Discussion

By means of combined GC and GC/MS, 14 components (99.98% of the oil) were identified in the essential oil of *F. vulgare* Miller. Results are shown in Table 1. The most important constituent was *trans*-anethole (64.08%), followed by a-phellandrene (14.54%) and a-pinene (9.38%). The monoterpene hydrocarbon fraction (29.30%) of the venezuelan oil was similar to the values reported for the essential oil from Italy, but the content of *trans*-anethole and estragole (66.85%) was higher (10, 11).

The pure essential oil of *F. vulgare* Miller showed inhibitory activity against Gram-negative bacilli of nosocomial origin. These bacteria show two or three resistance patterns due to at least two different antibiotics (bbb-lactams and aminoglycosides). The capacity of the essential oil to inhibit bacterial growth assessed by the tested inocula are shown in Table 2. Inhibition of bacterial growth was observed in all cases. While the essential oil of *F. vulgare* Miller exhibited slight differences of bacterial growth inhibition based on inoculum size between 10^7 and 10^5 cfu/mL, bacterial concentrations lower than 10^5 cfu/mL also showed considerably sensitivity to the inhibitory activity of the essential oil, regardless of bacterial species and resistance patterns, which was comparable to the strong activity of fleroxacin (4 μg/mL). Also, the inhibitory effect of the essential oil was related to the volumes used (Table 2). Results showed that between 10 to 30 μL the inhibitory activity increased the inhibition halos approximately by 0.5 mm per μL of essential oil.

The thirty strains of multiresistant Gram-negative bacilli studied were predominant nosocomial pathogens, ranking among the most frequently isolated bacteria in intensive care units. These pathogens showed wide resistance to conventional antibiotics (1, 9). Results obtained in this study indicate an excellent *in vitro* inhibitory activity against the nosocomial pathogens considered in this study. It is important to stress that the essential oil of *F. vulgare* showed inhibitory activity on several Gram-negative bacterial species with different types and number of resistance markers (9).

These findings suggest that the essential oil of aerial parts of *Foeniculum vulgare* Miller have inhibitory activity against multiresistant Gram-negative bacilli from patients with nosocomial infections. It seems plausible that

Table 1
Chemical composition of essential oil of *Foeniculum vulgare* Miller by GC/MS analysis.

RI	Compounds	%	Identification
931	α-Pinene	9.38	a-b-c
969	Camphene	0.08	a-b-c
971	Sabinene	0.12	b-c
981	β-Pinene	0.75	a-b-c
998	Myrcene	0.83	a-b-c
1004	α-Phellandrene	14.54	a-b-c
1022	*p*-Cymene	0.61	a-b-c
1030	β-Phellandrene	2.51	a-b-c
1037	β-Ocimene	0.25	b-c
1058	γ-Terpinene	0.23	a-b-c
1085	Fenchone	3.48	b-c
1190*	Estragole	2.77	-
1232	Fenchil acetate	0.35	b-c
1280	*trans*-Anethole	64.08	b-c

Percentage of identified compounds: 99.98%. RI: calculated retention indices. * RI reference value not found in the literature. a, Comparison with authentic compounds; b, Comparison of RI with literature values (8); c,Comparison of mass spectra with Wiley Library.

their main constituents, the *trans*-anethole and α-phellandrene, may play a significant role in the antibacterial activity of the oil tested. Future studies are needed to further acertain the antibacterial activity of these compounds, either pure or combined, to determine if the essential oil of *F. vulgare* Miller might offer new hopes to combat multiresistant Gram-negative pathogens.

Conclusions

The essential oil obtained from aerial parts of *Foeniculum vulgare* Miller have inhibitory activity against multiresistant Gram-negative bacilli from patients with nosocomial infections. Thus, the investigated essential oil has potential antibacterial activity, which can be further exploited to develop new drugs to treat infections produced by important multiresistant Gram-negative human pathogens.

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Table 2
Effect of inoculum size and different volumes on the *in vitro* antibacterial activity of essential oil of *Foeniculum vulgare* Miller

Microorganisms resistant to specific antibiotics (No.)	Average diameter (mm) of inhibition zone in Mueller-Hinton agar produced by different inoculum sizes (cfu/mL)[a]			Average diameter (mm) of inhibition zone in Mueller-Hinton agar produced by different volumes of essential oil[b]				Control Fleroxacin (4 μg/mL)[b]
	10^7	10^5	10^3	30 μL	25 μL	20 μL	15 μL	
Pseudomonas aeruginosa (*n*=15)								
CAR-PIP-GTM (6)	18	19	23	26	24	21	19	25
CAR-GTM-AMK (2)	17	19	22	26	23	21	19	25
CAR-PIP-CTZ-GTM-AMK (7)	18	20	23	25	23	22	20	23
P. aeruginosa ATCC 27853	20	26	28	28	28	26	26	30
Acinetobacter spp (*n*=10)								
GTM-AMK (5)	13	17	18	19	19	17	17	17
PIP-CTZ-GTM-AMK (5)	14	16	18	18	18	18	17	18
A. baumannii ATCC 19606	19	17	21	23	21	20	17	21
Alcaligenes faecalis (*n*=5)								
GTM-AMK (2)	17	18	22	24	23	20	18	22
PIP-CTZ-GTM (3)	17	19	23	25	22	20	19	25
A. faecalis ATCC 8750	20	26	29	30	29	29	26	30

[a]used volume of essential oil 15 μL; [b] used bacterial inoculum 10^5 cfu/mL. CAR: carbenicillin; PIP: piperacillin; GTM: gentamicin; AMK: amikacin; CTZ: ceftazidime. ATCC: American Type Culture Collection.

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