SMGroup

Annals of Chromatography and Separation Techniques

Research Article

Chemical Profiles and Antimicrobial Activity of *Piper caldense* Tissues

Luiz Alberto Barros Freitas[1], Fabiana da Silva Aquino[1], Janete Magaly de Araújo[2] and Clécio Souza Ramos[1]*

[1]*Department of Chemistry, Rural Federal University of Pernambuco, Recife-Pe, Brazil*
[2]*Antibiotics Department, Federal University of Pernambuco, Recife-Pe, Brazil*

Article Information

Received date: Sep 27, 2016
Accepted date: Oct 14, 2016
Published date: Oct 17, 2016

***Corresponding author**

Clecio Souza Ramos, Department of Chemistry, Rural Federal University of Pernambuco, Recife-Pe, Brazil, Tel: +55 81 33206379; Email: clecio.ufrpe@gmail.com

Keywords Piperaceae; *Piper caldense*; Prenylated benzoic acid; Antimicrobial

Abstract

The *Piper caldense* is a medicinal plant widely used to treat snake bites, as a sedative and for tooth pain. However, there are few reports about the biological potential of the plant and only two reports on its chemical composition. The objective of the present work was to determine the antimicrobial activity and chemical profiles of *P. caldense* tissues as well as to isolate their major compounds. The major compound 3-geranylgeranyl-4-hydroxybenzoic acid found in all plant tissues, showed antibacterial activity for all tested bacteria including those gram-positive and gram-negative, and especially against gram-positive *Staphylococcus aureus, Bacillus subtilis, Enterococcus faecalis* with minimum inhibitory concentration of 39.5 μg/mL. The compound was characterized based in the interpretations of spectra data of IR, MS, ^{1}H and ^{13}C NMR analysis and chemical profiles of plant tissues obtained by HPLC.

Introduction

Piper caldense C. DC., known popularly as the "pimenta d'arda" or "jaborandi" is a folk medicinal plant used as a sedative, antidote for snake-bite, and for toothache [1,2]. This specie belongs to the Piperaceae family, and is found in humid regions of the Atlantic Forest in Brazil. The Piperaceae family is a basal angiosperm family, estimated to contain more than 3000 species widely distributed in tropical and subtropical regions of the world. It is valued due to its biological, chemical, economic and ecological characteristics, attributed to its secondary metabolites such as amides, phenylpropanoids, lignans, neolignans, benzoic acids, chromenes, alkaloids and polyketides [3-8]. *Piper longum*, commonly known as the "long pepper", is a medicinal herb with diverse biological activities including antiamoebi, antifungal, antiasthmatic, antidiabetic and anti-cancer [9]. The piperine and piplartine amides isolated from the *Piper* species have been reported to exhibit anti-cancer, anti-pyretic and anti-inflammatory activities as well as effects of depression on the central nervous system [10]. Two chemical studies of *P. caldense* previously reported only revealed the isolation of one prenylated benzoic acid, named caldensinic acid, in the leaf tissues, and one *N*-aristolactam, named caldensin, in the root tissues [11,12]. Due to few reports about chemical investigations and about the biological potential of *P. caldense* tissues; the present work was addressed for chemical study and for an evaluation of the antimicrobial activity of the plant.

Materials and Methods

Material botanic

P. caldense was collected in Recife, Pernambuco state, in northeastern Brazil, in December 2014. Botanical identification was made by Dr. Ângela M. M. Freitas (Department of Forest Sciences - Pernambuco Federal Rural University) and a voucher specimen was deposited in the Sérgio Tavares Herbarium of that university (HST 18180).

Isolation and chromatographic analysis

Leaves, stems, fruits and roots were dried at 40°C and the dried materials were milled to a fine powder in a Macsalab mill (leaf 325 g, root 44 g, stem 188 g and fruit 2 g). All material was extracted by maceration with dichloromethane three times (3 × 300 mL) at room temperature for 48 h. The resulting solutions were concentrated in a vacuum to yield crude extracts (leaf 19.0 g, root 1.4 g, stem 1.1 g and fruit 0.2 g). Part of the extract from the leaves (6.0 g) was suspended in MeOH-H_2O (8:2), filtered in celite, and concentrated in vacuum to yield an extract of 2.9 g of the chlorophyll free leaves. This extract was subjected to fractionation on a silica gel column using hexane with increasing amounts of EtOAc as the eluent, yielding 40 fractions. Fractions 8 (348 mg) and 7 (337 mg) were applied again on a silica gel column and were eluted with hexane containing increasing amounts of EtOAc, yielding compound 1 (11.2 mg) and 2 (144.6 mg). HPLC analyses of extracts and the pure compound were performed using a Shimadzu LC10 instrument using a C_{18} column (250 mm, 4.6 mm, 5 μM) from Tupelo eluted in a gradient mode starting with CH_3OH:H_2O (3:7)

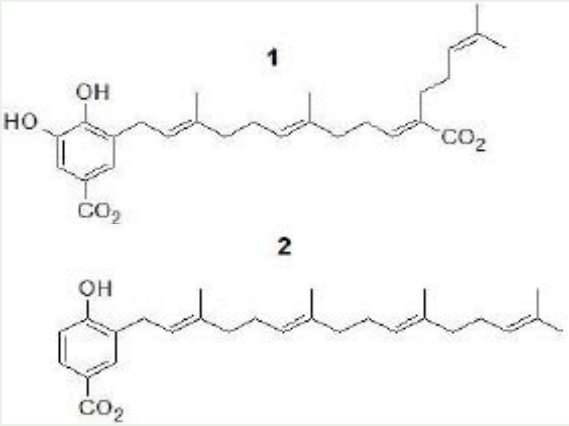

Figure 1: Structures of isolated compounds **1** and **2.**

for 10 min, raising to 100% of CH_3OH in 40 min, with detection at 254 nm and flow rate of 1.0 mL/min. Silica gel (Merck 230-400 mesh) was used for column chromatography and solvents were redistilled prior to use.

Characterization chemical

^{1}H and ^{13}C NMR spectra of 1 and 2 compounds were acquired in $CDCl_3$ on a Varian - Unity Plus 300 (300 MHz and 75 MHz, respectively) spectrometer with pulsed field gradient and signals referenced to the residual solvent signals ($CDCl_3$, at δ H 7.2 and δ C 77.0 pap). EI-MS (70 eV) was measured at on a Shimadzu spectrometer. IR spectra were recorded as glassy film on a Varian 640 FT-IR Spectrometer.

In vitro assay for antimicrobial activity

The antimicrobial activities of samples from the *P. caldense* tissues were tested against the following microorganisms: *Staphylococcus aureus* (UFPEDA02), *Bacillus subtilis* (UFPEDA82), *Enterococcus faecalis* (UFPEDA138), *Escherichia coli* (UFPEDA 224), *Klebsiella pneumonia* (ATCC29665), *Pseudomonas aeruginosa* (ATCC 27853), *Candida albicans* (UFPEDA1007), *Candida krusei* (URM5901), *Aspergillus niger* (URM6474) and *Malassezia furfur* (URM5890) using methods previously reported [7]. All strains were provided by the Antibiotics Department of Pernambuco Federal University (UFPEDA) and maintained in Nutrient Agar (NA), stored at 4°C. All experiments were carried out three times and repeated if the results differed. All sample having IDZs (inhibition diameter zones) greater than or equal to 10 mm were selected for Minimum Inhibitory Concentration (MIC) assay. Gentamicin and fluconazole were used as antibacterial and antifungal substances, respectively.

Table 1: Antimicrobial activity of the extracts obtained *P. caldense* tissues using disc diffusion.

Microorganisms	Diameter of inhibition zone (mm) Concentration of 20 mg/mL			
	Root	Leaf	Stem	Fruit
	Gram-positive bacteria			
S. aureus	15.5 ± 0.5	15.0 ± 0.0	17.5 ± 0.5	15.0 ± 0.0
B. subtilis	10.0 ± 0.0	11.5 ± 0.5	10.0 ± 0.0	12.5 ± 0.5
E. faecalis	0	13.0 ± 0.0	0	10.0 ± 0.0
	Gram-negative bacteria			
E. coli	0	3.0 ± 0.1	0	0
P. aeruginosa	0	2.0 ± 0.0	2.0 ± 0.1	0
K. pneumoniae	0	2.0 ± 0.0	0	0
	Fungi			
C. albicans	0	0	0	0
C. krusei	0	0	0	0
A. niger	0	0	0	0
M. furfur	0	0	0	0

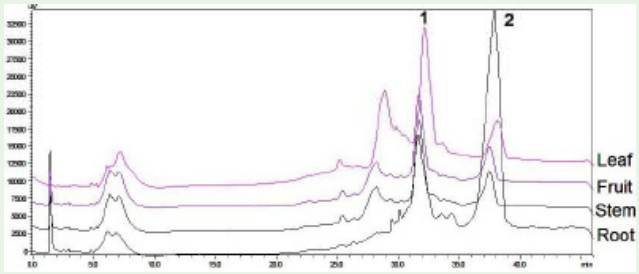

Figure 2: Chemical profiles obtained by HPLC of root, stem, fruit and leaf extracts of *P. caldense*.

Results and Discussion

The chemical profiles of dichloromethanic extracts from leaf, root, stem and fruit *P. caldense* obtained by HPLC were identical (Figure 1), with major peaks at 32 and 38 min. The extracts were tested against fungus and bacterium and showed inhibitory activity for Gram-positive bacterium. The leaf extract exhibited higher antibacterial activity than the other extracts with mean zones of inhibition between 11.5 to 25.0 mm, and with MIC concentrations ranging from 250 to 1000 μg/mL (Tables 1 and 2).

The leaf extract was submitted to purification steps by chromatographic methods resulting in the isolation of two compounds. The compound 1 was characterized as caldensinic acid with base on its ^{1}H NMR spectrum. Its ^{1}H NMR spectrum indicated the presence of aromatic hydrogen at δ 7.8, three signals at δ 6.8, 5.3 and 5.1, associated to four olefin hydrogen, one signal of benzylic methylene at δ 3.4, a signal set between 1.5 and 1.8 ppm, attributed to methyl groups linked to *sp*2 carbons, a broad group of signals between 2.0 and 2.4 ppm, assigned to twelve methylene hydrogen according to the literature [12-13]. The caldensinic acid has been previously reported as major compound of *P. caldense* leaf, and showed antifungal activity against the phytopathogenic fungi *Cladosporium cladosporioides* and *C. sphaerospermum* [12].

Table 2: MIC for extracts obtained of *P. caldense* tissues.

Microorganisms	Root	Leaf	Stem	Fruit
Gram-positive bacteria	μg/ml	μg/ml	μg/ml	μg/ml
S. aureus	1000	500	1000	500
B. subtilis	2000	1000	2000	1000
E. faecalis	-	1000	-	2000

Table 3: Antimicrobial activity of the compound **2** using disc diffusion.

Microorganisms	Diameter of inhibition zone (mm) Concentration of 10 mg/ml		
	Compound 2	Gentamicin	Fluconazole
Gram-positive bacteria			
S. aureus	20.5 ± 0.5	33.0 ± 0.0	-
B. subtilis	16.5 ± 0.5	43.0 ± 0.0	-
E. faecalis	12.5 ± 0.5	45.0 ± 0.0	-
Gram-negative bacteria			
E. coli	15.0 ± 0.0	25.0 ± 0.0	-
P. aeruginosa	10.0 ± 0.0	23.0 ± 0.0	-
K. pneumoniae	16.5 ± 0.5	24.0 ± 0.0	-
Fungi			
C. albicans	0	-	40 ± 0.0
C. krusei	0	-	39 ± 0.0
A. niger	0	-	34 ± 0.0
M. furfur	0	-	30 ± 0.0

The EI-MS spectra of compound 2, an amorphous solid, showed fragmentation ions at m/z 69 (100%), 81 (49%), 91 (18%), 107 (79%), 121 (22%), 161 (30%) and 297 (2%) Da. The IR spectrum showed absorption bands at 3520, 1692, and 1620 cm^{-1} assignable to a hydroxyl, conjugated carbonyl and aromatic ring, respectively. The ^{1}H NMR spectrum exhibited signals for three aromatic hydrogen at δ 6.85 (d, J = 8.9 Hz), 7.89 (d, J = 8.9 Hz), and 7.94 (br s), indicative of a 3,4-disubstituted benzoic acid derivative. The spectrum also displayed signals for five vinyl methyl groups at δ 1.61 (9H), 1.69 (3H), and 1.80, seven allylic methylene groups, six of them as multiplets at δ 2.31-1.98 (12H) and one as a doublet at δ 3.42 (2H, J = 7.1 Hz). These signals, associated with one triplet at δ 5.35 (J 7.1 Hz, 1H) and one multiplet at 5.17 (3H), suggested one geranyl-geranyl group as the side chain. The ^{13}C NMR spectrum exhibited twenty-seven signals: one corresponding to carboxylic carbons (δ 171.9), and six aromatic carbons at δ 121.6 (C-1), 131.2 (C-2), 130.4 (C-3), 115.7 (C-5) and 126.9 (C-6), and seven methylene carbons at δ 29.6 (C-1'), 39.7 (C-4'), 26.7 (C-5'), 39.7 (C-8'), 26.5 (C-9'), 39.6 (C-12') and 26.4 (C-13'), and five methyl groups at δ 25,6 (C-16'), 17.6 (C-17'), 15.9 (C-18'), 16,0 (C-19') and 16.9 (C-20'). Based in the interpretation of its spectra data, compound 2 was characterized as 3-geranylgeranyl-4-hydroxybenzoic acid (Figure 2), isolated first from the aerial parts of the *Piper saltuum* [14]. This is the first report of the occurrence of compound 2 in *P. caldense* leaf.

Table 4: MIC for compound **2**.

Microorganisms	Concentration μg/ml	
	Compound 2	Gentamicin
Gram-positive bacteria		
S. aureus	39.5	4.88
B. subtilis	39.5	4.88
E. faecalis	39.5	4.88
Gram-negative bacteria		
E. coli	78.15	4.88
P. aeruginosa	156.25	4.88
K. pneumoniae	78.15	4.88

Compound 2 was assayed against fungi, Gram-negative and Gram-positive bacteria. The results are shown in Tables 3 and 4. Compound 2 exhibited antibacterial activity against all the microorganisms tested, with potent activity against *S. aureus, B. subtilis* and *E. faecalis* with value of MIC of 39.5 μg/mL. The caldensinic acid was not evaluated for antimicrobial activity due to insufficient amount of compound isolated.

Conclusion

In summary, this study describes the first report of the occurrence of 3-geranylgeranyl-4-hydroxybenzoic acid in *P. caldense*. The potent antibacterial activity and broad-spectrum observed for compound 2 identifies this plant species as a promising candidate for the development of novel phytotherapic products.

Acknowledgments

This work was funded by grants provided by CNPq. LABF thanks CNPq for providing a scholarship. Dr. Ângela M. M. Freitas (Department of Forest Sciences - Pernambuco Federal Rural University) for identification of *P. caldense* species.

References

1. Pereira R, Guedes A, Da Silva GE. The hydroalcoholic extract of leaves of Piper caldense C. DC. decreases alcohol consumption in rats. Revista Brasileira de Plantas Medicinais. 2015; 17: 157-163.
2. Rocha DS, Silva JM, Navarro, DAF, Camara CAG, Ramos CS. Potential antimicrobial and chemical composition of essential oils from *Piper caldense* tissues. 2016; 60: 148-151.
3. Scott IM, Jensen HR, Philogene BJR, Arnason JT. A review of Piper spp. (Piperaceae) phytochemistry, insecticidal activity and mode of action. Phytochemical Review. 2008; 7: 65-75.
4. Xu WH, Li XC. Antifungal compounds from Piper species. Curr Bioact Compd. 2011; 7: 262-267.
5. Nascimento JC, De Paula VF, David JM, David JP. Occurrence, biological activities and ^{13}C NMR data of amides from Piper (Piperaceae). Quim Nova. 2012; 35: 2288-2311.
6. Santos RA, Ramos CS, Young CM, Pinheiro TG, Amorim AM, Kato MJ, et al. Antifungal Constituents from the Roots of Piper dilatatum Rich. Journal of Chemistry. 2013; 1-5.
7. Nascimento AS, Araujo EA, Da Silva JM, Ramos CS. Chemical study and antimicrobial activities of Piper arboreum (Piperaceae). J Chil Chem Soc. 2015; 60: 2837-2839.
8. Silva RJF, Aguiar-Dias ACA, Faial KCF, Mendonça MS. Pharmacognostical characterization of Piper arboreum var. arboreum and P. tuberculatum (Piperaceae). Acta Amaz. 2016; 46: 195-208.
9. Kanaki N, Dave M, Padh H, Rajani M. A rapid method for isolation of piperine from the fruits of Piper nigrum Linn. J Nat Med. 2008; 62: 281-283.
10. Bezerra DP, Pessoa C, Moraes MO, Saker-Neto N, Silveira ER, Costa-Lotufo LV. Overview of the therapeutic potential of piplartine (piperlongumine). Eur J Pharm Sci. 2013; 48: 453-463.
11. Cardozo EL, Chaves COM. Caldensin, a new natural n-methylaristolactam from Piper caldense. Pharmaceutical Biology. 2003; 41: 216-218.
12. Freitas GC, Kitamura ROS, Lago JHG, Young MCM, Guimaraes EF, Kato MJ. Caldensinic acid, a prenylated benzoic acid from Piper caldense. Phytochemistry Letters. 2009; 2: 119-122.
13. Alves HS, Souza MFV, Chaves MCO. Caldensinic acid, a benzoic acid derivative and others compounds from Piper carniconnectivum. Quím Nova. 2010; 33: 802-804.
14. Maxwell A, Rampersad D. Novel Prenylated Hydroxybenzoic Acid Derivatives from Piper saltuum. J Nat Prod. 1989; 52: 614-618.