Selection of Three-Phase Solvent System for Countercurrent Chromatography – A Practical Guide Using *Syzygium malaccense* Leaves Extract as an Example

Aline Gomes Lopes,[a,b,*] Fernanda das Neves Costa[a]

[a] Universidade Federal do Rio de Janeiro, CCS, Instituto de Pesquisas de Produtos Naturais, Bloco H, Ilha do Fundão, CEP 21941-590, Rio de Janeiro-RJ, Brasil.

[b]Instituto Federal de Educação, Ciência e Tecnologia do Estado de Rondônia, *Campus* Cacoal, Km 228, Lote 2A, BR-364, Zona Rural, CEP 76960-970, Cacoal-RO, Brasil.

[*]aline.gomes@ifro.edu.br

Supplementary Material

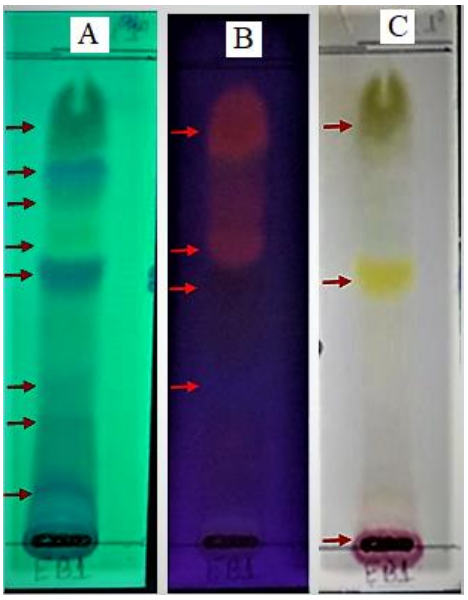

Fig. S1. Thin-layer chromatography plate with *Syzygium malaccense* crude extract. The plate was eluted with ethyl acetate–acetone–water, 25:15:10 (*v/v*). **A**: plate visualized under UV light (UV-254 nm). **B**: plate visualized under UV light (UV-365 nm). **C**: plate with the chemical developers, using spray-reagent H_2SO_4 10% in ethanol and vanillin 10% in ethanol before heating in a hot plate

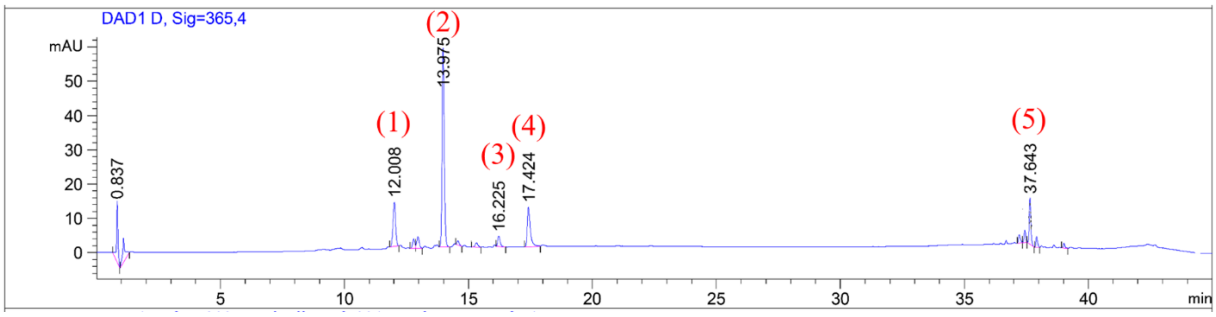

Fig. S2. *Syzygium malaccense* crude extract chromatographic profile was made by HPLC-DAD. The gradient was programmed as follows: 0 min, 5% B; 30 min, 50% B; 33 min, 100% B; 37 min, 100% B; 40 min, 5% B; and 45 min 5% B

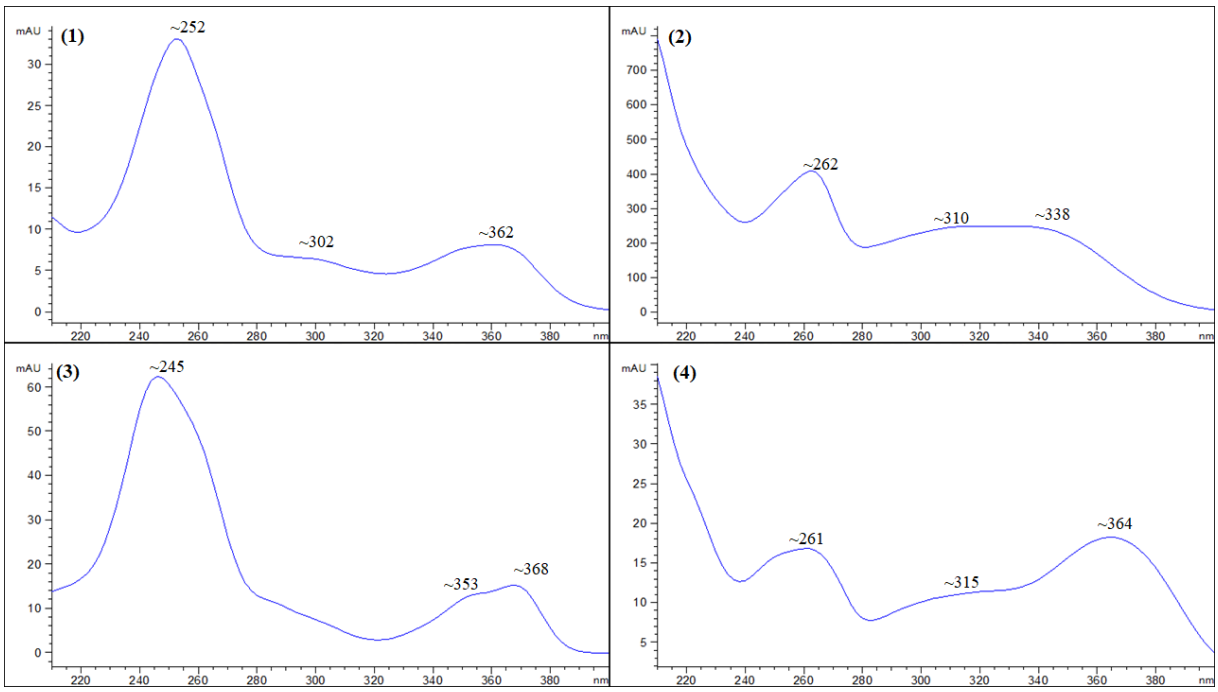

Fig. S3. UV spectra λ = 365 nm to four major peaks obtained by HPLC-DAD

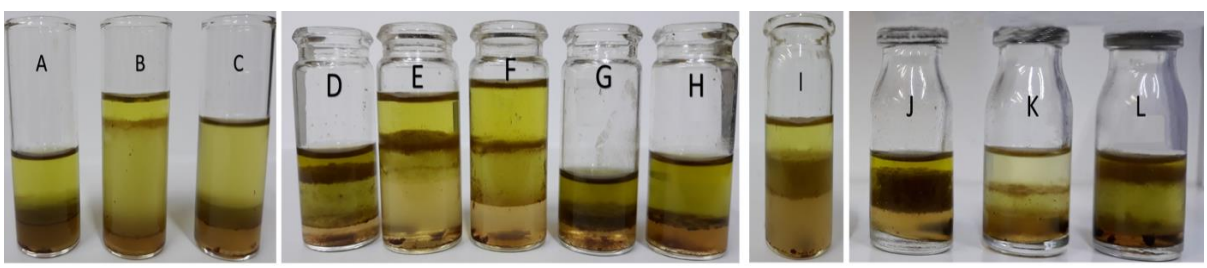

Fig. S4. TPSSs **A–C** belong to the same group of solvents *n*-hexane–ethyl acetate–acetonitrile–water, volume ratio (*v/v*) **A**: 2:1:1:1, **B**: 2:2:3:2 and **C**: 3:1:1:1. **D–H** belong to the same group of solvents *n*-hexane–methyl t-butyl ether–acetonitrile–water, volume ratio (v/v) **D**: 1:1:2:1; **E**: 2:1:3:2; **F**: 2:2:3:2, **G**: 2:3:3:2 and **H**: 3:5:5:3. **I**: *n*-hexane–dichloromethane–acetonitrile–water, 5:1:5:5 *v/v*