Taylor & Francis
Taylor & Francis Group

Pharmaceutical Biology

ISSN: 1388-0209 (Print) 1744-5116 (Online) Journal homepage: https://www.tandfonline.com/loi/iphb20

Antimicrobial activity of the crude ethanol extract from *Pimenta pseudocaryophyllus*

Joelma Abadia Marciano de Paula, José Realino de Paula, Fabiana Cristina Pimenta, Maria Helena Rezende & Maria Teresa Freitas Bara

To cite this article: Joelma Abadia Marciano de Paula, José Realino de Paula, Fabiana Cristina Pimenta, Maria Helena Rezende & Maria Teresa Freitas Bara (2009) Antimicrobial activity of the crude ethanol extract from *Pimenta pseudocaryophyllus*, Pharmaceutical Biology, 47:10, 987-993, DOI: 10.1080/13880200902969462

To link to this article: https://doi.org/10.1080/13880200902969462

Published online: 23 Sep 2009.

Submit your article to this journal

Article views: 340

View related articles

Citing articles: 12 View citing articles

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=iphb20

Pharmaceutical Biology, 2009; 47(10): 987–993

informa
healthcare

RESEARCH ARTICLE

Antimicrobial activity of the crude ethanol extract from *Pimenta pseudocaryophyllus*

Joelma Abadia Marciano de Paula[1], José Realino de Paula[2], Fabiana Cristina Pimenta[3], Maria Helena Rezende[4], and Maria Teresa Freitas Bara[2]

[1]*Curso de Farmácia, Universidade Estadual de Goiás, Anápolis, Goiás, Brazil,* [2]*Faculdade de Farmácia, Universidade Federal de Goiás, Goiânia, GO, Brazil,* [3]*Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, Goiás, Brazil, and* [4]*Instituto de Ciências Biológicas, Universidade Federal de Goiás, Goiânia, Goiás, Brazil*

Abstract

The antimicrobial activity of the crude ethanol extract from *Pimenta pseudocaryophyllus* (Gomes) L. R. Landrum (Myrtaceae), collected at two locations in Brazil, was investigated. Leaf samples were collected in April 2005 and in September 2005, both in Brasília, DF, Brazil, and in July 2000 in São Gonçalo do Abaeté, MG, Brazil. They were dried, crushed and used to obtain three crude ethanol extracts. The agar diffusion test was used for antimicrobial activity screening and the agar dilution method for determining the minimal inhibitory concentration (MIC). In assay conditions, extracts I, II and III demonstrated antimicrobial activity against Gram-positive *Staphylococcus aureus*, *Micrococcus luteus*, *M. roseus*, *Bacillus cereus*, *B. atrophaeus,* and *B. stearothermophilus* (MIC 0.39062 to 12.5 mg/mL, MIC 0.78125 to 1.5625 mg/mL and MIC 0.39062 to 1.5625 mg/mL, respectively), against Gram-negative *Pseudomonas aeruginosa* (MIC 0.39062 to 3.125 mg/mL, MIC 1.5625 mg/mL and MIC 0.78125 to 1.5625 mg/mL, respectively), against clinical isolates of *Pseudomonas stutzeri* (MIC 0.39062 to 0.78125 mg/mL, MIC 0.78125 to 1.5625 mg/mL, MIC 0.78125 to 1.5625 mg/mL, respectively) and also against *Candida albicans* fungi (MIC 0.19531 mg/mL for the extracts I, II and III). This study showed that the antimicrobial activity of *P. pseudocaryophyllus* might be considered sufficient to encourage further studies to isolate and identify its active principles. Pharmacological and toxicological studies are also necessary, followed by studies regarding the culturing and managing processes of this vegetable.

Keywords: *Agar diffusion test; agar dilution method; bacteria; minimal inhibitory concentration; fungi*

Copyright © 2009 Informa UK Ltd. Not for Sale or Commercial Distribution. Unauthorized use prohibited. Authorised users can download, display, view and print a single copy for personal use

Introduction

For the past five decades, the use of antibiotic or antimicrobial substances has represented one of the greatest advances in pharmacotherapy. These substances are among the most common pharmaceutical drugs used in hospital settings and have drastically reduced the risk of infection in the human body. However, microbial resistance is a major problem in the hospital environment, where the use of antimicrobials is imperative (Gales et al., 2005; Hsueh et al., 2005; Meyer et al., 2005; Zhanel et al., 2005). Many infections caused by emerging or multiresistant microorganisms remain without effective therapeutic options, not to mention the serious side effects of some of the drugs available (Lima, 2001; Tavares, 2001; Hardman et al., 2003).

Microorganisms acquire resistance to antimicrobial substances faster than new antimicrobial agents can be developed. Therapeutics have been strongly affected by knowledge concerning new standards of microbial resistance altering choice, dosage and substance combination. The discovery of new, safer and more specific drugs is, therefore, urgent (Helfand & Bonomo, 2005; Meyer, 2005; Thomson & Bonomo, 2005).

The development of new antimicrobial agents can be greatly aided by research involving natural products

Address for Correspondence: Joelma Abadia Marciano de Paula, Curso de Farmácia, Universidade Estadual de Goiás, Br. 153, Km 98, PO Box 459, 75001-970, Anápolis, Goiás, Brazil. Tel./Fax: (62) 3328-1156. E-mail: joelmapaula@uol.com.br

(Received 06 February 2008; revised 11 August 2008; accepted 11 August 2008)

ISSN 1388-0209 print/ISSN 1744-5116 online © 2009 Informa UK Ltd
DOI: 10.1080/13880200902969462

(Lima, 2001). The diversity of compounds found in plant species make these organisms promising sources of new antimicrobial agents, with general or specific effects. These findings have motivated several studies in different parts of the world (Souza et al., 2002; Cunico et al., 2004; Zuque et al., 2004; Hernández et al., 2005; Michelin et al., 2005; Yasunaka et al., 2005; Bugno et al., 2007; Oliveira et al., 2007; Nair & Chanda, 2007).

Brazil is recognized as a mega-diversity country (Mittermeier et al., 1997) and Brazilian folk medicine is substantial. Therefore, the plant *Pimenta pseudocaryophyllus* (Gomes) L.R. Landrum (Myrtaceae) was selected for this study using an ethno-pharmacological approach. This species is known as "pau-cravo" or "craveiro" and occurs in the Brazilian Atlantic Forest and also rarely in regions of the Brazilian savannah. It is a 4-10 m tall tree. Its leaves are elliptical, obovate or elliptic-oblanceolate, densely covered with long unicellular hairs below and glabrous above. Its inflorescence is a dichasium or a panicle (Landrum, 1986; Lorenzi, 2002).

In the folk medicine of São Goncalo do Abaeté, MG, Brazil, the leaves are used to make tea for influenza. In the county of Campos do Jordão, SP, Brazil, the leaves are infused to make a soothing tea as well as to regulate digestion and menstruation (Nakaoka-Sakita et al., 1994). According to Lima et al. (2006), the tea from the leaf is also used as refreshment and as a diuretic. The literature data supports that there is antimicrobial activity in the essential oils of two specimens of *P. pseudocaryophyllus*, collected at two locations in São Paulo state, Brazil, against *Candida albicans* (ATCC 10231), *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027) and *Staphylococcus aureus* (ATCC 6538) (Lima et al., 2006). The main purpose of this study is to assay the *in vitro* antimicrobial activity of the crude ethanol extract obtained from the leaves of three samples of *P. pseudocaryophyllus* collected in two locations in Brazil.

Material and methods

Plant material

P. pseudocaryophyllus leaves were collected in two counties: In São Gonçalo do Abaeté, MG, Brazil at 18°20'58.4" South; 45°55'23.4" West, 864 m altitude, in June 2000, in the fructification period (post-anthesis). The plant material was identified by Carolyn Elinore Barnes Proença, a specialist in Myrtaceae from the University of Brasilia and a voucher specimen was placed in the Herbarium of the Federal University of Goiás, number UFG-27.159.

In Brasília, DF, Brazil at 15°51'51.6" South; 47°49'43" West and at 767 m altitude, one sampling was taken in April 2005, during fructification (post-anthesis) and another in September 2005, during flowering (anthesis). These samplings were collected at about 10:00 am in sunny weather. A voucher specimen of the plant material was placed in the Ezechias Paulo Heringer Herbarium of Brasilla Botanical Garden, DF, Brazil, number 21, 745-0, and identified by specialists from this institution.

To obtain the pulverized plant material the leaves were oven dried with forced ventilation at 40°C and then crushed to a fine powder. The pulverized material was used to obtain the crude ethanol extract.

Crude ethanol extract preparation

The crude ethanol extract was obtained by cold maceration according to methodology adapted by Ferri (1996). Five parts of ethanol 95% (V/V) were added to one part pulverized plant material and submitted to mechanical agitation for 4 h. It was then filtered in filter paper and concentrated in a Rotary evaporator. The procedure was repeated twice more. The extracts obtained as previously described for each sample of both specimens were identified and stored at room temperature.

Antimicrobial activity screening by the agar diffusion method

The antimicrobial activity screening of the extracts was performed as recommended by the National Committee for Clinical Laboratory Standard (NCCLS, 2003), currently known as Clinical and Laboratory Standards Institute (CLSI). Standard American Type Culture Collection (ATCC) strains were used (Table 1).

The microorganisms stocked in simple inclined agar (SIA) at 4°C were subcultured in a thioglycollate broth and were incubated at 37°C for 24 to 48 h for reactivation of the cultures. Following this, they were subcultured

Table 1. Microorganism strains used in triage.

	ATCC
Gram-positive bacteria	
Staphylococcus aureus	29213
Micrococcus roseus	1740
Sporulated Gram-positive bacteria	
Bacillus cereus	14579
Bacillus stearothermophilus	1262
Bacillus atrophaeus	6633
Gram-negative bacteria	
Enterobacter cloacae	HMA/FT502
Enterobacter aerogenes	13048
Escherichia coli	8739
Escherichia coli	25922
Pseudomonas aeruginosa	9027
Pseudomonas aeruginosa	27253
Serratia marcescens	14756
Fungi	
Candida albicans	1023

in SIA plates and incubated at 37°C for 24 h. Microbial suspensions were then prepared, adding an inoculum to 2 mL of sterile saline solution 0.85% (W/V) until turbidity 1 on the MacFarland scale was obtained. Next, 100 µL of the microbial suspension obtained were added to 10 mL of Mueller Hinton liquefied agar, homogenized and transferred rapidly onto the plates that contained a base layer of 20 mL of Mueller Hinton agar. Orifices of 5-mm diameter were distributed in a circle at equidistant points on the plate leaving the center without orifice.

The crude ethanol extract of each sample was solubilized in ethanol 95% (V/V) at a 1:1 ratio and 10 µL was inoculated into each orifice. This step was performed in triplicate. A control was made with the solvent used in the dilution of the extract. The center of the Gram-positive bacteria plates received a disk of penicillin (Oxoid® 10µg) as positive control, the Gram-negative plates received a disk of erythromycin (Oxoid® 15µg) as positive control. The plates were incubated at 37°C for 24 h. The diameter of the inhibition halos was then measured.

Minimal inhibitory concentration (MIC) bioassay

The agar dilution method was used according to the NCCLS recommendation (2003). Double dilution series were made in 9 test tubes for each sample: 1 g of each crude ethanol extract was added to a first sterile test tube and the volume was completed to 2 mL with ethanol 95% (V/V), then 1 mL of sterile distilled water was added to a sequence of 8 sterile test tubes, an aliquot of 1 mL was transferred from the first test tube to the second and so on, successively, until the ninth tube, and at the end an aliquot of 1 mL was discarded.

Next, 19 mL of liquefied Mueller Hinton agar were added, then homogenized and quickly transferred onto the plate. In this manner the plates obtained contained the crude ethanol extract of the specimens under study in concentrations ranging from 25 to 0.0976 mg/mL. Control plates were prepared containing the solvent used in the extraction along with Mueller Hinton agar and also containing only Mueller Hinton agar.

The strains used were the standard American Type Culture Collection (ATCC) shown in Table 1 (except for *Serratia marcescens* ATCC 14756) and *Micrococcus luteus* ATCC 9341. Clinical isolates of *Staphylococcus aureus* and *Pseudomonas stutzeri* kept in the Bacterial Culture Collection in the Bacteriology Laboratory in the Tropical Pathology and Public Health Institute of the Federal University of Goiás, Brazil, were also used (Table 2).

The microorganisms were subcultured in thioglycollate broth and incubated at 37°C for 24 to 48 h for reactivation. Next, they were subcultured in SIA plates and once again incubated at 37°C for 24 h. After the microbial growth, inocula of each microorganism were prepared in 2 mL of sterile saline solution 0.85% (W/V) standardized to an opacity equivalent to 1 on the MacFarland scale. 100 µL of each inoculum was transferred to the Steers inoculator (Steers et al., 1959), applied on the surface of the plates containing the extract dilutions and their respective controls and subsequently incubated at 37°C for 24 h. The smallest concentration capable of inhibiting microbial growth was considered to be the MIC.

Table 2. Microorganisms obtained from clinical isolates used in MIC determination.

	Number
Gram-positive bacteria – clinical isolates	
Staphylococcus aureus	209
Staphylococcus aureus	223
Staphylococcus aureus	225
Staphylococcus aureus	234
Gram-negative bacteria – clinical isolates	
Pseudomonas stutzeri	130 B
Pseudomonas stutzeri	157 D
Pseudomonas stutzeri	230 B
Pseudomonas stutzeri	55 B
Pseudomonas stutzeri	90 A
Pseudomonas stutzeri	105 D

Results and discussion

Antimicrobial activity screening by the agar diffusion method

The crude ethanol extracts of the samples of *P. pseudocaryophyllus* analyzed by agar diffusion test presented antimicrobial activity against all Gram-positive bacteria tested. They presented traces of growth inhibition in *C. albicans* ATCC 1023. Regarding the Gram-negative bacteria, they presented antimicrobial activity against both strains of *P. aeruginosa* studied and against *S.marcescens* ATCC 14756, but did not inhibit the growth of *Enterobacter aerogenes* ATCC 13048, nor *E. cloacae* ATCC HMA/FT502 or both *E. coli* strains analyzed (Table 3).

Several authors have studied the antimicrobial activity of essential oils and plant extracts and have found activity against Gram-positive bacteria (Cunico et al., 2004; Zuque et al., 2004; Fernandes et al., 2005; Hernández et al., 2005; Michelin et al., 2005; Yasunaka et al., 2005; Bugno et al., 2007; Oliveira et al., 2007; Nair & Chanda, 2007). The antimicrobial activity against Gram-positive bacteria *B. cereus, B. atrophaeus* and *B. stearothermophilus* observed in this study was relevant, since these are spore-forming microorganisms, and can survive under adverse conditions for several years. *S. aureus* is the most common cause of pyogenic infections on the skin or in deeper regions

Table 3. Halo averages (mm) of microbial growth inhibition obtained in the agar diffusion test using crude ethanol extracts of *P. pseudocaryophyllus* specimens and controls.

Microorganisms	Extract I	Extract II	Extract III	Ethanol 95% (v/v)	Penicillin 10 µg	Erythromycin 15 µg
S. aureus ATCC 29213	17	19	17	0	18	NP
M. roseus ATCC 1740	27	29	28	0	NP	NP
B. cereus ATCC 14579	16	18	16	0	0	NP
B. stearothermophilus ATCC 1262	13	15	13	0	13	NP
B. atrophaeus ATCC 6633	14	16	14	0	14	NP
E. cloacae ATCC HMA/FT502	0	0	0	0	NP	0
E. aerogenes ATCC 13048	0	0	0	0	NP	0
E. coli ATCC 8739	0	0	0	0	NP	0
E. coli ATCC 25922	0	0	0	0	NP	0
P. aeruginosa ATCC 9027	13	12	13	0	NP	0
P. aeruginosa ATCC 27253	13	15	16	0	NP	10
S. marcescens ATCC 14756	15	14	15	0	NP	NP
Candida albicans ATCC 1023	T	T	T	0	NP	NP

Extract I, leaves collected in Brasília in April 2005; Extract II, leaves collected in Brasília in September 2005; Extract III, leaves collected in São Gonçalo do Abaeté in July 2000; NP, not performed; T, traces of microbial growth inhibition.

and commonly inhabits the nasal cavity of 40-50% of all human beings (Brooks et al., 2004). Considering the clinical importance of this microorganism, the selection of a few clinical isolates, beside the standard ATCC 29213 strains, was performed in order to determine the MIC of the crude ethanol extracts of *P. pseudocaryophyllus*.

None of the three extracts inhibited *E. coli* ATCC 8739 or ATCC 25922 strains. Analogous results were also observed in other plant species (Zuque et al., 2004; Fernandes et al., 2005; Yasunaka et al., 2005).

The *P. pseudocaryophyllus* extracts did not inhibit *E. aerogenes* ATCC 13048 and *E. cloacae* ATCC HMA/TF502, enterobacteria responsible for extra intestinal infections and the cause of a great number of hospital infections. Nair and Chanda (2007) found similar results for *E. aerogenes* ATCC 13048 when researching *Psidium guajava* L. leaf extracts. On the other hand, the enterobacteria *S. marcescens* ATCC 14756, which belongs to the same group, was inhibited by the tested extracts.

Although the group of Gram-negative bacteria mentioned above did not present inhibition in the agar diffusion test, all three extracts presented antimicrobial activity against *P. aeruginosa* ATCC 9027 and ATCC 27253 strains. This microorganism normally inhabits vegetables, water and soil and may be found on the skin, excrement and throat of normal individuals. It is opportunist and can be responsible for several infectious diseases such as localized and urinary infections, serious pneumonia and around 15% of bacteremia caused by Gram-negative germs. It is among the most frequent pathogens in hospital infection cases due to its various virulence factors and to its multi-resistance to several antimicrobial drugs (Thomson & Bonomo, 2005). Such findings justified the use of clinical isolates of this microorganism as well as standard strains for MIC determination.

Minimal inhibitory concentration (MIC) bioassay

Considering the results obtained from the screening tests performed previously, the determination of the minimal inhibitory concentration (MIC) for all three crude ethanol extracts was considered necessary and the results are shown in Table 4.

The results observed in the agar diffusion test are qualitative and present limitations for substances with low diffusion ability in the culture medium. The quantitative step occurred in the agar dilution test.

The antimicrobial activity evidenced by the agar diffusion test for Gram-positive bacteria was confirmed by the agar dilution test. Of all the Gram-positive bacteria tested, 60% (6 bacteria) were inhibited by the sample collected in April 2005 (extract I) at a MIC of 0.78125 mg/mL and 70% (7 bacteria) by the sample collected in September 2005 (extract II) at a MIC of 1.5625 mg/mL. The specimen from Minas Gerais (extract III) presented an inhibition pattern practically identical to the one collected in Brasilia in April 2005.

Similar observations can be made regarding the MIC of the three extracts against *P. aeruginosa* and *P. stutzeri*. Extract I of the Brasilia sample inhibited 50% of the *Pseudomonas* evaluated at a MIC of 0.78125 mg/mL and 37.5% at an even lower concentration (0.39062 mg/mL). Extract II of the Brasilia sample, on the other hand, inhibited 87.5% of the *Pseudomonas* at a MIC of 1.5625 mg/mL. Extract III of the Minas Gerais sample presented an inhibition pattern very similar to the one presented by extract I from Brasilia, inhibiting 87.5% of the *Pseudomonas* at a minimal concentration of 0.78125 mg/mL.

Table 4. Minimal inhibitory concentration (mg/mL) of the crude ethanol extracts of *P. pseudocaryophyllus* leaves.

Microorganisms	Extract I	Extract II	Extract III
Gram-positive			
S. aureus ATCC 29213	0.39062	0.78125	0.39062
S. aureus 209	0.78125	1.5625	0.78125
S. aureus 223	0.78125	1.5625	0.78125
S. aureus 225	12.5	1.5625	1.5625
S. aureus 234	0.78125	1.5625	0.78125
M. luteus ATCC 9341	0.78125	1.5625	0.78125
M. roseus ATCC 1740	0.78125	0.78125	0.78125
B. cereus ATCC 14579	0.39062	0.78125	0.78125
B. stearothermophilus ATCC 1262	0.78125	1.5625	0.78125
B. atrophaeus ATCC 6633	1.5625	1.5625	1.5625
Fungi			
Candida albicans ATCC 1023	0.19531	0.19531	0.19531
Gram-negative			
E. cloacae ATCC HMA/FT502	3.125	12.5	1.5625
E. aerogenes ATCC 13048	12.5	25	1.5625
E. coli ATCC 8739	12.5	1.5625	3.125
E. coli ATCC 25922	NI	25	25
P. aeruginosa ATCC 9027	3.125	1.5625	1.5625
P. aeruginosa ATCC 27253	0.39062	1.5625	0.78125
P. stutzeri 130 B	0.78125	1.5625	0.78125
P. stutzeri 157 D	0.78125	1.5625	0.78125
P. stutzeri 230 B	0.78125	1.5625	0.78125
P. stutzeri 55 B	0.39062	1.5625	0.78125
P. stutzeri 90 A	0.78125	1.5625	0.78125
P. stutzeri 105 D	0.39062	0.78125	0.78125

Extract I, leaves collected in Brasília in April 2005; Extract II, leaves collected in Brasília in September 2005; Extract III, leaves collected in São Gonçalo do Abaeté in July 2000; NI, no inhibition of microbial growth occurred with concentrations used in this test.

Such results may be explained by differences in extract compositions, since the plant materials were collected in different locations and times of the year. Plant material of the specimens analyzed is rich in phenolic compounds, such as tannins and flavonoids, and in essential oils (Paula et al., 2008). It is probable that these compounds are involved in the antimicrobial activity assessed in this study.

Concerning the essential oils, Paula (2006) verified a substantial variation in chemical yields and compositions, as much in terms of geographical location as in terms of stage of development for all three *P. pseudocaryophyllus* specimens studied. The fact that extract I presented an inferior MIC than extract II may be related to the higher yield of essential oil (0.8%) and to the higher content of methyl eugenol verified in the first sample. The Minas Gerais specimen (extract III), in spite of presenting an antimicrobial inhibition pattern similar to the Brasilia sample (extract I), had a completely different essential oil composition. Its antimicrobial action can be justified by the predominance of citral (neral and geranial) in its essential oil. It is important to highlight that citral has an intense antiseptic effect, even more potent than phenol (Santos & Mello, 2004; Simões & Spitzer, 2004; Zuanazzi & Montanha, 2004) and that eugenol is a well-known antimicrobial agent (Dorman & Deans, 2000). Recently, Lima et al. (2006) related the differences in the chemical composition of the essential oils of two specimens of *P. pseudocaryophyllus* to the different MIC observed against *E. coli* and *C. albicans*.

Data concerning the antimicrobial activity of extracts obtained from *P. pseudocaryophyllus* leaves were not found in the literature. However, other species of the Myrtaceae family have been studied regarding antimicrobial activity attributed to essential oils or tannins (Bara & Vanetti, 1998; Djipa et al., 2000; Estanislau et al., 2001; Cimanga et al., 2002; Saenz et al., 2004; Li et al., 2005; Oliveira et al., 2007).

Djipa et al. (2000) points out that tannins present in different plant extracts do not present a common pattern of microbial inhibition, since not all tannins have antimicrobial action and each extract can have different concentrations of antimicrobial tannins. This observation is important for the present study, since it is noteworthy that among the group of Gram-negative bacteria evaluated, the extracts proved to be more active against *Pseudomonas* ssp. Considering tannins are partly responsible for the antimicrobial activity of the extracts analyzed in this work, this fact may be related to the content of antimicrobial tannins in the extracts with specificities against *P. aeruginosa* and *P. stutzeri*. Therefore, more precise studies to clarify the chemical composition not only of tannins but also of the essential oil components responsible for the antimicrobial activity of *P. pseudocaryophyllus* leaves will soon be vital. According to Barreiro (2001), such compounds could serve as raw material for the production of pharmaceutical drugs or also serve as prototypes for the synthesis of new pharmaceutical drugs. Hence, the production of antimicrobial substances active against *P. aeruginosa,* a microorganism that presents multi-resistance to the most modern antimicrobial substances (Makedou et al., 2005) will be invaluable.

The activity of the three extracts against the Gram-positive bacteria studied and, especially against the Gram-negative bacteria *Pseudomonas* ssp. has significant clinical meaning. Such results indicate promising propositions for controlling microorganisms and for the production of new antimicrobial drugs as well as positively influencing the ethno-medicinal use of this plant. Nevertheless, in order for this plant to become raw material for the production of herbal medicines, phytochemical, pharmacological and toxicological studies are necessary, with a view to guaranteeing the effective and safe application of this plant. Our research group is now focused on these subjects. Besides, studies on the possibility of cultivation and domestication to enable the use

of this plant without causing negative environmental, economic and social impacts are also recommended.

Acknowledgements

The authors thank the Fundação de Apoio à Pesquisa (FUNAPE/UFG), the Faculdade de Farmácia /UFG, the Instituto de Ciências Biológicas/UFG and the Universidade Estadual de Goiás (UEG), all in Brazil. The authors thank Sharon Lois Vinaud for language assistance.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

Bara MTF, Vanetti MCD (1997/1998): Estudo da atividade antibacteriana de plantas medicinais, aromáticas e corantes naturais. *Rev Bras Farmacogn* 7/8:23–34.

Barreiro EJ (2001): Desenho de fármacos a partir de produtos naturais, in: Yunes RA, Calixto JB, eds., *Plantas Medicinais sob a Ótica da Química Medicinal Moderna*. Chapecó, Argos, pp. 237–296.

Brooks GF, Butel JS, Morse SA (2004): Jawetz, Melnick & Adelberg's *Medical Microbiology*. 23rd edition, New York, Lange/McGraw-Hill Publishing, pp. 202–229.

Bugno A, Nicoletti MA, Almodóvar AAB, Pereira TC, Auricchio MT (2007): Antimicrobial efficacy of *Curcuma zedoaria* extract as assessed by linear regression compared with commercial mouth rinses. *Braz J Microbiol* 38: 440–445.

Cimanga K, Kambu K, Tona L, Apers S, De Bruyne T, Hermans N, Totté J, Pieters L, Vlietinck AJ (2002): Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. *J Ethnopharmacol* 79: 213–220.

Cunico MM, Carvalho JLS, Kerber VA, Higakino CEK, Cruz Almeida SC, Miguel MD, Miguel OG (2004): Atividade antimicrobiana do extrato bruto etanólico de raízes e partes aéreas de *Ottonia martiana* Miq. (Piperaceae). *Rev Bras Farmacogn* 14: 97–103.

Djipa CD, Delmée M, Quetin-Leclercq J (2000): Antimicrobial activity of bark extracts of *Syzygium jambos* (L.) Alston (Myrtaceae). *J Ethnopharmacol* 71: 307–313.

Dorman HJD, Deans SG (2000): Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *J Appl Microbiol* 88: 308–316.

Estanislau AA, Barros FAS, Pena AP, Santos SC, Ferri PH, Paula JR (2001): Composição química e atividade antibacteriana dos óleos essenciais de cinco espécies de *Eucalyptus* cultivadas em Goiás. *Rev Bras Farmacogn* 11: 95–100.

Fernandes TT, Santos ATF, Pimenta FC (2005): Atividade antimicrobiana das plantas *Plathymenia reticulata, Hymenaea courbaril* e *Guazuma ulmifolia*. *Revista de Patologia Tropical* 34: 113–122.

Ferri PH (1996): Química de produtos naturais: métodos gerais, in: Distasi LC, ed., *Plantas Medicinais: Arte e Ciência*. São Paulo, Editora da Universidade Estadual Paulista, pp.129–156.

Gales AC, Jones RN, Andrade SS, Sader HS (2005): Antimicrobial susceptibility patterns of unusual non fermentative Gram-negative bacilli isolated from Latin America: Report from the SENTRY Antimicrobial Surveillance Program (1997-2002). *Mem Inst Oswaldo Cruz* 100: 671–677.

Hardman JG, Limbird LE, Gilman AG (2003). *As Bases Farmacológicas da Terapêutica,* tenth edition, Rio de Janeiro, McGraw-Hill, pp. 859–1033.

Helfand MS, Bonomo RA (2005): Current challenges in antimicrobial chemotherapy: the impact of extended-spectrum β-lactamases and metallo-β-lactamases on the treatment of resistant Gram-negative pathogens. *Curr Opin Pharmacol* 5: 452–458.

Hernández T, Canales M, Avila JG, García AM, Martínez A, Caballero J, Romo de Vivar A, Lira R (2005): Composition and antibacterial activity of essential oil of *Lantana achyranthifolia* Desf. (Verbenaceae). *J Ethnopharmacol* 96: 551–554.

Hsueh P, Chen W, Luh K (2005): Relationships between antimicrobial use and antimicrobial resistance in Gram-negative bacteria causing nosocomial infections from 1991–2003 at a university hospital in Taiwan. *Int J Antimicrob Agents* 26: 463–472.

Landrum LR (1986): Monography 45: Campomanesia, Pimenta, Blepharocalyx, Legrandia, Acca, Myrrhinium, and Luma (Myrtaceae). *Flora Neotropica* 4: 72–115.

Li Y, Xu C, Zhang Q, Liu JY, Tan RX (2005): *In vitro* anti-*Helicobacter pylori* action of 30 Chinese herbal medicines used to treat ulcer diseases. *J Ethnopharmacol* 98: 329–333.

Lima EO (2001): Plantas e suas propriedades antimicrobianas: Uma breve análise histórica, in: Yunes RA, Calixto JB, eds, *Plantas Medicinais sob a Ótica da Química Medicinal Moderna*. Chapecó, Argos, pp. 481–501.

Lima MEL, Cordeiro I, Young MCM, Sobra MEG, Moreno PRH (2006): Antimicrobial activity of the *Pimenta pseudocaryophyllus* (Gomes) L. R. Landrum (Myrtaceae) native from São Paulo State – Brazil. *Pharmacologyonline* 3: 589–593.

Lorenzi H (2002): *Árvores Brasileiras. Manual de Identificação e Cultivo de Plantas Arbóreas do Brasil*, second edition, *Nova Odessa, Instituto Plantarum de Estudos da Flora*, pp. 262–262.

Makedou KG, Tsiakiri EP, Bisiklis AG, Chatzidimitriou M, Halvantzis AA, Ntoutsou K, Alexiou-Daniel S (2005): Changes in antibiotic resistance of most common Gram-negative bacteria isolated in intensive care units. *J Hosp Infect* 60: 245–248.

Meyer AL (2005): Prospects and challenges of developing new agents for tough Gram-negatives. *Curr Opin Pharmacol* 5: 490–494.

Meyer E, Schwab F, Jonas D, Ruden H, Gastmeier P, Daschner FD (2005): Temporal changes in bacterial resistance in German intensive care units, 2001-2003: data from the SARI (surveillance of antimicrobial use and antimicrobial resistance in intensive care units) project. *J Hosp Infect* 60: 348–352.

Michelin DC, Moreschi PE, Lima AC, Nascimento GGF, Paganelli MO, Chaud MV (2005): Avaliação da atividade antimicrobiana de extratos vegetais. *Rev Bras Farmacogn* 15: 316–320.

Mittermeier RA, Gil PR, Mittermeier CG (1997): *Megadiversity: Earth's Biologically Wealthiest Nations*. Agrupacíon Sierra Madre, Mexico City, CEMEX, pp. 39–49.

Nair R, Chanda S (2007): *In-vitro* antimicrobial activity of *Psidium guajava* L. leaf extracts against clinically important pathogenic microbial strains. *Braz J Microbiol* 38: 452–458.

Nakaoka-Sakita M, Aguiar OT, Yatagai M, Igarashi T (1994): Óleo essencial de *Pimenta pseudocaryophyllus* var. *pseudocaryophyllus* (Gomes) Landrum (Myrtaceae) I: Cromatografia a gás/espectrometria de massa (CG/EM). *Rev Inst Flor* 6: 53–61.

NCCLS (National Committee for Clinical Laboratory Standards) (2003): *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*: *Approved Standard M7-A6*, sixth edition. Wayne, PA, NCCLS. pp. 1–47.

Oliveira GF, Furtado NAJC, Silva Filho AA, Martins CHG, Bastos JK, Cunha WR, Silva MLA (2007): Antimicrobial activity of *Syzygium cumini* (Myrtaceae) leaves extract. *Braz J Microbiol* 38: 381–384.

Paula JAM (2006): *Estudo Farmacognóstico e Avaliação da Atividade Antimicrobiana das Folhas de* Pimenta pseudocaryophyllus *(Gomes) L. R. Landrum – Myrtaceae. Goiânia, Universidade Federal de Goiás*, pp. 91–110.

Paula JAM, Paula JR, Bara MTF, Rezende MH, Ferreira HD (2008): Estudo farmacognóstico das folhas de *Pimenta pseudocaryophyllus* (Gomes) L.R. Landrum – Myrtaceae. *Rev Bras Farmacogn* 18: 265–278.

Saenz MT, Tornos MP, Alvarez A, Fernadez MA, García MD (2004): Antibacterial activity of essential oils of *Pimenta racemosa* var. *terebinthina* and *Pimenta racemosa* var. *grisea*. *Fitoterapia* 75: 599–602.

Santos SC, Mello JCP (2004): Taninos, in: Simões CMO, Schenkel EP, Gosmann G, Mello JCP, Mentz LA, Petrovick PR, eds, *Farmacognosia: da Planta ao Medicamento*. *Florianópolis*, Ed. da UFSC, pp. 615–656.

Simões CMO, Spitzer V (2004): Óleos voláteis, in: Simões CMO, Schenkel EP, Gosmann G, Mello JCP, Mentz LA, Petrovick PR, eds., *Farmacognosia: da Planta ao Medicamento*. *Florianópolis*, Ed. da UFSC, pp. 467–495.

Souza LKH, Oliveira CMA, Ferri PH, Santos SC, Oliveira Júnior JG, Miranda ATB, Lião LM, Silva MRR (2002): Antifungal properties of Brazilian cerrado plants. *Braz J Microbiol* 33: 247–249.

Steers E, Foltz EL, Graaves VS (1959): An inocula replicating apparatus for continued testing of bacterial susceptibility to antibiotics. *Antibiot Chemother* 9: 307–311.

Tavares W (2001): *Manual de Antibióticos e Quimioterápicos Antiinfecciosos*, third edition. São Paulo, Atheneu, pp. 40–520.

Thomson JM, Bonomo RA (2005): The treatment of antibiotic resistance in Gram-negative pathogenic bacteria: β-lactams in peril! *Curr Opin Microbiol* 8: 518–524.

Yasunaka K, Abe F, Nagayama A, Okabe H, Lozada-Pérez L, López-Villafranco E, Muñiz EE, Aguilar A, Reyes-Chilpa R (2005): Antibacterial activity of crude extracts from Mexican medicinal plants and purified cumarins and xanthones. *J Ethnopharmacol* 97: 293–299.

Zhanel GG, Hisanaga TL, Laing NM, DeCorby MR, Nichol KA, Palatnick LP, Johnson J, Noreddin A, Harding GKM, Nicolle LE, Hoban DJ (2005): Antibiotic resistance in outpatient urinary isolates: final results from the North American Urinary Tract Infection Collaborative Alliance (NAUTICA). *Int J Antimicrob Agents* 26: 380–388.

Zuanazzi JAS, Montanha JA (2004): Flavonóides, in: Simões CMO, Schenkel EP, Gosmann G, Mello JCP, Mentz LA, Petrovick PR, eds., *Farmacognosia: da Planta ao Medicamento*. Florianópolis, Ed. da UFSC, pp. 577–614.

Zuque ALF, Watanabe ES, Ferreira AMT, Arruda ALA, Resende UM, Bueno NR, Castillo RO (2004): Avaliação das atividades antioxidante, antimicrobiana e citotóxica de *Couepia grandiflora* Benth. (Chrysobalanaceae). *Rev Bras Farmacogn* 14: 129–136.