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RESEARCH ARTICLE

Antifungal activity of extracts of some plants used in Brazilian traditional medicine against the pathogenic fungus *Paracoccidioides brasiliensis*

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Abstract

Paracoccidioidomycosis (PCM) is a systemic granulomatous disease caused by *Paracoccidioides brasiliensis* Almeida (Onygenales) that requires 1–2 years of treatment. In the absence of drug therapy, the disease is usually fatal, highlighting the need for the identification of safer, novel, and more effective antifungal compounds. With this need in mind, several plants employed in Brazilian traditional medicine were assayed on *P. brasiliensis* and murine macrophages. Extracts were prepared from 10 plant species: *Inga* spp. Mill. (Leguminosae), *Schinus terebinthifolius* Raddi (Anacardiaceae), *Punica granatum* L. (Punicaceae), *Alternanthera brasiliana* Kuntze (Amaranthaceae), *Piper regnellii* CDC. (Piperaceae), *P. abutiloides* Kunth (Piperaceae), *Herissantia crispa* L. Briz. (Malvaceae), *Rubus urticaefolius* Poir (Rosaceae), *Rumex acetosa* L. (Polygonaceae), and *Baccharis dracunculifolia* DC. (Asteraceae). Hexane fractions from hydroalcoholic extracts of *Piper regnellii* and *Baccharis dracunculifolia* were the most active against the fungus, displaying minimum inhibitory concentration (MIC) values of 7.8 µg/mL and 7.8–30 µg/mL, respectively. Additionally, neither of the extracts exhibited any apparent cytotoxic effects on murine macrophages at 20 µg/mL. Analyses of these fractions using gas chromatography-mass spectrometry (GC-MS) showed that the major components of *B. dracunculifolia* were ethyl hydrocinnamate (14.35%) and spathulenol (16.02%), while the major components of the hexane fraction of *Piper regnellii* were 1-methoxy-4-(1-propenyl) benzene (21.94%) and apiol (21.29%). The activities of these fractions against *P. brasiliensis* without evidence of cytotoxicity to macrophages justify their investigation as a potential source of new chemical agents for the treatment of PCM.

Keywords: *Antifungal activity; GC-MS;* P. brasiliensis*; traditional medicinal plants*

Introduction

Paracoccidioidomycosis (PCM) is the most prevalent systemic mycosis in Latin America (Coutinho et al., 2002). The etiologic agent of this mycosis is the thermodimorphic fungus *Paracoccidioides brasiliensis* Almeida (Onygenales). The annual incidence rate of PCM in Brazil, the country with the highest disease endemicity, is 10–30 per million inhabitants, and the mean mortality rate estimated for the period from 1980 to 1995 was 1.4 deaths per million inhabitants per year (Coutinho et al., 2002).

In the absence of drug therapy, the disease is usually fatal. The treatment of PCM is usually long, with many patients receiving therapy for 1–2 years or even more (Shikanai-Yasuda et al., 2006). Although azoles

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and other drugs can arrest the progression of PCM, the fibrosis sequelae persist, probably constituting a source of *P. brasiliensis* that could lead to a relapse in the disease following termination of treatment (Onishi et al., 2000; Borges-Walmsley et al., 2002). The strong toxicity of amphotericin B makes the effective management of severe disease difficult (Lorthay et al., 1999). This situation highlights the need for the advent of safe, novel, and effective antifungal compounds. In this regard, plants provide abundant resources of antimicrobial compounds and have been used for centuries to inhibit microbial growth (Nicoletti, 2002).

There are some literature reports on the antimicrobial activity of *Punica granatum* L. (Punicaceae), *Schinus terebinthifolius* Raddi (Anacardiaceae), *Herissantia crispa* L. Briz. (Malvaceae), *Rubus urticaefolius* Poir (Rosaceae), *Baccharis dracunculifolia* DC. (Asteraceae), and *Piper regnellii* CDC. (Piperaceae) (Martinez et al., 1996; Prashanth et al., 2001; Holetz et al., 2002; Voravuthikunchai et al., 2004; Guerra et al., 2005; Schomourlo et al., 2006; Johann et al., 2007b). Silva and Siqueira (2000) observed that the extracts of *R. urticaefolius* were capable of inhibiting the growth of Gram-positive and Gram-negative bacteria. Johann et al. (2007b) showed that *P. granatum*, *S. terebinthifolius*, *R. urticaefolius*, *B. dracunculifolia*, and *P. regnellii* display activity against *Candida* spp., *Cryptococcos neoformans*, and *Sporothrix schenkii*. Souza et al. (2004) and Johann et al. (2007b) found that *Staphylococcus aureus*, *S. epidermidis*, *Escherichia coli*, *Bacillus subtilis*, and *Candida* spp. were resistant to the extracts of *Alternanthera brasiliana*. However, other authors reported that extracts of this plant act as inhibitors of lymphocyte proliferation (Moraes et al., 1994) and as an antiviral agent against virus herpes simplex 1 (Lagrota et al., 1994). Johann et al. (2008) reported that extracts of *S. terebinthifolius* and *P. granatum* were able to inhibit the growth of three isolates of *Candida albicans*. These plant species also showed the best results as inhibitors of adhesion of *C. albicans* to buccal epithelial cells. Otherwise, *Rumex acetosa* was active against *C. tropicalis* whereas *Inga* spp. were inactive against all the fungal species tested (Johann et al., 2007b). The aims of this work were to evaluate extracts of plants used in Brazilian popular medicine against *P. brasiliensis* and to verify the toxicity of these extracts on murine macrophages.

Materials and methods

Plant materials

Different parts of 10 plant species were sampled for use in this study. The samples included leaves (312 g) of *Inga* spp. Mill. (Leguminosae), leaves (400 g) and stem (250 g) of *Schinus terebinthifolius* Raddi (594 g), and fruit peel (546 g) and stems (1520 g) of *Punica granatum*. The aerial parts (a mix of leaves, stems, and flowers) of the following plants were collected: *Alternanthera brasiliana* Kuntze (Amaranthaceae), 149 g; *Piper regnellii*, 191 g; *P. abutiloides* Kunth (Piperaceae), 301 g; *Herissantia crispa*, 95 g; *Rubus urticaefolius*, 141 g; *Rumex acetosa* L. (Polygonaceae), 45 g; and *Baccharis dracunculifolia*, 483 g. The plant materials were collected in different regions of Santa Catarina State, Brazil from December 2003 to January 2004. *Inga* spp. and *P. granatum* were identified by Professor Barcellos Falkenbergat from the Department of Botany, Federal University of Santa Catarina (UFSC), and voucher specimens were deposited in the FLORA-UFSC herbarium. The remaining plant materials were furnished by EPAGRI (Empresa Agropecuária e Extensão Rural de Santa Catarina) germoplasm bank, Itajaí, SC, Brazil. Botanical names and voucher specimens are listed in Table 1.

Preparation of the extracts

Plant materials were extracted by maceration with 80% ethanol during 10 days at room temperature. After filtration, the hydroalcoholic extract (EtOH) was concentrated under reduced pressure to afford the crude extracts. These extracts were suspended in distilled water and extracted successively with hexane (HEX), dichloromethane (DCM), and ethyl acetate (AcOEt) (Johann et al., 2008). After separation of the phases, the solvents were removed in a rotary evaporator at 45°C under vacuum. All extracts and fractions were submitted to biological assays.

Analysis by gas chromatography-mass spectrometry

Hexane extracts of *B. dracunculifolia* and *P regnellii* were analyzed by gas chromatography coupled to nass spectrometry (GC-MS) using the solid phase microextraction (SPME) analysis mode.

Collection of volatiles by SPME

The volatiles of the hexane fractions of *B. dracunculifolia* and *P regnellii* were adsorbed in an SPME sampling device and analyzed by GC-MS. Thus, 1 mg of each hexane fraction was transferred to a 2 mL glass vial, which was closed with a cap sealed with a Teflon coated septum (Supelco, USA) and placed in a heat block adjusted to 90°C. An SPME fiber (PDMS/DVB™ 65 µm; Supelco, USA) was inserted through the septum and left in the headspace for 5 min. Before use, the fiber was preconditioned at 230°C for 30 min in the GC injector port (Siqueira et al., 2007).

GC-MS analysis

Gas chromatography-mass spectrometry (GC-MS) analyses were performed on a Shimadzu QP-5050A (Shimadzu, Japan) instrument, equipped with a PTE™-5 column (30 mm, 0.25 mm, 0.25 μm; Supelco, USA), using helium as the carrier gas. The following conditions were employed for all analysis: carrier gas, helium at 22.3 mL/min; injector temperature, maintained at 230°C; column temperature, 3 min at 80°C, 80–300°C at 7°C/min, maintained at 300°C for 5 min, and kept at this temperature for 5 min. The split valve was closed during the first minute of injection and then opened, with a 1:10 ratio. The mass detector was set to scan from 50 to 500 atomic mass units, at a rate of two scans per second. Data acquisition and handling were done via CLASS 5000 Shimadzu software. Raw data files were analyzed by Automated Mass Deconvolution and Identification System software (AMDIS), version 2.1, supplied by the National Institute of Standards and Technology (NIST, USA), and compound identification was performed by comparison of the experimental spectra with those stored in the NIST/EPA/NIH library version 2.0 using the NIST Mass Spectral Search Program.

Paracoccidioides brasiliensis *strain maintenance*

Three clinical *P. brasiliensis* strains, Pb01 (ATCC MYA-826), Pb339 (ATCC 32069), and Pb18 (from the fungal collection of the Faculty of Medicine of the University of São Paulo, São Paulo, SP, Brazil), were used in the biological assays.

Inoculum preparation

The strains were maintained at the Laboratory of Mycology, ICB, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil, by weekly passage in solid Fava-Netto medium (Lacaz et al., 2002) at 37°C and were used after 7–10 days of fungus growth. Yeast cells in the exponential phase were collected aseptically with a platinum loop and resuspended in a tube containing 5 mL of sterile saline. If large aggregates existed, they were allowed to settle for several minutes, and the supernatants were collected. The suspensions were then diluted in synthetic RPMI medium (Sigma, St. Louis, MO, USA) with L-glutamine buffered to pH 7.0 with 0.165 morpholine propanesulfonic acid (MOPS; Sigma), and prepared according to Clinical and Laboratory Standards Institute (CLSI) document M27-A_2 (NCCLS, 2008) to obtain a final inoculum size suitable for the strains (Nakai, 2003). After homogenization by vortexing, transmittance was measured at 520 nm and adjusted to 69–70% (Hahn & Hamdan, 2000).

Susceptibility test of **Paracoccidioides brasiliensis**

The plant extracts were dissolved in dimethylsulfoxide (DMSO). Serial dilutions were then performed, using RPMI as a diluent, maintaining a constant volume of 1 mL per tube. The extracts were tested at eight concentrations that ranged from 1000 to 7.8 μg/mL. Volumes of 100 μL of each dilution were distributed in sterile flat-bottom 96-well microplates (Difco Laboratories, Detroit, MI, USA).

Susceptibility was determined by the broth microdilution method. Broth microdilution testing was performed in accordance with the guidelines in the CLSI M27-A_2 document (NCCLS, 2008) and Nakai (2003). RPMI medium was used without compounds or solvents as a control for growth and sterility. Solvent DMSO at the same volume as used in the assay was used as a control for toxicity. Amphotericin B (Sigma) was included as a positive antifungal control, as stock solutions prepared in DMSO and water, respectively. Two-fold serial dilutions were prepared exactly as outlined in CLSI document M27-A_2 (NCCLS, 2008). After inoculation of fungal strains the plates were incubated at 37°C for 72 h. The tests were performed in triplicate in at least two independent experiments. The endpoints were determined visually by comparison with the drug-free growth control well. MIC is expressed in μg/mL and defined as the lowest compound concentration for which the well was optically clear.

Toxicity to murine macrophages

Murine macrophages were obtained as previously described by Paulnock (2000), and the cytotoxicity assay was performed according to Soto et al. (2007).

Results

The three *P. brasiliensis* strains presented similar susceptibility profiles in relation to the plant extracts tested (Table 1). Hexane fractions of the extracts of *S. terebinthifolius*, *R. urticaefolia*, *B. dracunculifolia*, *P. abutiloides*, *P. regnellii*, and *H. crispa* were most active against the fungus when compared with the other fractions of these plants. All *P. brasiliensis* isolates tested were resistant to the EtOH extract of *A. brasiliana* at a concentration of 1000 μg/mL.

The hexane fractions of *B. dracunculifolia* and *P. regnellii* showed MIC values of between 7.8 and 30 μg/mL and 7.8 μg/mL, respectively, against the three strains of *P. brasiliensis* (Table 1). The chromatogram of the hexane fraction of *B. dracunculifolia* under the conditions described in the "Materials and methods" section showed 87 peaks. The major components were: ethyl hydrocinnamate (14.35%), caryophyllene (14.45%), δ-cadinene (4.83%), nerolidol (5.95%), spathulenol

(16.02%), and viridiflorol (4.37%) (Figure 1, Appendix). Analysis of the hexane fraction of *P. regnellii* showed 24 peaks, and seven of them were identified by comparison with the NIST library (v. 2.0) (Figure 2, Appendix). The major components were 1-methoxy-4-(1-propenyl) benzene (21.94%), α-copaene (8.91%), aromadendrene (12.92%), δ-cadinene (9.82%), dillapiole (11.31%), apiol (23.22%), and β-eudesmol (3.48%). Extracts and fractions of *P. granatum*, *S. terebinthifolius*, *R. urticaefolius*, *B. dracunculifolia*, *P. abutiloides*, and *Inga* spp. did not show cytotoxic activity against murine macrophages (Table 1).

Discussion

There are a few reports describing the search for new compounds with the ability to inhibit *P. brasiliensis*.

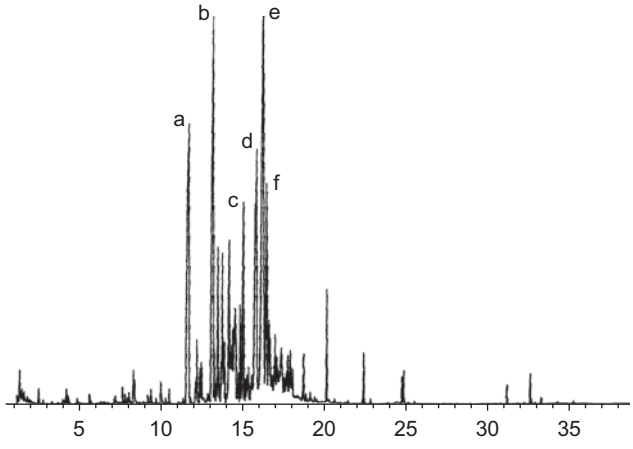

Figure 1. Total ion chromatogram of the hexane fraction of *Baccharis dracunculifolia*. a, ethyl hydrocinnamate, m/z = 178; b, caryophyllene, m/z = 204; c, δ-cadinene, m/z = 204; d, nerolidol, m/z = 222; e, spathulenol, m/z = 220; f, viridiflorol, m/z = 222.

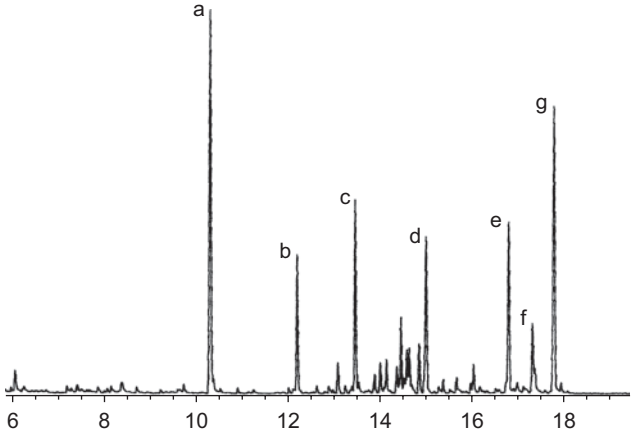

Figure 2. Total ion chromatogram of the hexane fraction of *Piper regnellii*. a, 1-Methoxy-4-(1-propenyl) benzene, m/z = 148; b, α-copaene, m/z = 204; c, aromadendrene, m/z = 204; d, δ-cadinene, m/z = 204; e, dillapiole, m/z = 222; f, β-eudesmol, m/z = 222, g, apiol, m/z = 222.

San-Blas et al. (1989) demonstrated that ajoene, an alicin derivative isolated from garlic (*Allium sativum* L., (Liliaceae)), was active against the yeast and filamentous forms of *P. brasiliensis*. These authors observed a lytic effect when the fungal cells were treated with 200 μM of ajoene.

In our work, we observed that extracts from six out of 10 plants examined exhibited antifungal activity against *P. brasiliensis*. For *P. granatum* specifically, the hexane fraction from stems (MIC: 40–71.6 μg/mL) exhibited better antifungal activity against the three clinical isolates of *P. brasiliensis* than extracts from leaves and fruits, indicating that the plant is a potential source of new antimicrobial compounds (Table 1). The presence of flavonoids and tannins in the Punicaceae species is responsible for several biological activities (Hussein et al., 1997). However, these substances were in more polar, ethanol or aqueous fractions. On the other hand, Purwantini and Wahyuono (2003) isolated terpenoids, steroids with a stigmastane skeleton esterified by a long-chain fatty acid, from the hexane fraction of *P. granatum*, which showed antifungal activity against *Candida albicans*. This finding is in agreement with our observations that the hexane fraction is the most active.

The hexane fraction of the extract from the aerial parts of *R. urticaefolius* inhibited *P. brasiliensis* growth at a concentration of 60-500 μg/mL. The fractions of the hydroalcoholic extracts of *A. brasiliana* did not present any activity against the three clinical isolates of *P. brasiliensis*. This result contrasts with that of Biavatti et al. (2003), which showed inhibitory activity of the extracts of this plant against oocysts of the protozoa *Eimeira acervulina*.

We could not find data on the biological activity and phytochemical investigation of the species *Inga* spp. and *H. crispa*. Extracts of these traditional medicinal plants were active on *P. brasiliensis*, and could represent new sources of antifungal compounds.

As the hexane fractions of *B. dracunculifolia* and *P. regnellii* showed the best MIC values (7.8–30 and 7.8 μg/mL, respectively) against the three strains of *P. brasiliensis* (Table 1), these extracts were chosen for chromatographic analysis. Pessini et al. (2005) showed that the EtOAc extract of *P. regnellii* presented significant activity against *C. albicans* and moderate activity against both *C. krusei* and *C. parapsilosis*. The compounds eupomatenoid-6, eupomatenoid-5, eupomatenoid-3, and conocarpan were isolated, but only the latter was active against the yeasts (Pessini et al., 2005). Constantin et al. (2001) analyzed the essential oil obtained by hydrodistillation of leaves of *P. regnellii* by GC-MS and found that it was active against *Staphylococcus aureus* and *C. albicans*. The analysis showed the presence of mircene (52.6%), linalol (15.9%), β-caryophyllene

Table 1. Minimum inhibitory concentrations (μg/mL) of extracts of selected Brazilian medicinal plants against *Paraccocidioides brasiliensis* and cytotoxic effects on murine macrophages.

Plant species (plant part)	*Extract/fraction*	Pb01	Pb18	Pb339	% Cytotoxic effect on murine macrophages[†]
*P. granatum (*leaves*) FLOR 7141**	EtOH	500	750	833	0
	Hexane	1000	166	416	0
	AcOEt	500	1000	750	0
	DCM	666	666	250	0
	Aqueous	1000	883	1000	0
*P. granatum (*stems*)*	EtOH	1000	416	1000	0
	Hexane	71.6	50	40	0
	AcOEt	333	500	500	0
	DCM	500	166	1000	0
	Aqueous	500	500	1000	0
*P. granatum (*fruit peel*)*	EtOH	500	500	1000	0
	Hexane	1000	708	1000	0
	AcOEt	500	833	1000	0
	DCM	1000	1000	1000	0
	Aqueous	500	500	1000	0
S. terebinthifolius (leaves) 44*	EtOH				0
	Hexane	27.6	125	15.2	0
	AcOEt	1000	1000	583	0
	DCM	1000	1000	186	0
	Aqueous	625	1000	1000	0
S. terebinthifolius (stem)	EtOH	30	30	30	0
	Hexane	1000	1000	1000	0
	AcOEt	125	500	1000	0
	DCM	30	30	30	0
	Aqueous	1000	1000	1000	0
R. urticaefolius (aerial parts) 31*	EtOH	125	500	500	0
	Hexane	30	125	125	0
	AcOEt	250	250	1000	0
	DCM	60	500	500	0
	Aqueous	500	1000	1000	0
B. dracunculifolia (aerial parts) 138*	EtOH	125	250	250	0
	Hexane	7.8	7.8	30	0
	AcOEt	250	1000	500	0
	DCM	60	250	500	0
	Aqueous	500	500	1000	0
P. regnellii (aerial parts) 550*	EtOH	250	500	250	100[†]
	Hexane	7.8	7.8	7.8	0
	AcOEt	500	250	500	0
	DCM	30	30	30	0
	Aqueous	500	500	1000	0
P. abutiloides (aerial parts) 102*	EtOH	30	250	30	9
	Hexane	15	60	60	0
	AcOEt	500	500	500	0
	DCM	30	60	30	0
	Aqueous	–	–	–	0
Inga spp. (aerial parts) FLOR 33887*	EtOH	125	500	125	8
	AcOEt	250	1000	1000	5
	DCM	125	125	1000	0
	Aqueous	1000	1000	1000	0
H. crispa. (aerial parts) 316*	EtOH	125	500	500	18
	Hexane	30	250	125	0

Table 1. Continued on next page

Table 1. Continued.

Plant species (plant part)	*Extract/fraction*	Pb01	Pb18	Pb339	% Cytotoxic effect on murine macrophages†
	AcOEt	250	500	1000	0
	DCM	60	250	250	0
R. acetosa (aerial parts) 514*	EtOH	60	500	125	0
A. brasiliana (aerial parts) 382*	EtOH	1000	1000	1000	0
Amphotericin-B		0.03	0.01	0.03	0.6
DMSO		–	–	–	0.7

**Voucher number;* †≥20% is cytotoxic; –, not active.

(8.5%), (*E*)-nerolidol (4.2%), and limonene (4.1%). In our studies, the chromatographic profile of the volatiles obtained in the GC-MS analysis was different because the samples were obtained using different methodologies (fresh leaves vs. hexane fraction of the hydroalcoholic extract), and because we used solid phase extraction instead of hydrodistillation to capture the volatiles. Further studies will be needed to isolate and characterize the antifungal compounds from extracts of *P. regnellii* and *P. abutiloides*. The present study confirms the antifungal properties of the *P. regnellii* extract and its potential as a source of useful bioactive compounds.

The extract of *B. dracunculifolia* and its hexane fraction were active at low concentrations against the three isolates of *P. brasiliensis*. This fraction was also analyzed by GC-MS and showed the presence of diterpenes and sesquiterpenes (δ-cadinene, spathulenol, caryophyllene, viridiflorol, and nerolidol). These compounds could be responsible for the observed activity against *P. brasiliensis*, since terpenoids are known for their antimicrobial activities (Nicoletti, 2002). Terpenoids as both optical isomers of carvone were found to be active toward many kinds of human pathogenic fungi. The development of *C. albicans*, *C. krusei*, and *C. tropicalis* was also inhibited by a combination of monoterpenes, including terpenin-4-ol, α-pinene, 1,8-cineole, linalool, and α-terpineol. These monoterpenes also inhibited the development of dermatophytes such as *Trichophyton mentagrophytes*, *T. rubrum*, and *Microsporum gypseum*. α-Terpinene also exhibited antifungal activity similar to that of commonly used antifungal drugs (Paduch et al., 2007).

The mechanism of action of terpenoids has not been explained completely, but is speculated to involve the rupture of the cellular membrane and modification of the structure of enzymes (Lima et al., 1992). Additionally, it may involve inhibition of the synthesis of 1,3-β-D-glucan, which participates in the synthesis of the cellular wall of fungi (Onishi et al., 2000). According to Santos et al. (1966), nerolidol is the major constituent of the essential oil of *B. dracunculifolia*. However, in our work, this compound represented only 6% of the total area in the hexane fraction of *B. dracunculifolia*. This discrepancy could be due to many factors, including differences in chemotype, seasonality, and methods of collection and extraction. Loayza et al. (1995) described the presence of alloaromadendrene and nerolidol in the essential oil of *B. dracunculifolia*, which is in accordance with our findings. However, bioassay-guided isolation using an assay with *P. brasiliensis* is necessary to verify the compounds responsible for the antifungal activity.

Macrophages are professional phagocytes that act as the first line of defense, provided by the innate immune system. Resident macrophages are widely distributed in tissues and are one of the primary cell types to sense and respond to microbial invaders. The antifungal activity of macrophages is an interesting and important effector mechanism that involves intercellular and/or intracellular killing of parasites (Paulnock, 2000). In the search for plant extracts with antimicrobial activities, it is very important to show that the extracts are not toxic to these cells, which should be included as a control in the screening procedure (Johann et al., 2008; Santos et al., 2008). Taking this into consideration, our extracts were tested in a bioassay with murine macrophages. Our results show that in spite of their ability to inhibit the isolates of the pathogenic fungus *P. brasiliensis*, the fractions of extracts of *P. granatum*, *S. terebinthifolius*, *R. urticaefolius*, *B. dracunculifolia*, *P. abutiloides*, and *Inga* spp. are not toxic to these cells. Thus, the results obtained in the present work will encourage future studies characterizing the impact of these extracts or compounds isolated from them on human cells in the quest for new antifungal drugs. This is especially true for the hexane fractions of *P. regnellii* and *B. dracunculifolia*, which were the most active against this pathogenic fungus and without apparent cytotoxicity to murine macrophages. Further investigations aimed at identifying their bioactive constituents are under way.

Declaration of interest

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References

Biavatti MWO, Bellaver MH, Volpato LO, Costa C, Bellaver C (2003): Preliminary studies of alternative feed additives for broilers: *Alternanthera brasiliana* extract, propolis extract and linseed oil. *Rev Bras Cienc Avic* 5: 147–151.

Borges-Walmsley MI, Chen D, Shu X, Walmsley AR (2002): The pathobiology of *Paracoccidioides brasiliensis*. *Trends Microbiol* 10: 80–87.

Constantin MB, Sartorelli P, Limberger R, Henriques AT, Steppe M, Ferreira MJP, Ohara MT, Emerenciano VP, Kato MJ (2001): Essential oils from *Piper cernuum* and *Piper regnellii*: Antimicrobial activities analysis by GC/MS and ^{13}C-NMR. *Planta Med* 67: 771–773.

Coutinho ZF, da Silva D, Lazéra M, Petri V, Oliveira RM, Sabrosa PC, Wanke B (2002): Paracoccidioidomycosis mortality in Brazil (1980-1995). *Cad Saúde Pública* 18: 1441–1454.

Guerra MJM, Barreiro ML, Rodríguez ZM, Rubalcaba Y (2005): Actividad antimicrobiana de um extracto fluido al 80% de *Schinus terebinthifolius* Raddi (Copal). *Rev Cubana Med Trop* 5: 23–25.

Hahn RC, Hamdan JS (2000): Effects of amphotericin B and three azole derivatives on the lipids of yeast cells of *Paracoccidioides brasiliensis*. *Antimicrob Agents Chemother* 44: 1997–2000.

Holetz FB, Pessini GL, Sanches NG, Cortez DAG, Nakamura CV, Dias Filho BP (2002): Screening of some plants used in the Brazilian folk medicine for the treatment of infectious diseases. *Mem Inst Oswaldo Cruz* 97: 1027–1031.

Hussein SAM, Barakat HH, Merfort I, Nawwar MAM (1997): Tannins from the leaves of *Punica granatum*. *Phytochemistry* 45: 819–823.

Johann S, Pizzolatti MG, Donnici CL, Resende MA (2007b): Antifungal properties of plants used in Brazilian traditional medicine against clinically revelant fungal pathogens. *Braz J Microbiol* 38: 632–637.

Johann S, Silva DL, Martins CVB, Zani CL, Pizzolatti MG, Resende MA (2008): Inhibitory effect of extracts from Brazilian medicinal plants on the adhesion of *Candida albicans* to buccal epithelial cells. *World J Microbiol Biotechnol* 24: 2459–2464.

Lacaz CS, Porto E, Martins JEC, Heins-Vaccari EM, Melo NT (2002): *Tratado de Micologia Médica Lacaz*. São Paulo, Sarvier, pp. 639–729.

Lagrota MHC, Wigg MD, Santos MMG, Miranda MMFS, Camara FP, Couceiro JNSS, Costa SS (1994): Inhibitory activity of extracts of *Alternanthera brasiliana* (Amaranthaceae) against the herpes simplex virus. *Phytother Res* 8: 358–361.

Lima EOG, Paulo MQ, Giesbrecht AM (1992): *In vitro* antifungal activity of essential oils against clinical isolates of dermatophytes. *Rev Microbiol* 23: 235–238.

Loayza I, Abujder D, Aranda R, Jakupovic J, Collin G, Deslauriers H, Jeand FI (1995): Essential oils of *Baccharis salicifolia*, *B. latifolia* and *B. dracunculifolia*. *Phytochemistry* 38: 381–389.

Lorthay O, Denning DW, Dupont B (1999): Endemic mycoses: A treatment update. *J Antimicrob Chemother* 43: 321–331.

Martinez MJ, Betancourt J, Alonso-Gonzfilez N, Jauregui A (1996): Screening of some Cuban medicinal plants activity for antimicrobial. *J Ethnopharmacol* 52: 171–174.

Moraes VL, Santos LF, Castro SB, Loureiro LH, Lima AO, Souza ML, Yien LM, Rossi-Bergmann B, Costa SS (1994): Inhibition of lymphocyte activation by extracts and fractions of *Kalanchoe*, *Alternanthera*, *Paullinia* and *Mikania* species. *Phytomedicine* 1: 199–204.

Nakai T, Uno J, Ikeda F, Jauregui A (2003): *In vitro* antifungal activity of micafungin (FK463) against dimorphic fungi: Comparison of yeast-like and mycelial forms. *Antimicrob Agents Chemother* 47: 1376–1381.

NCCLS (2008): *Performance Standards for Antimicrobial Susceptibility Testing; Ninth Informational Supplement*. NCCLS document M100-S9. Wayne, PA, National Committee for Clinical Laboratory Standards, pp. 120–126.

Nicoletti M (2002): Fitoterapia: *Curcuma zedoaria* (Christm.) var. *roscoe*, uma possibilidade terapêutica como antifúngica de uso tópico. *Infarma* 14: 60–65.

Onishi MM, Thompson J, Curotto S, Dreikorn M, Rosenbach C, Douglas G, Abruzzo A, Flattery L, Kong A, Cabello F, Vicente F, Pelaez MT, Diez I, Martin G, Bills R, Giacobbe A, Dombrowski R, Schwartz S, Morris G, Harris A, Tsipouras K, Wilson M, Kurtz. B (2000): Discovery of novel antifungal (1,3)-β-D-glucan synthase inhibitors. *Antimicrob Agents Chemother* 44: 368–377.

Paduch R, Kandefer-Szerszen M, Trytek M, Fiedurek J (2007): Terpenes: Substances useful in human healthcare. *Arch Immunol Ther Exp* 55: 315–327.

Paulnock DM (2000): *Macrophages: A Practical Approach*. New York, Oxford University Press, pp. 211.

Pessini GL, Dias BP, Nakamura CV, Cortez DAG (2005): Antifungal activity of the extracts and neolignans from *Piper regnellii* (Miq.) C.DC. var. *pallescens* (C.DC.) Yunck. *J Braz Chem Soc* 16: 1130–1133.

Prashanth D, Asha MK, Amit A (2001): Antibacterial activity of *Punica granatum*. *Fitoterapia* 72: 171–173.

Purwantini I, Wahyuono S (2003): Isolation and identification of antifungal (*Candida albicans*) compound from the hull of delima fruits (*Punica granatum* L.). *Baglan Biologi Farmasi* 14: 150–159.

San-Blas G, San-Blas F, Gil F, Mariño L, Apitiz-Castro R (1989): Inhibition of growth of the dimorphic fungus *Paracoccidioides brasiliensis* by ajoene. *Antimicrob Agents Chemother* 33: 1641–1644.

Santos AO, Ueda-Nakamura T, Dias Filho BP, Veiga Junior VF, Pinto AC, Nakamura CV (2008): Effect of Brazilian copaiba oils on *Leishmania amazonensis*. *J Ethnopharmacol* 120: 204–208.

Santos SR, Terence RMM, Pinto AJDA, Brilho CC, Donalísio MR, Souza CJ (1966): A new Brazilian essential oil from *Baccharis dracunculifolia*. *Rivista Ital Essenze* 98: 428–429.

Schomourlo G, Mendonça-Filho RR, Alviano CS, Costa SS (2006): Screening of antifungal agents using ethanol precipitation and bioautography of medicinal and food plants. *J Ethnopharmacol* 96: 563–568.

Shikanai-Yasuda MA, Telles-Filho FQ, Mendes RP, Colombo AL, Morreti ML (2006): Guideliness in paracoccidioidomycosis. *Rev Soc Bras Med Trop* 39: 279–310.

Silva JP, Siqueira AM (2000): Accíon antibacteriana de extratos hidroalcohólicos de *Rubus urticaefolius*. *Rev Cubana Plant Med* 5: 26–29.

Siqueira EP, Alvez TMA, Zani CL (2007): Fingerprint of volatiles from plant extract based on SPME-GC-MS. *Rev Bras Farmacogn* 17: 565–571.

Soto K, Garza KM, Murr LE (2007): Cytotoxicity effects of aggregated nanomaterials. *Acta Biomater* 3: 351–358.

Souza GC, Haas APSG, Poser L, Schapoval ES, Elisabetsky E (2004): Ethnopharmacological studies of antimicrobial remedies in the south of Brazil. *J Ethnopharmacol* 90: 135–143.

Voravuthikunchai S, Lortheeranuwat A, Jeeju W, Sririrak T, Phongpaichit S, Supawita T (2004): Effective medicinal plants against enterohaemorrhagica *Escherichia coli* O157:H7. *J Ethnopharmacol* 94: 49–54.

Appendix

List of peaks with percentage relative abundance

B. dracunculifolia

Ethyl hydrocinnamate (*m/z*; % relative abundance): (41; 1.1), (42; 2.4), (43; 3.0), (44; 0.2), (45; 1.3), (50; 4.4), (51; 14.0), (52; 3.2), (53; 1.5), (54; 0.1), (55; 1.2), (61; 0.2), (62; 1.0), (63; 3.9), (64; 1.2), (65; 9.8), (66; 1.7), (74; 1.1),

(75; 1.2), (76; 2.2), (77; 20.6), (78; 14.0), (79; 18.6), (80; 1.3), (87; 0.2), (89; 2.4), (91; 56.0), (92; 5.6), (102; 2.3), (103; 15.2), (104; 99.9), (105; 40.4), (107; 40.8), (108; 3.0), (131; 1.8), (132; 1.9), (133; 10.6), (134; 1.1), (149; 1.2), (178; 26.8), (179; 3.0), (180; 0.2).

Caryophyllene (*m/z*; % relative abundance): (40; 7.1), (41; 76.9), (42; 4.6), (43; 8.5), (44; 3), (50; 1.7), (51; 8.2), (52; 6.3), (53; 29.9), (54; 4.0), (55; 35.6), (56; 7.6), (57; 5.8), (63; 2.5), (64; 1.0), (65; 18.3), (66; 5.7), (67; 38.7), (68; 9.5), (69; 75.4), (70; 4.7), (71; 3.1), (77; 43.9), (78; 12.0), (79; 76.3), (80; 19.2), (81; 38.6), (82; 11.4), (83; 3.5), (89; 1.0), (91; 85.8), (92; 25.9), (93; 99.9), (94; 20.6), (95; 21.6), (96; 3.0), (97; 1.2), (103; 5.1), (104; 2.5), (105; 62.3), (106; 34.2), (107; 48.3), (108; 11.8), (109; 20.5), (110; 3.2), (111; 3.4), (112; 5), (113; 1), (115; 5.0), (116; 1.9), (117; 6.9), (118; 1.9), (119; 40.6), (120; 44.7), (121; 30.5), (122; 8.7), (123; 6.0), (128; 1.6), (129; 1.5), (130; 6), (131; 4.2), (133; 92.1), (134; 24.4), (135; 13.2), (136; 6.6), (137; 1.8), (145; 1.5), (146; 1.1), (147; 28.9), (148; 26.7), (149; 7.4), (150; 1.0), (151; 1), (159; 4), (160; 1.0), (161; 33.0), (162; 9.4), (163; 2.8), (175; 9.9), (176; 6.1), (177; 8); (189; 16.6), (190; 2.5), (204; 6.5), (205; 1.1).

δ-*Cadinene* (*m/z*; % relative abundance): (41; 20.8), (42; 1.5), (43; 6.5), (51; 2.0), (52; 1.1), (53; 6.5), (55; 12.2), (56; 1.1), (57; 1.5), (65; 4.3), (66; 1.4), (67; 10.0), (68; 1.8), (69; 12.2), (70; 1.2), (77; 13.0), (78; 3.4), (79; 22.2), (80; 4.3), (81; 19.6), (82; 2.2), (83; 1.4), (91; 24.4), (92; 12.2), (93; 33.5, (94; 12.2), (95; 7.4), (96; 1.0), (103; 1.9), (104; 1.7), (105; 38.2), (106; 7.8), (107; 9.6), (108; 2.5), (109; 3.1), (115; 3.1), (116; 1.3), (117; 4.1), (118; 2.2), (119; 34.8), (120; 10.0), (121; 10.0), (122; 2.7), (123; 1.5), (127; 1.3), (128; 1.6), (129; 1.6), (131; 3.1), (132; 1.4), (133; 18.3,), (134; 10.0), (135; 7.4), (136; 7.0), (145; 2.7), (147; 6.1), (148; 6.1), (149; 2.1), (150; 1.4), (159; 2.0), (160; 2.7), (161; 99.9), (162; 14.3), (163; 1.4), (175; 2.1), (176; 1.6), (189; 11.3), (190; 1.6), (204; 33.0), (205; 5.1).

Nerolidol (*m/z*; % relative abundance): (40; 2.7), (41; 58.6), (42; 4.1), (43; 41.0), (44; 1.3), (45; 1.1), (51; 1.3), (53; 9.6), (54; 1.5), (55; 27.2), (56; 2.1), (57; 4.6), (58; 1.1), (65; 3.6), (66; 1.3), (67; 24.9), (68; 9.1), (69; 99.9), (70; 8.1), (71; 37.0), (72; 2.3), (77; 7.2), (78; 1.2), (79; 19.2), (80; 9.7), (81; 24.1), (82; 7.6), (83; 5.4), (84; 1.1), (91; 11.9), (92; 3.4), (93; 56.0), (94; 7.2), (95; 10.9), (96; 1.8), (97; 3.7), (105; 6.5), (106; 1.5), (107; 27.5), (108; 3.9), (109; 11.8), (110; 1.3), (111; 2.2), (119; 9.4), (120; 3.2), (121; 13.1), (122; 4.2), (123; 13.9), (124; 1.6), (133; 4.5), (134; 3.2), (135; 5.4), (136; 18.3), (137; 3.0147; 1.8), (148; 2.6), (149; 1.1), (161; 12.7), (162; 2.2).

Spathulenol (*m/z*; % relative abundance): (40; 3.5), (41; 68.8), (42; 3.2), (43; 99.9), (44; 1.7), (45; 3.7), (51; 5.5), (52; 2.8), (53; 15.3), (54; 1.8), (55; 27.4), (56; 1.4), (57; 5.2), (58; 3.8), (59; 3.1), (63; 1.4), (64; 1.0), (65; 9.6), (66; 3.1), (67; 24.4), (68; 2.7), (69; 34.9), (70; 2.4), (71; 20.1), (72; 2.4), (77; 19.5), (78; 6.2), (79; 31.6), (80; 7.1), (81; 22.8), (82; 15.9), (83; 8.3), (84; 1.2), (85; 3.9), (91; 42.2), (92; 10.9), (93; 39.7), (94; 10.6), (95; 19.6), (96; 3.7), (97; 5.0), (99; 2.2), (103; 2.9), (104; 4.7), (105; 34.1), (106; 20.0), (107; 29.1), (108; 6.2), (109; 10.6), (110; 2.7), (111; 1.5), (115; 3.4), (116; 1.5), (117; 14.0), (118; 4.8), (119; 42;1), (120; 14.2), (121; 17.5), (122; 4.7), (123; 6.3), (124; 1.1), (125; 2.4), (128; 2.3), (129; 2.7), (130; 1.8), (131; 20.8), (132; 5.9), (133; 16.7), (134; 10.3), (135; 11.2), (136; 2.7), (137; 2.4), (143; 1.7), (144; 1.8), (145; 17.2), (146; 15.2), (147; 21.7), (148; 3.5), (149; 12.7), (150; 4.5), (151; 1.6), (159; 33.5), (160; 9.4), (161; 3.8), (162; 23.5), (163; 5.0), (164; 1.4), (173; 2.9), (174; 2.4), (177; 8.6), (178; 1.1), (187; 17.1), (188; 2.5), (202; 16.1), (203; 3.1), (205; 33.2), (206; 5.5), (220; 5.6).

Viridiflorol (*m/z*; % relative abundance): (41; 60.9), (43; 99.9), (53; 13.0), (55; 33.7), (57; 5.3), (59; 7.3), (65; 5.8), (67; 3.07), (68; 3.9), (69; 53.8), (70; 4.3), (71; 19.6), (77; 12.9), (79; 21.0), (80; 5.0), (81; 33.8), (82; 18.5), (83; 9.5), (91; 17.2), (93; 25.6), (94; 7.2), (95; 25.7), (96; 10.3), (97; 4.7), (105; 24.6), (106; 6.5), (107; 25.9), (108; 15.4), (109; 49.8), (111; 4.7), (119; 14.0), (120; 4.3), (121; 21.4), (122; 21.7), (123; 8.2), (133; 10.0), (135; 11.3), (136; 5.7), (139; 7.8), (147; 11.3), (148; 8.7), (149; 9.3), (161; 30.6), (162; 7.1), (164; 5.2), (189; 14.5), (204; 17.0).

P. regnellii

1-Methoxy-4-(1-propenyl) benzene (*m/z*; % relative abundance): (41; 2.2), (50; 6.4), (51; 11.6), (52; 4.1), (53; 4.0), (55; 5.1), (62; 2.9), (63; 8.2), (64; 2.4), (65; 6.4), (74; 3.7), (75; 2.8), (77; 24.6), (78; 11.2), (79; 15.9), (80; 1.1), (89; 5.1), (90; 1.8), (91; 16.0), (92; 2.3), (93; 1.6), (102; 4.0), (103; 15.9), (104; 5.1), (105; 22.3), (106; 2.4), (107; 1.9), (115;18.1), (116; 7.2), (117; 28.7), (118; 3.7), (119; 2.5), (121; 17.1), (122; 1.7), (131; 4.9), (132; 4.4), (133; 24.0), (134; 2.2), (147; 55.2), (148; 99.9), (149; 10.1).

α-*Copaene* (*m/z*; % relative abundance): (41; 33.6), (42; 2.8), (43; 1.9), (44; 3.8), (51; 3.2), (52; 1.3), (53; 8.0), (55; 23.4), (56; 6.4), (57; 2.6), (63; 1.5), (65; 6.3), (66; 1.3), (67; 7.0), (69; 14.5), (70; 3.0), (73; 1.0), (77; 13.1), (78; 4.2), (79; 11.2), (80; 4.2), (81; 32.0), (82; 2.7), (83; 1.2), (91; 29.2), (92; 18.8), (93; 25.9), (94; 3.9), (95; 4.5), (103; 3.4), (104; 2.5), (105; 97.6), (106; 11.3), (107; 14.4), (108; 5.2), (109; 1.6), (115; 4.8), (116; 2.0), (117; 6.4), (118; 4.2), (119; 93.3), (120; 28.6), (121; 4.9), (122; 1.5), (127; 1.3), (128; 2.7), (129; 2.6), (130; 1.2), (131; 3.9), (132; 1.5), (133; 8.3), (134; 2.9), (145; 2.2), (146; 1.1), (147; 4.2), (148; 1.1), (159; 3.2), (161; 99.9), (162; 13.6), (189; 1.7), (204; 20.4), (205; 3.5).

Aromadendrene (*m/z*; % relative abundance): (40; 12.1), (41; 99.9), (42; 8.3), (43; 16.2), (44; 1.4), (50; 2.4), (51; 10.6), (52; 5.9), (53; 25.6), (54; 5.3), (55; 35.4), (56; 3.7), (57; 3.7), (63; 3.7), (64; 2.7), (65; 16.5), (66; 6.4), (67; 35.8), (68; 5.7), (69; 34.8), (70; 2.2), (71; 1.8), (76; 9), (77; 36.5), (78; 12.8), (79; 50.7), (80; 10.4), (81; 32.3), (82; 10.2), (83; 4.3), (89; 1.8), (91; 67.8), (92; 16.5), (93; 45.5), (94; 13.1), (95; 15.9), (96; 3.3), (102; 1.8), (103; 5.4), (105; 50.2), (106; 16.5), (107; 36.5), (108; 9.1), (109; 7.1), (115;

6.0), (116; 3.3), (117; 8.9), (119; 32.3), (120; 13.2), (121; 16.9), (122; 13.3), (123; 2.7), (127; 1.7), (128; 3.3), (129; 2.2), (131; 1.7), (132; 4.5), (133; 31.6), (134; 9.5), (135; 7.4), (145; 2.6), (147; 22.1), (148; 15.9), (149; 4.2), (159; 1.4), (161; 33.3), (162; 5.6), (163; 1.2), (175; 3.2), (176; 2.1), (189; 9.3), (204; 11.6), (205; 2.4).

δ-*Cadinene* (*m/z*; % relative abundance): (41; 20.8), (42; 1.5), (43; 6.5), (51; 2.0), (52; 1.1), (53; 6.5), (54; 8), (55; 12.2), (56; 1.1), (57; 1.5), (65; 4.3), (66; 1.4), (67; 10.0), (68; 1.8), (69; 12.2), (70; 1.2), (77; 13.0), (78; 3.4), (79; 22.2), (80; 4.3), (81; 19.6), (82; 2.2), (83; 1.4), (91; 24.4), (92; 12.2), (93; 33.5), (94; 12.2), (95; 7.4), (96; 1.0), (103; 1.9), (104; 1.7), (105; 38.2), (106; 7.8), (107; 9.6), (108; 2.5), (109; 3.1), (110; 5), (115; 3.1), (116; 1.3), (117; 4.1), (118; 2.2), (119; 34.8), (120; 10.0), (121; 10.0), (122; 2.7), (123; 1.5), (124; 5), (127; 1.3), (128; 1.6), (129; 1.6), (130; 9), (131; 3.1), (132; 1.4), (133; 18.3), (134; 10.0), (135; 7.4), (136; 7.0), (145; 2.7), (147; 6.1), (148; 6.1), (149; 2.1), (150; 1.4), (159; 2.0), (160; 2.7), (161; 99.9), (162; 14.3), (163; 1.4), (175; 2.1), (176; 1.6), (189; 11.3), (190; 1.6), (204; 33.0), (205; 5.1).

Dillapiole (*m/z*; % relative abundance): (40; 1.2), (50; 1.7), (51; 3.0), (52; 2.1), (53; 3.3), (55; 1.2), (59; 1.0), (62; 1.3), (63; 2.7), (64; 1.1), (65; 6.8), (66; 3.8), (67; 1.3), (69; 1.9), (76; 1.1), (77; 7.4), (78; 4.0), (79; 3.7), (81; 1.8), (83; 2.2), (90; 1.9), (91; 6.0), (92; 1.6), (93; 4.2), (94; 1.4), (95; 1.3), (103; 2.2), (105; 1.9), (106; 4.9), (107; 2.0), (117; 1.9), (118; 1.3), (119; 2.2), (121; 6.8), (131; 1.6), (133; 4.5), (134; 3.9), (135; 1.8), (145; 1.3), (147; 1.6), (149; 16.3), (150; 2.3), (151; 1.4), (161; 8.5), (162; 1.9), (163; 2.9), (165; 1.6), (175; 1.7), (176; 1.0), (177; 18.1), (178; 2.4), (179; 1.4), (180; 2.4), (191; 12.1), (192; 2.8), (193; 1.7), (195; 10.5), (196; 1.0), (207; 26.1), (221; 3.0), (222; 99.9), (223; 12.9).

β-*Eudesmol* (*m/z*; % relative abundance): (41; 44.4), (43; 25.2), (52; 4.7), (55; 22.3), (59; 99.9), (65; 10.5), (67; 30.5), (69; 10.8), (77; 12.9), (78; 8.5), (79; 20.6), (81; 23.7), (83; 10.6), (88; 2.6), (89; 2.0), (93; 14.3), (102; 1.2), (103; 2.5), (105; 13.7), (106; 5.3), (107; 10.3), (109; 16.5), (121; 13.1), (127; 1.3), (133; 7.6), (135; 6.0), (151; 1.5), (160; 1.6), (161; 12.7), (164; 10.8), (191; 1.4), (202; 1.1), (212; 9), (223; 8).

Apiol (*m/z*; % relative abundance): (40; 1.2), (50; 1.7), (51; 3.0), (52; 2.1), (53; 3.3), (55; 1.2), (59; 1.0), (62; 1.3), (63; 2.7), (64; 1.1), (65; 6.8), (66; 3.8), (67; 1.3), (69; 1.9), (76; 1.1), (77; 7.4), (78; 4.0), (79; 3.7), (81; 1.8), (82; 5), (83; 2.2), (90; 1.9), (91; 6.0), (92; 1.6), (93; 4.2), (94; 1.4), (95; 1.3), (103; 2.2), (105; 1.9), (106; 4.9), (107; 2.0), (117; 1.9), (118; 1.3), (119; 2.2), (120; 5), (121; 6.8), (131; 1.6), (132; 4), (133; 4.5), (134; 3.9), (135; 1.8), (145; 1.3), (147; 1.6), (149; 16.3), (150; 2.3), (151; 1.4), (161; 8.5), (162; 1.9), (163; 2.9), (165; 1.6), (175; 1.7), (176; 1.0), (177; 18.1), (178; 2.4), (179; 1.4), (180; 2.4), (191; 12.1), (192; 2.8), (193; 1.7), (194; 1), (195; 10.5), (196; 1.0), (207; 26.1), (221; 3.0), (222; 99.9), (223; 12.9).