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Antimicrobial activity of Lippia lacunosa and Lippia rotundifolia essential oils.

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The genus Lippia (Verbenaceae) comprises ca. 200 species occurring mainly in Central and South America and also in some areas of Tropical Africa. One of the main diversity centers of the genus is located in the State of Minas Gerais, Brazil. As part of our continuing study on Brazilian Lippia species, L. lacunosa Mart. & Schauer and L. rotundifolia Cham. were selected for investigation. Both form a complex of very difficult taxonomic delimitation. Additionally, no previous studies concerning their chemistry or biological activity have been published. Fresh leaves and flowers of L. lacunosa and L. rotundifolia, cultivated, from original clones brought from Diamantina (MG - Brazil), were collected at the campus of Federal University of Juiz de Fora, Brazil. Their essential oils were obtained separately by hydrodistillation in a Clevenger-type apparatus for 2 hours. Essential oils analyses were performed in a HP 5890 gas chromatograph equipped with a FID detector and a HP5 fused silica capillary column (30mX 0.25mmX0.25μm), using H₂ as carrier gas. The oven temperature was programmed from 60 to 240°C at 3°C/min. The constituents of the oils were identified by comparing their mass spectra with those in a spectral database (Wiley 6th ed) and by their retention indices (RI). The antimicrobial assay was carried out using the drop agar diffusion method. The microorganisms tested were the fungi Candida albicans Serotype B (ATCC 36802), C. albicans (ATCC 2949) and the bacteria Escherichia coli and S. aureus MRSA (BMB9393). Microorganisms were spread over Petri dishes containing solid medium and, after 10 minutes, a µL drop of the essential oil diluted 1:1 with Tween 80 was placed in the center of each plate. Reference antibiotics were: amphotericin B, methicillin and vancomycin. Plates were incubated at 37°C (incubation time depending on the microorganism tested), after which the diameter (mm) of the inhibition zone was measured. The major components of the essential oils of flowers and leaves of L. lacunosa were myrcene (14.7% and 11.9%), myrcenone (45.2% and 64.2%), (Z)-ocimenone (5.7% and 5.2%), and (E)ocimenone (14.7% and 4.1%), respectively; whereas in the essential oils of flowers and leaves of L. rotundifolia those were α -pinene (8.7% and 1.8%), myrcene (5.1% and 3.6%), limonene (26.0% and 7.9 %), cis-pinocamphone (4.5% and 3.1%) and myrtenal (22.3% and 16.7%), respectively. All the assayed essential oils were active against the microorganisms assayed (bacteria and fungi), with inhibition haloes ranging from 9 to 25 mm. Fractionation of L. lacunosa leaves essential oil by silica gel column chromatography afforded pure myrcene and myrcenone (99% purity by GC). Myrcenone was assayed by the drop test (diluted 1:2 in DMSO and then 1:2 in water) against the same microorganisms, with inhibition haloes of 8, 7, 10 and 10 mm, respectively.