Micromorphological, phytochemical profile and antibacterial evaluation of two Rutaceae species

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INTRODUCTION

Throughout their evolution plants have developed several functional and metabolic mechanisms to survive. One of the major adaptations is the biosynthesis of a large diversity of secondary metabolites which includes terpenic compounds, alkaloids, flavonoids and phenolics, among others. The objective of this work was to carry out preliminary studies on the micromorphology, the phytochemical profile and the antibacterial activity of two Rutaceae species, *Zanthoxylum zanthoxyloides* and *Zanthoxylum leprieurii*.

Zanthoxylum zanthoxyloides

Zanthoxylum leprieurii

RESULTS AND DISCUSSION

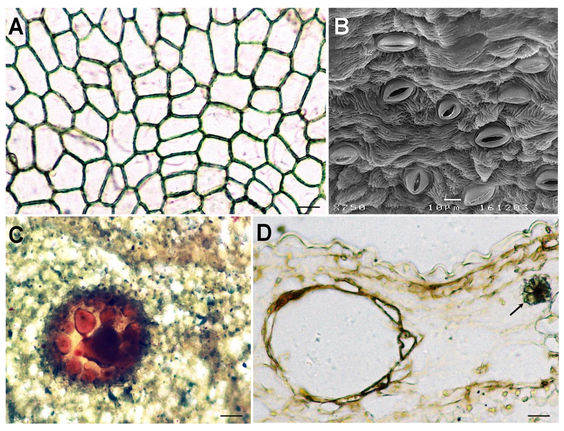


Fig. 1 Micrographs of foliar epidermal surfaces of *Z. zanthoxyloides* (A and C) and *Z. leprieurii* (B). A - adaxial epidermal cells (scale bar 15 µm); B - abaxial epidermal cells and stomata; C - adaxial surface view of a secretory structure with lipidic content (Sudan III histochemical test), (scale bar 10 µm); D - *Z. leprieurii* leaf cross section with a large internal secretory structure and a druse calcium oxalate crystal (arrow), (scale bar 12 µm).

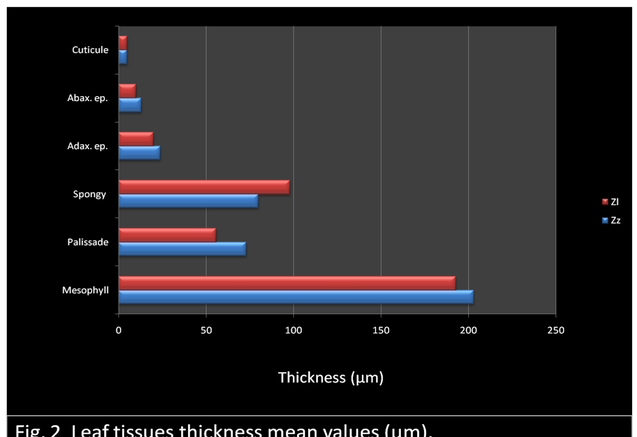
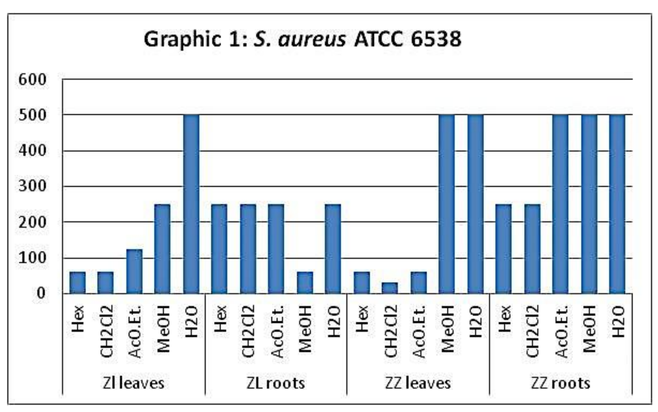


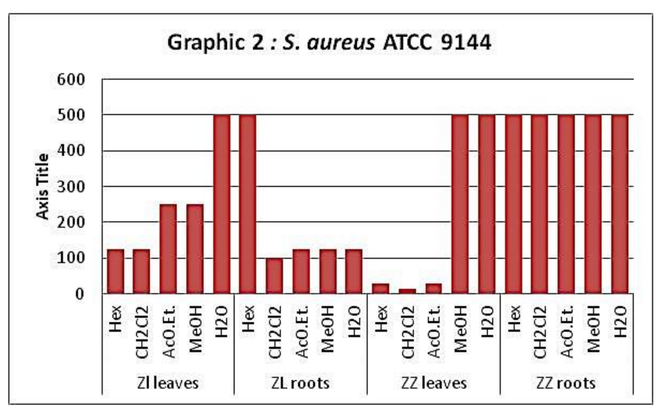
Fig. 2 Leaf tissues thickness mean values (µm).
(Zl, *Z. leprieurii; Zz, Z. zanthoxyloides;* Adax. ep., Adaxial epidermis; Abax. ep., Abaxial epidermis).

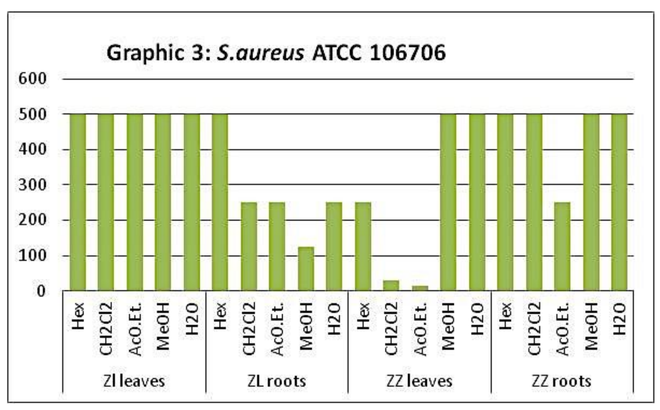
The leaves of both species show similar microcharacters: i) polyhedral epidermal cells on the adaxial (Fig. 1A) and abaxial surfaces; ii) hypostomatic leaves (Fig. 1B); iii) internal secretory structures with lipidic content (Fig. 1C). Considering the foliar anatomy some differences were found (Fig. 2): the mesophyll and the palissade parenchyma is thicker in *Z. zanthoxyloides;* the spongy parenchyma is higher in *Z. leprieurii;* the internal secretory structures might occupy the entire mesophyll in *Z. leprieurii;* idioblasts with druse calcium oxalate crystals are common in *Z. leprieurii* parenchyma cells (Fig. 1D). Both species show no significant differences in the thickness of adaxial and abaxial epidermal tissues as well as in the cuticle (Fig. 2).

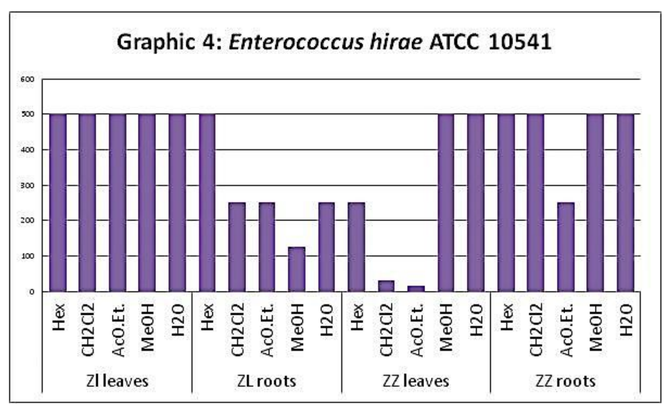
Table 1: Phytochemical results

Plant	Part	Extract	Alkaloids	Terpenes	Flavonoids	Phenolics
Zanthoxylum zanthoxyloides	leaves	*n*-Hexane	—	+++	++	—
		CH_2Cl_2	—	++	+++	—
		AcOEt	—	++	+++	—
		MeOH	—	—	+	++
		H_2O	—	—	+	++
	roots	*n*-Hexane	+	++	—	—
		CH_2Cl_2	+	++	—	+
		AcOEt	+	++	—	+
		MeOH	+	+	+	+++
		H_2O	+	—	+	+++
Zanthoxylum leprieurii	leaves	*n*-Hexane	—	+++	++	—
		CH_2Cl_2	—	++	++	—
		AcOEt	—	++	+++	++
		MeOH	—	—	+++	++
		H_2O	—	—	+	+
	roots	*n*-Hexane	+	++	+++	—
		CH_2Cl_2	+	++	+++	+
		AcOEt	++	++	+++	+
		MeOH	++	—	++	++
		H_2O	+	—	+	+









As can be observe on graphics 1-4, the best results were obtained with *Z. zanthoxyloides* non-polar leaves extracts that presented the lowest MIC values (7,5-30 µg /mL) against the Gram-positive *S. aureus* and *E. hirae* strains. This may be related to the high content of terpenes and flavonoids detected in those extracts (Table1.). None of the tested extracts was active against the Gram negative strains.

MATERIALS AND METHODS

Plant Material: Plant material was collected in the Bijagós archipelago, Guinea-Bissau, during 2016. Plant vouchers are housed at the Herbarium of the University of Lisbon (LISC). Plant manipulation followed the usual methods for microscopy observations (1). For anatomical studies the plant material was processed with the paraffin micro technique (2).

Preparation of Extracts: The plant extracts were obtained by a sequential extraction of the dry plant powder with 100mL of *n*-hexane, CH_2Cl_2, EtOAc, MeOH and water. The extracts were filtered and concentrated in a rotary evaporator and stored at -20ºC.

Evaluation of the phytochemical profile: Semi quantitative phytochemical analysis was carried out thought TLC on silica gel. developed with appropriated mixtures of solvents. Spots were revealed with appropriated revelators. prepared according to Wagner and Blader (1996). Results were displayed semi quantitatively in a range between absence (−) and strongly present(+++).

Screening for Antimicrobial Activity: Reference bacteria: *S. aureus* (ATCC 6538, 9144, CIP 106706), *E. hirae* (ATCC 10541), *P. aeruginosa* (ATCC 9027) and *E. coli* (ATCC 8739). The minimum inhibitory concentrations (MIC) were determined by the serial broth microdilution method (3). The MIC values were considered negative when > 100 µg/mL.

References

(1)Teixeira, G.; Monteiro, A. &Pepo, C. (2008) - Leaf morphoanatomy in *Hakea sericeae* and *H. salicifolia*. *Microscopy and Microanalysis*, vol. 14, p. 109-110. (2) Ruzin, S.E. (1999) -*Plant microtechnique and microscopy.* 1st ed.New York, Oxford University Press. (3) Clinical and Laboratory Standards Institute, Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard, Document M27-A3, CLSI, Wayne, Pa, USA, 3rd edition, 2008.

These are preliminary results that point to the validation of the use of these plant species in traditional medicine and emphasize the worthwhile of additional studies.