Isolation and identification of acylphloroglucinols in the seedlings of the medicinal plant, *Melaleuca alternifolia* (Australian tea tree)

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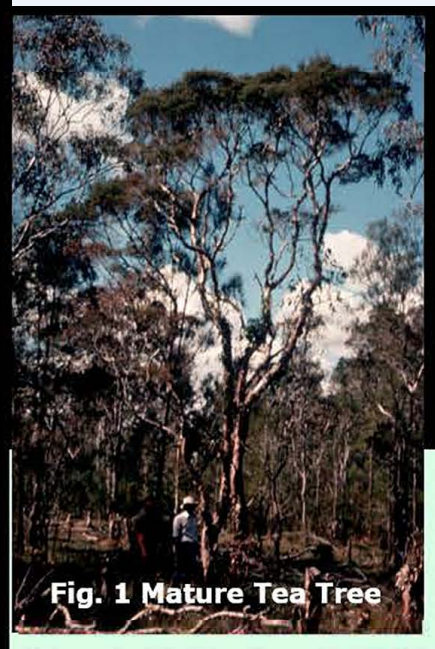

Fig. 1 Mature Tea Tree

Introduction

Melaleuca alternifolia (Australian tea tree) is a popular antiseptic medicinal plant. While the volatile oils have been well-researched, the low-volatile compounds have been overlooked[1,2]. Our aim was to purify and identify four compounds in tea tree (Fig. 1) seedling leaf (Fig. 2).

Fig. 2 Tea tree seedling

Materials & methods

Methanolic extracts of tea tree seedlings when subject to GC-MS (Hewlett Packard HP6890 GC-system coupled with a Hewlett- Packard 5973 mass selective detector) and prep-HPLC-MS (Agilent 1260 Infinity preparative HPLC, coupled with a 1260 binary preparative pump, using a Phenomenex Luna C18 column (150 mm x 21.20 mm x 5 µm)) separated 4 acylphloroglucinols (Fig. 3). Structures were elucidated by accurate mass and a Bruker Avance III HDX 800 MHz nuclear magnetic resonance (NMR) spectrometer equipped with a TCI probe, in CD_3OD observed at 200 MHz for ^{13}C and 800 MHz for ^{1}H. Bruker TopSpin 4.0.7 software was used to analyse ^{13}C, ^{1}H, Heteronuclear Single Quantum Coherence (HSQC), ^{1}H-^{1}H Correlation Spectroscopy (^{1}H-^{1}H-COSY) and Heteronuclear Multiple Bond Correlation (HMBC) (Fig. 5) to determine structures for comparison with the literature.

Results Four acylphloroglucinols were isolated from Australian tea tree seedlings:

- 1-(2,6-dihydroxy-4-methoxy-3-methylphenyl) -2-methylpropan-1-one 1 (**1**)
- Callisalignone A [3] (**2**)
- 1-(2,6-dihydroxy-4-methoxyphenyl)-3-methylbutan -1-one [4] (**3**)
- Pulverulentone B[1] syn. Aspidinol D[5] (**4**)

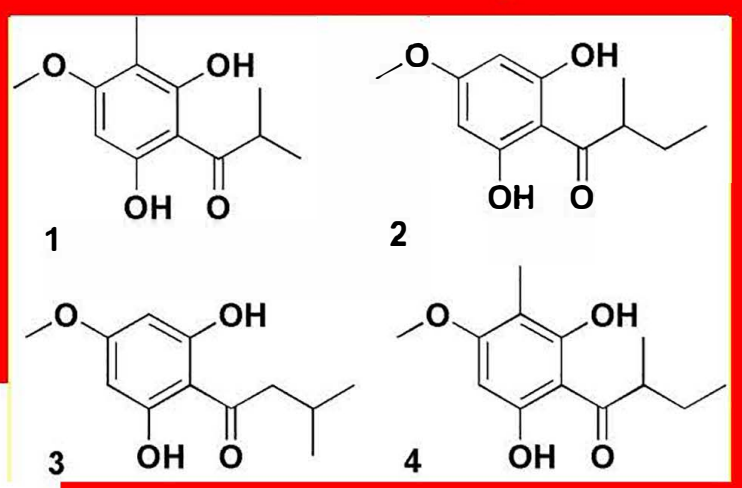

Fig. 3 Structures of tea tree seedling isolates

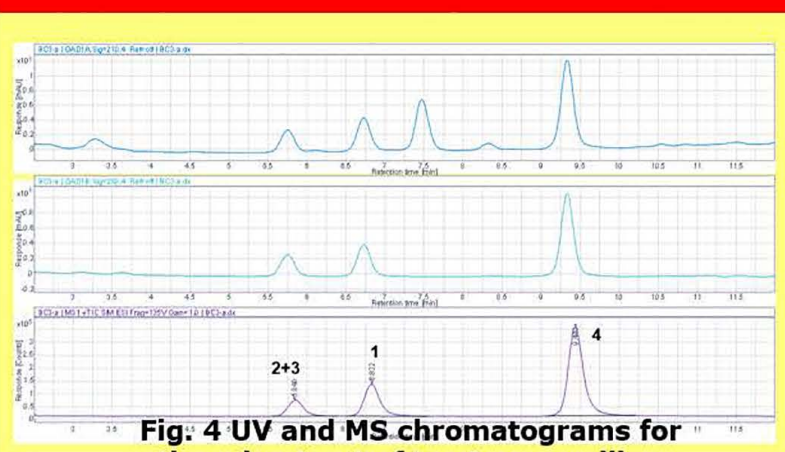

Fig. 4 UV and MS chromatograms for ethanol extract of tea tree seedlings.

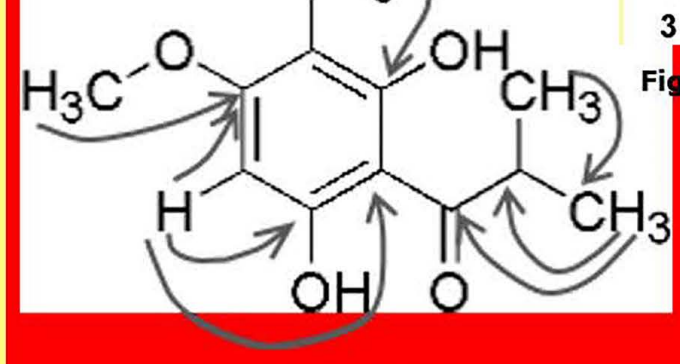

Fig. 5 HMBC correlations for (1)

Conclusions

This is the first time acylphloroglucinols have been isolated from Australian tea tree.

These compounds have previously been found to possess antifungal,[3] antinociceptive,[4] antibacterial,[5] and plant growth regulatory activity.[6]

Should similar bioactivity be found in *M. alternifolia*, then they provide a wealth of opportunities for the commercial development of new tea tree industries.

Currently we are looking for volatile compounds in fresh tea tree leaf using LCQTOF.

Literature cited: **1** Brophy, J.J. et al. J. Agric. and Food Chem. 1989, **37**, 1330-1335. **2** I.A. Southwell and R.F. Lowe (eds.), Tea Tree, the Genus *Melaleuca.* Vol 9 in Series Medicinal and Aromatic Plants - Industrial Profiles (ed. R.Hardman), Harwood Academic Publishers, Amsterdam, 1999. **3** Qin, X.-J et al.; Tetrahedron 2017, 73 (14), 1803-1811, **4** Radulović, N. S. et al.; Food Res. Int. 2015, 77, 280-289, **5** Wang, W. et al.; J. Nat. Prod. 2010, 73 (11), 1815-1820, **6** Bolte, M. L. et al.; Agr Biol Chem 1984, 48 (2), 373-376.

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