

Composition and Antimicrobial Activity of Essential Oils from *Poiretia bahiana* C. Müller (Papilionoideae-Leguminosae)

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A espécie *Poiretia bahiana*, popularmente conhecida como arrudinha, arruda da serra ou arruda brava, tem uma ocorrência restrita aos campos rupestres da Chapada Diamantina, BA. Suas partes aéreas apresentam um aroma pungente e é usada pelo povo do local no controle de infestações por pulgas e no tratamento de hemorróidas, dores nas articulações e sinusite. Neste trabalho, relatamos a composição dos óleos voláteis obtidos das partes aéreas de cinco espécimes e do óleo volátil obtido dos frutos de um espécime de *P. bahiana*, os quais foram coletados em diferentes regiões da Chapada Diamantina. Nos óleos voláteis que foram analisados por CG-FID e CG-EM foram identificados vinte e nove componentes sendo que os dois principais foram a umbelulona, em concentrações entre 55,0% e 75,3%, e sabineno, em concentrações entre 10,3% e 25,2%. O óleo das partes aéreas de um espécime e o óleo dos frutos de outro espécime foram avaliados quanto às suas propriedades antimicrobianas contra seis bactérias, duas leveduras e cinco fungos filamentosos pelos ensaios de difusão em ágar e microdiluição. Os óleos exibiram atividade contra *Escherichia coli*, *Salmonella choleraesuis*, *Proteus mirabilis*, *Staphylococcus aureus*, *Staphylococcus aureus* resistente à meticilina (MRSA), *Candida albicans*, *Cryptococcus neoformans*, *Cladosporium herbarum*, *Microsporium canis*, *Trichophyton rubrum* e foram inativos contra *Pseudomonas aeruginosa*, *Aspergillus fumigatus* e *Penicillium notatum*. A toxicidade do óleo das partes aéreas foi determinada contra *Artemia salina* apresentando uma LC₅₀ de 459,03 µg mL⁻¹.

Poiretia bahiana known as “arrudinha”, “arruda da serra” or “arruda brava”, has its occurrence restricted to the rock fields (campo rupestre) areas in Chapada Diamantina, Bahia, northeastern region of Brazil. Its aerial parts present a pungent smell and have been used by local communities as fumigant for the control of infestation of fleas and in the treatment of hemorrhoids, articulations pain and sinusitis. This work reports the composition of the volatile oils from aerial parts of five specimens and of the essential oil from the fruits of one specimen of *Poiretia bahiana* that were harvested in four different places of Chapada Diamantina. Twenty nine components were identified from the oils analyzed by GC-FID and GC-MS. Umbellulone, in concentrations between 55.0% and 75.3% and sabinene, in concentrations between 10.3% and 25.2% are the major components. The oil from the aerial parts of one specimen and the oil of fruits of another specimen had their antimicrobial properties evaluated against six bacteria, two yeasts and five filamentous fungi by the agar diffusion method and microdilution assay. The *in vitro* results demonstrated that these essential oils exhibit antimicrobial activity against *Escherichia coli*, *Salmonella choleraesuis*, *Proteus mirabilis*, *Staphylococcus aureus*, methicilin resistant *Staphylococcus aureus* (MRSA), *Candida albicans*, *Cryptococcus neoformans*, *Cladosporium herbarum*, *Microsporium canis*, *Trichophyton rubrum* and are inactive against *Pseudomonas aeruginosa*, *Aspergillus fumigatus* and *Penicillium notatum*. The oil obtained from the aerial parts of one specimen displayed moderate toxicity against *Artemia salina* with a LC₅₀ value of 459.03 µg mL⁻¹.

Keywords: *Poiretia bahiana*, essential oil, umbellulone, sabinene, antimicrobial activity

Introduction

The genus *Poiretia* comprises 12 species that are widespread in the tropical and subtropical America from México and Antilhas to Argentina.¹ In Brazil, the people use some species with therapeutic purposes. Thus, *P. tetraphylla* is used as stomachal tonic, *P. latifolia* is used to input aroma for tea and it is considered as an aphrodisiac, and another *Poiretia* species is used in the state of Mato Grosso as anti-emetic against fever and meningitis.¹ *P. bahiana* was first reported in 1984¹ and its occurrence is restricted to the rock fields (campo rupestre) areas in Chapada Diamantina, Bahia, northeastern region of Brazil. The aerial parts of *P. bahiana*, known as “arrudinha”, “arruda da serra” or “arruda brava”, present a pungent smell and have been used by local communities as fumigant to kill fleas, in the treatment of hemorrhoids, articulation pain and as nasal decongestant in sinusitis.² In the treatment of sinusitis the plant is used by inhalation of the vapors released in boiled water and it is possible that, besides a decongestant action, there is also an antimicrobial action against bacteria and fungi generally associated to sinusitis. As a fumigant to kill fleas, the aerial parts of the plant are dispersed by the infested places.

The aim of this work is the study of the composition of the volatile oils from aerial parts of five specimens and of the volatile oil from fruits of one specimen of *P. bahiana*, in addition to the evaluation of the antimicrobial properties of the oil from two specimens against six bacteria, two yeasts and five filamentous fungi and of the toxicity of the oil from the aerial parts against *Artemia salina*. The specimens were harvested in four different places of Chapada Diamantina named Morro do Pai Inácio, Mucugê, Morro do Chapéu and Chapadinha.

Experimental

Plant material

The aerial parts of three specimens of *P. bahiana* were collected in July 2004 in Morro do Pai Inácio (MPIFI), Mucugê (MFI) and Chapadinha (CFI) when the plants were flowering. The fruits were collected from the specimen found in Chapadinha (CFr). In September 2004, when the plants presented no flowers, the aerial parts of two specimens were collected in Mucugê (MWFI) and Morro do Chapéu (MCWFI). The species were identified by Prof. Maria Lenise da Silva Guedes and voucher specimens were deposited in the herbarium Alexandre Leal Costa at Instituto de Biologia, UFBA, under the numbers 032231, 032232,

032233, 032244 and 032245, respectively for MPIFI, MFI, CFI, MWFI and MCWFI.

Essential oils analysis

The aerial parts and fruits were dried on air at room temperature and reduced into small pieces. The volatile oils were obtained by 3 h hydrodistillation in a Clevenger-type apparatus. The identification of compounds was performed by comparison of their retention indices and mass spectra with those reported in the literature^{3,4} and stored in the NIST libraries (Mass Spectral Library, 1998). The retention indices were calculated by co-injection with a standard saturated *n*-alkanes homologous series.

GC analyses were performed as described previously.⁵

Microbial strains

Thirteen microbial strains were used to access the antimicrobial properties of the test samples: six bacteria, two yeasts and five filamentous fungi. Microorganisms were obtained from the culture collections of Instituto Nacional de Controle de Qualidade em Saúde- INCQS/ Brasil as follows: *Escherichia coli* ATCC 25922, *Salmonella choleraesuis* ATCC 10708, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* ATCC 29906, *Staphylococcus aureus* ATCC 25923, methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 33591, *Candida albicans* ATCC 90028, *Cryptococcus neoformans* ATCC 32045, *Aspergillus fumigatus* ATCC 16913, *Cladosporium herbarum* ATCC 26362, *Microsporum canis* ATCC32903, *Penicillium notatum* ATCC 9478 and *Trichophyton rubrum* ATCC 28189. Bacteria were grown on Mueller Hinton Agar (MHA) and fungi on Sabouraud Dextrose Agar (SDA) (Difco Laboratoires). Inocula were prepared as described previously.⁵

Essential oils dilution

The essential oils were tested pure and at dilutions ranging from 8% (72.5 mg mL⁻¹) to 0.25% (2.3 mg mL⁻¹) for agar diffusion method and for the determination of Minimum Inhibitory Concentration by microdilution assays. The dilutions were prepared in sterile distilled water with addition of Tween 80 (Merck) to the first dilution in a concentration of 10% (v/v), each dilution being mixed on Vortex apparatus.⁶ Due to the similarity of the oils composition, only the oils from the aerial parts collected in Morro do Pai Inácio (MPIFI) and from the fruits collected in Chapadinha (CFr) in July 2004 had their antimicrobial properties and toxicity tested.

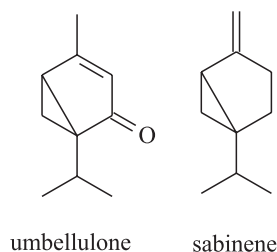


Figure 1. Chemical structure of umbellulone and sabinene.

Agar diffusion method

To evaluate antimicrobial activity, assays were performed in duplicate as described previously.⁵ The bioassay results (Table 2) were expressed in terms of mean of inhibition zone diameters: < 9 mm, inactive; 9-12 mm, partially active; 12-18 mm, active; > 18 mm, very active.⁷

Minimum Inhibitory Concentration (MIC)

MIC assays were performed by Broth Microdilution Method as described previously.⁵

Toxicity against *Artemia salina*

The brine shrimp lethality assay was performed by the method of McLaughlin and co-workers.⁸ Brine shrimp eggs (*Artemia salina*) were hatched in saline solution of NaCl (38 g L⁻¹). The essential oil was tested at concentrations of 300, 500 and 1000 µg L⁻¹. Survival was measured after 24 h incubation at 10 °C. The collected data were computerized and LC₅₀ values determined by Probit analysis.

Results and Discussion

Twenty-nine compounds were identified in the essential oils from aerial parts and fruits of *P. bahiana* (Table 1). Umbellulone is the major constituent in the essential oils from the aerial parts of all specimens of *P. bahiana* with flowers or without flowers, with a mean concentration of 60.6%. The next most concentrated component was sabinene, with a mean concentration of 16.0%. The essential oil from fruits showed umbellulone (58.2%) and sabinene (25.2%) as predominant compounds too. In addition to umbellulone and sabinene, myrcene, with a mean concentration 2.5%, *Z*-ocimene, with a mean concentration 3.3%, and germacrene D, with a mean concentration 3.7%, were presented in all samples analyzed. A complete study of seasonal variation in oil composition was not carried out, although in a general way, the oils collected in different geographical areas in July and September demonstrated

high monoterpene content, specially oxygenated ones and variations in concentrations of the main components could be noted. Thus, the MPIFI sample, collected in July, was considerably richer in umbellulone content (75%) than the other samples collected. The MFI and CFI samples, harvested in July, presented the highest content in sabinene among the aerial parts samples; however, the highest concentration of sabinene was found in the fruits (25.2%). The essential oils from the flowery specimens presented larger yield of oil than those from the specimens without flowers; however, the best yield was presented by the essential oil from the fruits.

The assays with agar diffusion method revealed that the pure oils presented activity to most of the microorganisms tested except to *P. aeruginosa*, *A. fumigatus* and *P. notatum* (Table 2). The results of diluted oils showed that with regard to Gram-negative bacteria, only the oil from aerial parts presented partial activity to *E. coli* and the oil from fruits presented partial activity to *P. mirabilis*, both at 8% dilution. With regard to Gram-positive bacteria, the oil from aerial parts was active against MRSA and partially active against *S. aureus* at 8% while the oil from fruits presented partial activity to MRSA and was inactive against *S. aureus* at 8% dilution. The oil from aerial parts presented good activity while the oil from fruits presented partial activity to *C. albicans* at 8%. Both oils showed activity to *C. neoformans* although the oil from aerial parts presented inhibition until 2% dilution with greater zones than those of the oil from fruits. Both oils showed to be partially active to the phytopathogenic specie *C. herbarum* at 8% dilution. The greatest inhibition zones were presented by both oils against *M. canis* although the oil from aerial parts showed good activity until 2% and the oil from fruits until 4% dilution. The oil from aerial parts presented good activity until 2% and the oil from fruits until 4% dilution to *T. rubrum*.

The minimum inhibitory concentrations of the essential oil obtained from the aerial parts were determined against six microbial species (Table 3). The results indicated that the oil presents strong activity against *C. neoformans*, *C. albicans* and *E. coli* with MIC values of 2.3, 2.3 and 9.1 mg mL⁻¹, respectively and weaker activity against *P. mirabilis*, *S. aureus* and methicilin resistant *S. aureus*. Although agar diffusion and broth microdilution are commonly used methods to evaluate antimicrobial activity, the results obtained from each of these methods may differ as many factors vary between assays, which include differences in microbial growth, exposure of microorganisms to plant oils, the solubility of oils or oil components, the use and quantity of emulsifier and other elements.^{6,9,10} It is well known that agar diffusion method has its accuracy impaired as the hydrophobic nature of most essential oils prevents

Table 1. Volatile compounds of the essential oils from *Poiretia bahiana*

Compound	KI	MPIFI	MFI	CFI	MWFI	MCWFI	CFr
α -thujene	930	-	0.5	-	0.3	0.4	-
α -pinene	938	-	0.7	0.7	0.5	0.4	-
thuja-2,4(10)-diene	948	-	0.4	-	0.2	0.3	-
sabinene	977	10.3	17.6	19.4	11.6	11.8	25.2
β -pinene	980	0.7	1.8	1.6	-	1.3	1.8
myrcene	992	1.3	2.9	2.8	2.2	2.8	3.1
α -terpinene	1019	-	0.9	0.8	0.8	1.3	1.1
o-ocimene	1028	-	-	-	-	0.1	-
limonene	1032	0.9	2.0	1.9	1.7	1.6	-
Z-ocimene	1040	3.0	3.9	3.6	3.0	3.3	3.0
E-ocimene	1051	1.3	0.9	0.8	2.0	2.6	-
γ -terpinene	1062	-	1.4	1.3	1.2	2.1	1.8
cis-hydrate sabinene	1070	-	-	-	0.3	0.2	-
terpinolene	1090	-	-	-	0.3	0.5	-
linalool	1101	-	-	-	0.8	1.0	-
thujone	1118	1.3	1.4	1.3	1.4	1.6	-
p-menth-2-en-1-ol	1123	-	-	-	0.3	0.4	-
umbellulone	1177	75.3	55.0	58.3	60.4	56.4	58.2
terpinen-4-ol	1179	1.7	2.9	3.1	-	-	4.0
α -terpineol	1191	-	0.6	-	0.7	0.9	-
methyl salicylate	1195	-	-	-	0.5	0.4	-
thymol	1294	-	-	-	1.6	1.9	-
E-caryophyllene	1417	1.3	0.7	-	1.9	1.0	-
allo-aromadendrene	1459	-	-	-	0.3	0.3	-
germacrene D	1480	2.9	4.6	4.4	4.3	4.0	1.8
bicyclogermacrene	1494	-	1.0	-	0.9	0.9	-
δ -cadinene	1522	-	-	-	0.2	-	-
spathulenol	1576	-	-	-	0.5	0.4	-
caryophyllene oxide	1580	-	-	-	0.6	-	-
Total		100.0	99.2	100.0	98.5	97.9	100.0
monoterpene hydrocarbons		17.5	33.0	32.9	23.8	28.5	36.0
oxygenated monoterpenes		78.3	59.9	62.7	63.9	60.5	62.2
sesquiterpene hydrocarbons		4.2	6.3	4.4	7.6	6.2	1.8
oxygenated sesquiterpenes		-	-	-	1.1	0.4	-
Others		-	-	-	2.1	2.3	-
yield / (% , m/m)		1.4	0.8	1.4	0.7	0.4	2.1
mass of dry material / g		124.0	148.0	150.0	119.0	139.0	61.0

MPIFI: Morro do Pai Inácio with flowers; MFI: Mucugê with flowers; CFI: Chapadinha with flowers; MWFI: Mucugê without flowers; MCWFI: Morro do Chapéu without flowers; CFr: Chapadinha fruits.

the uniform diffusion of these substances through the agar medium that may account for differences on the obtained results.

These *in vitro* results demonstrated that essential oils from *P. bahiana* exhibit antimicrobial activity against *E. coli*, *P. mirabilis*, *Staphylococcus* species, yeasts and filamentous fungi, presenting greater inhibition zones to fungi than to bacteria. The oil obtained from aerial parts was shown to be more active than the one obtained from fruits due to quantitative and qualitative differences in their constituents, mainly in the content of umbellulone.

Probably, the biological properties of *P. bahiana* oils are associated to their high monoterpene content, specially umbellulone and sabinene, their major components. The strong activity depicted by the essential oils against *M. canis* and *T. rubrum*, the most common causative agent of dermatophytoses (onychomycosis, tinea or ringworm) worldwide infecting the hair, skin, and nails of patients with diabetes, peripheral vascular diseases or immunosuppressed individuals¹² suggests that they have a potential use as an alternative or complementary method to combat these microorganisms. Nevertheless, the literature reports that

Table 2. Mean zones of growth inhibition (mm) showing antimicrobial activities of aerial parts (AP) and fruits (Fr) essential oil from *Poiretia bahiana* collected in July 2004

Microorganism	Pure Oil		Sample Concentration (v/v)								GE 10µg	OX 1µg	VA 30µg	KT 50µg
	AP	Fr	8%		4%		2%		1%					
			AP	Fr	AP	Fr	AP	Fr	AP	Fr				
<i>E. coli</i>	14.0	11.5	9.2	8.8	8.5	8.2	NI	NI	NI	NI	15.0			
<i>S. choleraesuis</i>	11.0	10.0	8.8	NI	8.0	NI	NI	NI	NI	NI	16.0			
<i>P. mirabilis</i>	12.0	11.0	8.8	9.0	8.5	8.2	NI	NI	NI	NI	20.0			
<i>S. aureus</i>	12.0	10.0	10.0	8.2	9.5	NI	NI	NI	NI	NI		17.0		
MRSA ^a	13.0	10.0	12.2	10.8	10.8	8.2	9.0	NI	NI	NI			19.0	
<i>C. albicans</i>	16.0	14.0	13.0	11.8	11.0	10.5	9.5	9.5	NI	NI				23.0
<i>C. neoformans</i>	19.0	14.0	18.0	13.0	15.0	11.0	11.5	9.0	NI	NI				26.0
<i>C. herbarum</i>	13.0	12.0	12.0	11.0	NI	NI	NI	NI	NI	NI				23.0
<i>M. canis</i>	33.0	19.0	32.0	18.0	24.0	12.5	17.5	NI	NI	NI				22.0
<i>T. rubrum</i>	24.0	16.0	22.0	14.0	16.5	10.0	12.5	NI	NI	NI				20.0

^amethicilin resistant *Staphylococcus aureus*; NI: no inhibition; GE: Gentamicin; OX: Oxacilin; VA: Vancomycin; KT: Ketoconazol.

Table 3. Minimum inhibitory concentration of aerial parts essential oil from *Poiretia bahiana* against six microbial species

Microorganism	Sample / (mg mL ⁻¹)	Controls / (µg mL ⁻¹)			
		GE	OX	VA	KT
<i>E. coli</i>	9.1	1.00	-	-	-
<i>P. mirabilis</i>	18.1	1.00	-	-	-
<i>S. aureus</i>	36.3		0.25	-	-
MRSA ^a	36.3		-	0.50	-
<i>C. albicans</i>	2.3		-	-	0.13
<i>C. neoformans</i>	2.3		-	-	0.25

^amethicilin resistant *Staphylococcus aureus*. GE: Gentamicin; OX: Oxacilin; VA: Vancomycin; KT: Ketoconazol.

umbellulone, the major component of the *Poiretia* oils, interferes strongly with respiration, heartbeat, and blood circulation, causing death in laboratory animals. Its inhalation by guinea pigs irritated mucous membranes of eyes and nose and caused irregular respiration at times, but no failure of respiration.¹³ In the present work, the essential oil from aerial parts of *P. bahiana* displayed moderate toxicity against *Artemia salina* with an LC₅₀ of 459.03 µg mL⁻¹. This result suggests the necessity of more accurate toxicity evaluation of the essential oils obtained from this species.

Studies showed that California bay (*Umbellularia californica*) is used by wood rats (*Neotoma fuscipes*) for fumigation of nest-borne ectoparasites.¹⁴ Leaves of this plant significantly reduced survival of flea larvae to 26.4%. The biocidal activity of this plant has been associated with its high content in umbellulone (39%), which has been shown

to be toxic when fed to laboratory mice.¹¹ Therefore, the high content of umbellulone in aerial parts of *P. bahiana* might be responsible for its reputed effectiveness to kill fleas.

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Supplementary Information

Supplementary data are available free of charge at <http://jbscs.sbq.org.br> as PDF file.

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