Experimental design applied in UPLC-DAD method development of Malpighiaceae species extracts and metabolite identification

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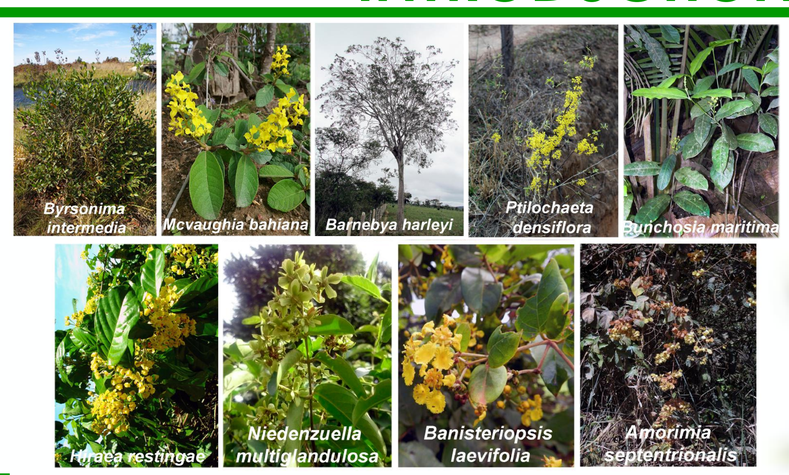
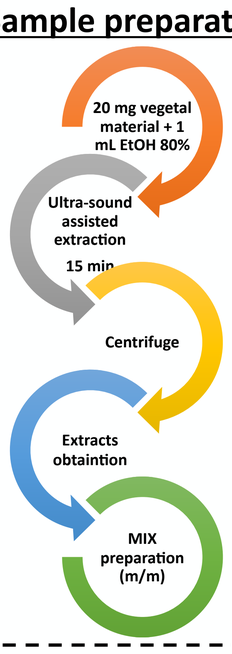
INTRODUCTION

Malpighiaceae species present important biological activities, such as cytotoxic, hallucinogen, AChE inhibition and antifungal[1-3]. However, this family can still be considered chemically underexplored. Therefore, our goals were to evaluate the chemical diversity of Malpighiaceae species extracts by preparing a representative MIX of nine species from diferente phylogenetic groups found in Brazil (Figure 1)[4]. An experimental design for UPLC-DAD method development was applyed with the assist of na automated tool, which assisted in the identification of their secondary metabolites by UPLC-QToF and Molecular Networking algorithm.



METHODOLOGY

Sample preparation



UPLC method development

Parameters – abrupt variations
- ✓ **6 columns:** Cortecs C8; Cortecs C18+; Acquity HSS T3; Acquity HSS PFP; Acquity BEH C18; Acquity BEH Shield C18
- ✓ **3 pHs:** 2.54 (H_2O + 0.1% HCOOH v/v); 6.60 (H_2O); 10.45 (H_2O + 0.1% NH_4OH v/v)
- ✓ **2 organic solvents:** ACN; MeOH
- ✓ **2 gradient times:** 15 min; 30 min

Matrix with 57 runs
Quadratic model – A- and G- optimal

Screening → Optimization → Robustness simulator

Parameters – slight variations (1st)
- ✓ **3 pHs:** 2.26 (H_2O + 0,4% HCOOH v/v); 2.54 (H_2O + 0.1% HCOOH v/v); 3.07 (H_2O + 0.013% HCOOH v/v)
- ✓ **2 temperatures:** 35 ℃; 45 ℃
- ✓ **Final % organic solvent:** 60%; 80%
- ✓ **ACN % in organic solvent:** 70%; 100%
- ✓ **Constant:** Acquity BEH Shield C18; ACN; 30 min

Matrix with 30 runs

Parameters – slight variations (2nd)
- ✓ **Final % organic solvent:** 40%; 70%
- ✓ **ACN % in organic solvent:** 60%; 100%
- ✓ **Constant:** Acquity BEH Shield C18; ACN; 30 min; 45 ℃; pH 2.26

Matrix with 11 runs
Quadratic model – Central composite

Responses selected for each step:
- ✓ Nº of total peaks
- ✓ Nº of peaks with resolution ≥ 1.5
- ✓ Nº of peaks with resolution ≥ 2.0
- ✓ Nº of peaks with tailing ≤ 1.2

Compounds identification

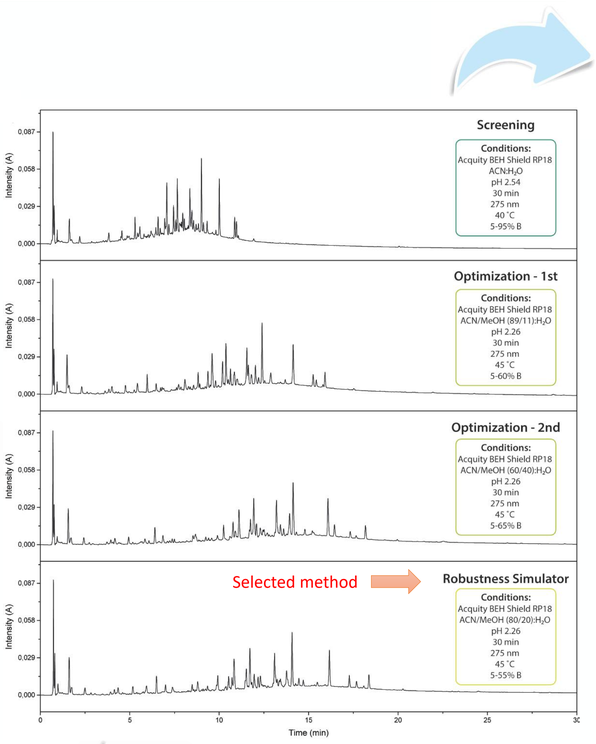
UPLC method developed

UPLC-QToF analysis – MIX and separated extracts

Compounds identification by Molecular Networking algorithm and library search

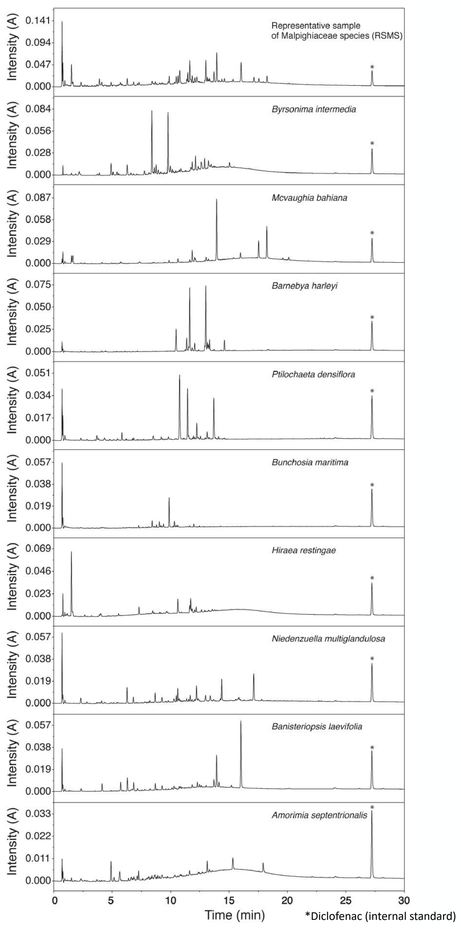
RESULTS

• UPLC-DAD method development



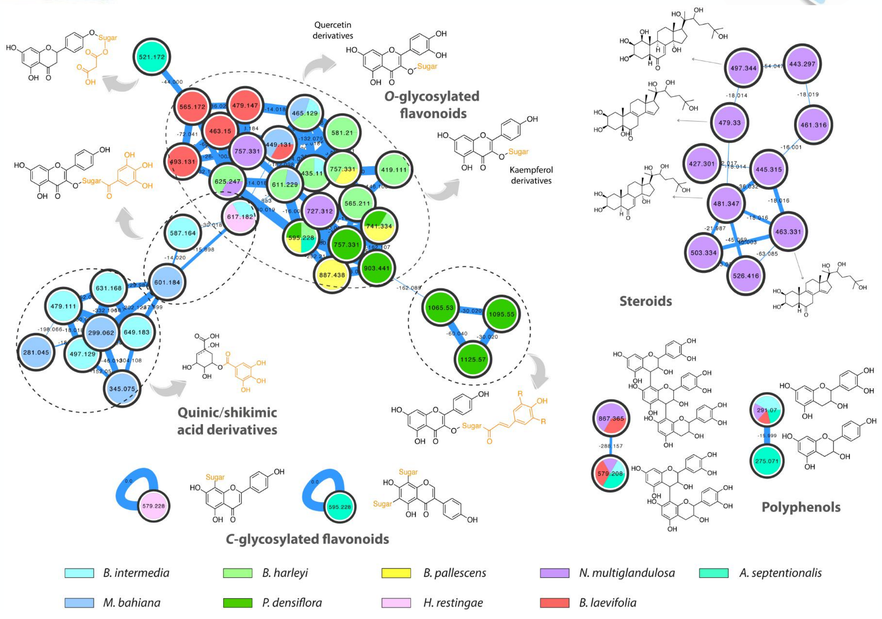
Method validation

MIX and 9 samples separately



• UPLC-QToF analyses – metabolites annotation

Developed chromatographic method → Untargeted MS/MS analysis → GNPS Molecular Networking algorithm → **61 compounds annotated**



CONCLUSIONS

In conclusion, the experimental design allowed the development of a precise and robust chromatographic method both for the quantitative analysis and metabolomic profiling of Malpighiaceae species. Phenolic, steroids and alkaloids compounds, along with several unknown structures were annotated.

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