Phytochemical investigation of hexanic extract of the *Melaleuca leucadendra* (l) l. Myrtaceae

Investigação fitoquímica do extrato hexânico da *Melaleuca leucadendra* (l) l. Myrtaceae

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ABSTRACT
Studies on the effectiveness of species of the genus *Melaleuca* (Myrtaceae) against mastitis-causing bacteria, particularly *Staphylococcus aureus*, have focused on the chemical components of the essential oil. The goal of this work was to use GC-MS analysis to characterize the phytochemical composition of the hexane extract from *Melaleuca leucadendra* (L) L. leaves. There was evidence of 37 secondary metabolites, including 2-Hexadecen-1-ol, Nerolidol B (Cis or Trans), Heneicosan, Vitamin E, 3, 7, 11, and 15-Tetramethyl-, and [R-[R*,R*-(E)]]. Squalene, Cyclopropanemethanol, Gamma-Sitosterol, (-)-Caryophyllene Oxide, and Neophytadiene. The plant *Melaleuca leucadendra* (L) L. can be regarded as a source of nonpolar compounds with promise for the treatment of mastitis because of the significance and the wide range of biological activities documented in the literature on the compounds found in this study.

Keywords: Myrtaceae, *Melaleuca leucadendra*, mastitis, hexanic extract, GC-MS.

RESUMO
Estudos sobre a eficácia de espécies do gênero Melaleuca (Myrtaceae) contra bactérias causadoras de mastite, principalmente Staphylococcus aureus, têm se concentrado nos componentes químicos do óleo essencial. O objetivo deste trabalho foi utilizar a análise GC-MS para caracterizar a composição fitoquímica do extrato hexânico das folhas de Melaleuca leucadendra (L) L.. Houve evidência de 37 metabólitos secundários, incluindo 2-Hexadecen-1-ol, Nerolidol B (Cis ou Trans), Heneicosan, Vitamina E, 3, 7, 11 e 15-Tetrametil-, e [R-[R*, RÉ)]]. Esqualeno, Ciclopropanometanol, Gama-Sitosterol, Óxido de (-)-Cariofileno e Neofitadieno. A planta Melaleuca leucadendra (L) L. pode ser considerada uma fonte de compostos apolares promissores para o tratamento da mastite devido à importância e ampla gama de atividades biológicas documentadas na literatura sobre os compostos encontrados neste estudo.

Palavras-chave: Myrtaceae, *Melaleuca leucadendra*, mastite, extrato hexânico, CG-EM.

1 INTRODUCTION

The genus *Melaleuca* belongs to the Myrtaceae family, native to Australia and the Indian Ocean Islands,[1] and has been used in the treatment of infections by *Staphylococcus aureus*, the main etiologic agent of mastitis.[2]

Dairy production in Brazil represents a large portion of agricultural production and mastitis is responsible for considerable losses in this productive sector, mainly with annual expenses with antibiotics due to the emergence of multidrug-resistant strains.[2]

Many reports on activity against *S. aureus* of extracts and oils of *Melaleuca alternifolia* (L) L. are found in the literature, however *M. leucadendra* is poorly studied for this purpose and in relation to chemical compounds of the nonpolar fraction that may represent a source of compounds with therapeutic properties.[3] Therefore, a more comprehensive understanding of the nonpolar components of *Melaleuca* spp., both volatile and fixed fractions, is required.

2 OBJECTIVE

The aim of this study was the phytochemical characterization of hexanic extract from the leaves of *Melaleuca leucadendra* (L) L. using analysis by GC-MS and identify substances with promise for treating mastitis.

3 MATERIALS AND METHODS

3.1 BOTANICAL MATERIAL

Melaleuca leucadendra was collected in the Universidade Federal Rural de Pernambuco, Recife campus, state of Pernambuco, Northeast of Brazil (8°00'59.8"S 34°56'44.3"W). Identification and deposition at PEUFR-Hebário Professor Vascocelos Sobrinho/UFRPE, under PEUFR- n. 56001 and PEUFR- n.56002.

3.2 HEXANIC EXTRACT OBTENTION

Leaves of *Melaleuca leucadendra* were ground in a Willey mill and then 40 mg of the material was subjected to extraction using 2 mL of hexano (HPLC grade - Merck®) for 30 minutes in an ultrasonic bath (Unique brand, model USC-1400). The samples were evaporated in an N_2 gas dryer and 2 mg of the extracts were dissolved in 2 mL of hexano for analysis with gas chromatography coupled to mass spectroscopy (GC-MS).

3.3 CG-MS ANALYSIS

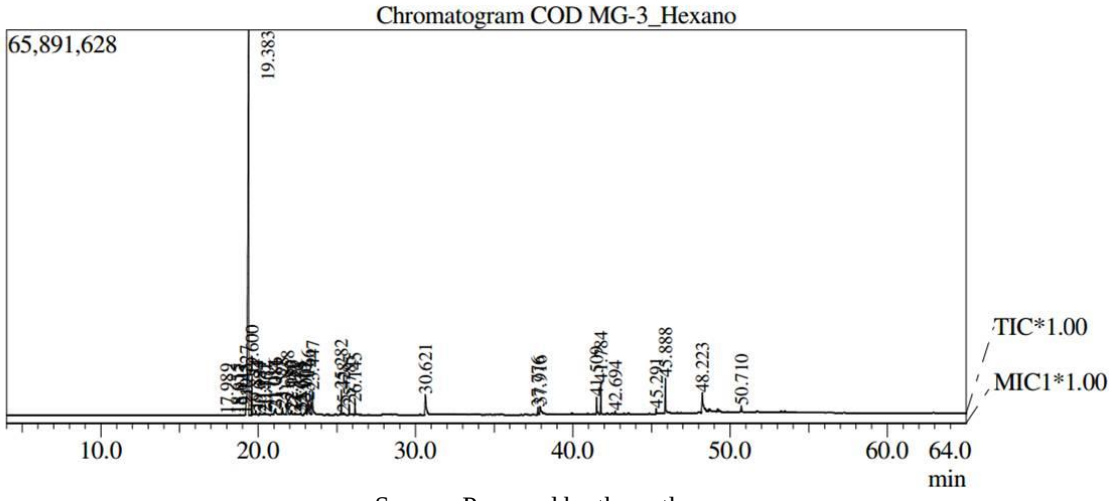
The hexanic extracts from the leaves were analyzed by gas chromatography-mass spectrometry (GC-MS) using a Shimadzu GCMS model QP2010 instrument in a system operated by electron impact (70 eV) and the injector temperature was set at 260 °C with a split ratio of 1:5. A DB5-MS column [30 m × 0.25 mm i.d., film thickness 0.25 µm (5% cross-linked phenyl-methylpolysiloxane)] was used (Agilent J & W GC Columns), with helium as the carrier gas, a column flow of 1.3 mL/min, an injection volume of 1 µL, the injector temperature at 260 °C and pressure of 97.4 kPa. A mixture of (C_9-C_{20},

C_{21}-C_{40}) linear hydrocarbons was injected under the same conditions to identify the components. The spectra obtained were compared with the equipment database (FFNSC1.3.lib, WILEY7.LIB, NIST08s.LIB, MY LIBRARY.lib).

4 RESULTS AN DISCUSSION

In the GC-MS chromatogram (Fig. 1), 37 peaks were found, corresponding to 37 chemical compounds (Tab. 1), that contain antipyretic, analgesic, anti-inflammatory, antibacterial, and antioxidant properties.[1,4] The larger areas were: Nerolidol B (Cis or Trans) (55.84%), that enhances antibiotic activity against Staphylococcus aureus and Escherichia coli, [5] antileishmanial Activity; [6] Heneicosane (42,69%), that possesses antimicrobial activity; [7] Vitamin E (4.44%), a powerful antioxidant; [8,9,10] 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- (4.53%), with anticancer activity reports; [11,12,13]gamma-sitosterol (3.79%), It has anti-diabetic properties; [14] Cyclopropanemethanol (3.51%), anti-amoebic agents[15] and features of anesthetic; [16] Squalene (3.36%), It has been shown to have antioxidative action, anti-neurodegenerative disease activity, immune system potentiation, anti-hepatic steatosis activity, and antimutagenic activity; [17,18] (-)-Caryophyllene oxide (3.21%), which has anti-inflammatory and analgesic properties and is effective against onychomycosis[19,20] and Neophytadiene (2.79%), which exhibits antibacterial properties.[21, 22]

Fig. 1 - GC-MS chromatogram of the hexanic extract from the leaves of *Melaleuca leucadendra* (L.) L.



Source: Prepared by the authors

Tab. 1- Compounds identified in the hexanic extract from the leaves of *Melaleuca leucadendra* (L) L. by GC-MS

Peak	Retention time (min.)	Area%	Compound
1	17.989	0.04	Farnesol
2	18.655	0.06	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethy (MW222) C_{15} $H_{26}O$
3	18.817	0.10	Bisabolol oxide A - (MW238) C_{15} $H_{26}O_2$
4	19.145	0.01	Epoxy-inalooloxide (MW186) $C_{10}H_{18}O_3$
5	19.227	0.90	Citronellylacetone - (MW196) $C_{13}H_{24}O$
6	19.383	55.84	Nerolidol B (Cis or Trans) (MW222) $C_{15}H_{26}O$
7	19.600	3.51	Cyclopropanemethanol, .alpha.,2-dimethyl-2-(4-methyl-3 (MW182) $C_{12}H_{22}O$
8	19.854	0.59	(-)-Caryophyllene oxide (MW220) $C_{15}H_{24}O$
9	20.084	0.15	Veridiflorol (MW222) $C_{15}H_{26}O$
10	20.372	0.20	Nerolidol-Epoxyacetate (MW296) $C_{17}H_{28}O_4$
11	20.461	0.10	Humulene epoxide II (MW220) $C_{15}H_{24}O$
12	21.014	0.46	Citronellylacetone (MW196) $C_{13}H_{24}O$
13	21.086	0.72	alpha.-Bisabolol (MW222) $C_{15}H_{26}O$
14	21.528	0.92	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl- (MW222) $C_{15}H_{26}O$
15	21.858	2.29	(-)-Caryophyllene oxide (MW220) $C_{15}H_{24}O$
16	22.089	0.25	alpha.-Bisabolol (MW222) $C_{15}H_{26}O$
17	22.136	0.31	alpha.-Bisabolol (MW222) $C_{15}H_{26}O$
18	22.321	0.15	Octadecanoic acid, 2-oxo-, methyl ester (MW312) $C_{19}H_{36}O_3$
19	22.679	0.20	2,7-Octadiene-1,6-diol, 2,6-dimethyl- (MW170) $C_{10}H_{18}O_2$
20	22.915	0.23	Nerolidol (MW222) $C_{15}H_{26}O$
21	23.002	0.51	(-)-Caryophyllene oxide (MW220) $C_{15}H_{24}O$
22	23.166	2.37	Nerolidol A (Cis or Trans) (MW222) $C_{15}H_{26}O$
23	23.447	3.21	(-)-Caryophyllene oxide (MW220) $C_{15}H_{24}O$
24	25.282	2.79	Neophytadiene (MW278) $C_{20}H_{38}$
25	25.428	0.21	2-Pentadecanone, 6,10,14-trimethyl- ((MW268) $C_{18}H_{36}O$
26	25.785	0.92	3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Phytol Trans) (MW296) $C_{20}H_{40}O$
27	26.145	1.23	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- (MW296) $C_{20}H_{40}O$
28	30.621	4.53	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- (Phytol) (MW296) $C_{20}H_{40}O$
29	37.776	0.85	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (MW390) $C_{24}H_{38}O_4$
30	37.916	1.40	Flemichapparin (MW270) $C_{16}H_{14}O_4$
31	41.509	1.69	Decanedioic acid, bis(2-ethylhexyl) ester (MW426) $C_{26}H_{50}O_4$
32	41.784	3.36	Squalene (MW410) $C_{30}H_{50}$
33	42.694	42.69	Heneicosane (MW296) $C_{21}H_{44}$
34	45.291	0.60	Hexacosane (MW366) $C_{26}H_{54}$
35	45.888	4.44	Vitamin E (MW430) $C_{29}H_{50}O_2$
36	48.223	3.79	gamma.-Sitosterol (MW414) $C_{29}H_{50}O$
37	50.710	0.78	Phytol acetate (MW338) $C_{22}H_{42}O_2$

Source: Prepared by the authors

5 CONCLUSIONS

In the present study, chemical constituents that have activity against *Staphylococcus aureus* proven in the literature were evidenced, such as: Nerolidol B (Cis or Trans), Heneicosane, Neophytadiene, among others. Thus, the results will serve as a

basis for future studies aimed at biomonitored fractionation for the isolation of apolar compounds effective in the treatment of mastitis.

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