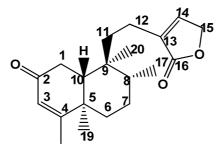
ANTIMICROBIAL CLERODANE DITERPENE FROM THE LEAVES OF *Echinodorus scaber* RATAJ.

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E. scaber Rataj. (Alismataceae) known as "chapéu de couro" is used in Brazilian folk medicine mainly as anti-inflammatory, antirheumatic and diuretic. Chemical studies reported the presence of clerodane and labdane diterpenes as well as flavonoids as the main constituents of this specie [1]. The scientific literature presents many reports about the pharmacological properties of E. scaber extracts/preparations[1]. Antimicrobial and antinflamatory properties have been previously reported to clerodane/abietane-like diterpenes [2]. The aim of this work is to carry out antimicrobial assays and to isolate and identify diterpenes from the leaves of E. scaber. The leaves (150.00g) were extracted with methanol (7x500mL) to afford the crude methanolic extract (ESME; 27.52g; 18.34 %). The ESME was suspended in MeOH/H₂O (1:1; 500 mL) solution and extracted with CHCl₃ (3x500 mL). The CHCl₃ fraction (2.0 g; 7,44%) was chromatographed in sílica gel 60 (70-230 mesh) column, affording 5 fractions (A-E). Fraction D (450 mg; 22.5 %) afforded an impure majoritary compound which was submitted to column chromatography on sephadex LH-S 20, yielding the clerodane diterpene 1 (350mg; 13.5%). The identification was carried out through ¹H and ¹³C NMR analysis (uni and bidimensional) and the ¹H NMR data is in agreement with the literature [3]. The antimicrobial assays were performed employing the disc diffusion method (Bauer et al. 1966), with the following pathogens: Escherichia coli (ATCC 25928), Staphylococcus aureus (ATCC 25923), Pseudomonas aeruginosa and Escherichia coli ESBL+. Tetracycline (5 mg/mL) was used as a positive control, and pure MeOH was the negative control. The clerodane diterpene 1 was effective, inhibiting pathogens E. coli (2 cm) and S. aureus (3 cm) when assays were performed at a concentration of 20 ug/ml of 1. E. coli and P. aeruginosa were multidrug-resistant, with zone of inhibition lower than those obtained with tetracycline (1.5 cm). Therefore the compound 1 was considered active against the pathogens tested.



15-hydroxy-2-oxo-kolavenic-16-oic acid lactone (1).

References

- [1] Tanus-Rangel, E., Santos, SR., Lima, JC., Lopes, L., Noldin, V., Monache, FD., Cechinel-Filho, V., Martins, DT. 2010. Topical and systemic anti-inflamatory effects of *E. macrophyllus*. J. of Med. Food. 5: 1161-1166.
- [2] Zhang, J., Rahman, A. R., Jain, S., Jacob, M. R., Khan, S., Tekwani, B. L., Muhammad, I. 2012. Antimicrobial and antiparasitic diterpenoids. Res. Rep. Med. Chem. 2:1–6.
- [3] Bohlmann, F., Gupta, R. K., King, R. M., Robinson, H. 1980. Diterpenes and Tetranorditerpenes from *Acritopappus* species. Phytochem. 19: 2695-2705.