ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL FROM TWO SPECIMENS OF *Pimenta pseudocaryophyllus* (GOMES) L. R. LANDRUM (MYRTACEAE) NATIVE FROM SÃO PAULO STATE – BRAZIL

Marcos Enoque Leite Lima[1], Inês Cordeiro[2], Maria Cláudia Marx Young[2], Marcos E. G. Sobra[3], Paulo Roberto H. Moreno[1]

1. Instituto de Química, USP, Av. Lineu Prestes 748 B11 T 05508-900 São Paulo, Brasil (E-mail:prmoreno@iq.usp.br; enoque@iq.usp.br)
2. Instituto de Botânica – Secretaria do Meio Ambiente - Av. Miguel Stéfano, 3687 – CEP 04301-902 - Água Funda - São Paulo SP-Brasil
3. Instituto de Biociências - UFMG- Av. Antônio Carlos, 6627 – Pampulha Caixa Postal 486 31270-910, Belo Horizonte.

Summary

The antimicrobial activity of the essential oils of two specimens of *Pimenta pseudocaryophyllus,* collected at two locations in São Paulo state-Brazil (Cardoso Island and Paranapiacaba), was determined. The antimicrobial activity was evaluated using the microdilution method against *Candida albicans* (ATCC 10231), *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027) and *Staphylococcus aureus* (ATCC 6538). The best results against *E.coli* (CIM = 48mL/L; CBM = 390μL/L) were obtained with the specimen collected in the Cardoso Island while against *P. aeruginosa* and *S. aureus* both specimens showed similar results. However, against *C. albicans*, the best results were found with the specimen collected in Paranapicaba (MIC=24μL/L and MBC=97μL/L). The difference in the sensitivity observed might be due to the different chemical composition, in the Cardoso Island specimen the major component was eugenol while in the other one of 4-methyl-eugenol.

Key-words**:** *Pimenta pseudocaryophyllus*, antimicrobial activity, MIC, MBC, eugenol, 4-methyl-eugenol

Introduction

Nowadays, the research towards the discovery of new antimicrobial agents has increased, mainly due to the development of resistance and the appearance of fatal opportunistic infections associated with the immune system depression caused by AIDS, chemotherapy and transplants. Additionally, there is a consensus among natural products pharmacologists that substances showing an antimicrobial activity have a great probability to possess other pharmacological activities. Myrtaceae is one of the families of highest occurrence in the Brazilian Atlantic Rain Forest, especially in the Southern and Southeastern regions of the country, and it is recognized by its great potential of producing volatile oils of economic interest.

Pimenta pseudocayophyllus (Gomes) Landrum (Myrtaceae) is a native species from the Brazilian Atlantic Rain Forest dispersed from southern Bahia until Rio Grande do Sul. This plant is popularly known by the names of ‘‘Cataia’’, “Craveiro” and ‘‘Louro-do-Mato’’; it is used for culinary and medicinal purposes. Its leaves are used by the population in the production of teas used as refreshments or in the healing process of colds, fatigue and as a diuretic. Furthermore, this leaves are very appreciated for some traditional populations from the Southern Coast of São Paulo State and the Northern Seashore of Paraná State as flavoring agent in the cachaça – traditional Brazilian alcoholic drink produced from the distillation of fermented sugar cane sap (1,2). The present work aims to study the antimicrobial activity of the essential oils obtained from the leaves of two specimens of *Pimenta pseudocaryophyllus* collected at two locations in São Paulo State, Brazil (Cardoso Island and Paranapiacaba).

Methods

Plant material

The two specimens of Pimenta pseudocaryophyllus were collected from State Park Ilha do Cardoso (SPIC) 25º03’05” - 24º18’18”S e 47º53’48” - 48º03’05”W. in Municipallity of Cananéia - SP and from the Municipal Park Nascentes de Paranapiacaba in the Municipality of Santo André - SP in 2005 and 2006. The specimens were identified by Dr. Inês Cordeiro (Instituto de Botânica-SP) and a voucher specimen was deposited at the Herbarium of the Instituto de Botânica de São Paulo (SP).

Essential oil extraction

The essential oils from the dried leaves (100g) of two species of *P. pseudocaryophyllus* were obtained by hydrodistillation in a Clevenger-type apparatus

for 4 h. The oil was collected, dried over anhydrous sodium sulfate, weighted and then stored at -25°C until testing.

Determination of the minimum inhibitory concentration (MIC), minimum bactericidal and fungicidal concentration (MBC, MFC)

The essential oils were tested with Gram-positive model bacteria, *Staphylococcus aureus* subsp. *aureus* (ATCC 25923), two Gram-negative models, *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 9027) and yeast *Candida albicans* (ATCC 10231).

A broth microdilution method was used to determine the MIC and MBC (3,4,5). All tests were performed in Tryptose soya broth (TSB - Difico®), with the exception of the yeast which employed Sabouraud dextrose broth (SDB - Difico®). A serial doubling dilution of the oils was prepared with a solution of Methanol: Dimethylsulfoxide 1:1 in a 96-well microtiter plate over the range of 7 – 3125 μL/L. Overnight broth cultures of each strain were prepared and the final concentration of the microbe in each well was adjusted to 2 x 10^3 cfu/ml. Plates were incubated at 37°C for 24 h for bacteria and at 25°C for 48 h for the yeast. The MIC is defined as the lowest concentration of the essential oil at which the microorganism does not demonstrate visible growth and the absorbance of each well was determined using an automatic Elisa tray reader adjusted at 630nm (SLT Spectra). The samples were analyzed in duplicate and the assay was repeated twice. The antibiotics Chloramphenicol, Amikacin and Nystatin were employed as positive controls.

The wells showing complete absence of growth were identified and 10 μL of each well were transferred to agar plates (TSA and SDA) and incubated at previously mentioned times and temperatures. The concentration where complete absence of growth was observed was considered as the minimum bactericidal and fungicidal concentration.

Results

The results of the antimicrobial assay of the essential oils of the two specimens of *P. pseudocaryophillus* are shown in Table 1. The specimen collected in the Cardoso Island showed the best results against *E.coli* (MIC = 48μL/L; MBC = 390μL/L) while against *P. aeruginosa* both specimens showed similar results (MIC = 48 μL/L; MBC = 390μL/L) and against *S. aureus* both oils were very active. However, against *C. albicans*, the best results were found with the specimen collected in Paranapicaba (MIC=24μL/L and MFC=97μL/L)

Table 1 – Determination of the antimicrobial activity from the essential oils obtained from two specimens of *P. pseudocaryophyllus* against different microorganisms

	P. aeruginosa		*E. coli*		*S. aureus*		*C. albicans*	
Specimen	MBC* (μL/L)	MIC** (μL/L)	MBC (μL/L)	MIC (μL/L)	MBC (μL/L)	MIC (μL/L)	MFC*** (μL/L)	MIC (μL/L)
Cardoso Island	390	48	390	48	390	0.047	190	48
Paranapiacaba	390	48	1560	48	190	1.5	97	24

* MBC = minimum bactericidal concentration
** MIC = minimum inhibitory concentration
*** MFC = minimum fungicidal concentration

Discussion

These results may be related to the chemical composition of the oils. The volatile oil from the specimen collected in the Cardoso Island contained eugenol (71.9%) as the major compound, while the specimen from Paranapiacaba presented 4-methyl-eugenol (94.6%) as the major oil component and a small amount of estragole (1%). Eugenol has been recognized as a strong antimicrobial agent against Gram (-) bacteria, such as *E. coli* and *Salmonella enterica* (6), as well as against Gram (+), such as *S. aureus*. The specimen rich in eugenol presented indeed the lowest MIC value for *S. aureus*. However, the lowest bactericidal concentration was obtained with the oil rich in 4-methyl-eugenol. The antifungal properties from eugenol have also been described against several pathogenic strains of C. *albicans* and *Cryptococcus neoformans*, with MIC values of 625 e 293 μg/mL and MFC de 1209 e 5210 μg/mL, respectively (7). The literature data supports the antifungal results obtained for both specimens. As observed for *S. aureus*, the specimen collected in Paranapiacaba displayed lower values for MIC and MFC against *C. albicans* than those obtained with the Cardoso Island specimen. These results indicate that the simple methylation of eugenol for the Paranapiacaba population increased its toxic effects against *S. aureus* and *C. albicans* and decreased the toxicity against *E. coli*. There no reports in the literature about the antimicrobial properties of 4-methyl-eugenol. On the other hand, the presence of estragole may also have contributed for the results by synergism with 4-methyl-eugenol due to its reported antimicrobial activity (8).

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