SHORT COMMUNICATION

Bioactivity and chemical characterisation of *Lophostemon suaveolens* – an endemic Australian Aboriginal traditional medicinal plant

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*Lophostemon suaveolens* is a relatively unexplored endemic medicinal plant of Australia. Extracts of fresh leaves of *L. suaveolens* obtained from sequential extraction with *n*-hexane and dichloromethane exhibited antibacterial activity in the disc diffusion and MTT microdilution assays against *Streptococcus pyogenes* and methicillin sensitive and resistant strains of *Staphylococcus aureus* (minimum bactericidal concentration, 63 \(\mu\)g/mL). The dichloromethane extract and chromatographic fractions therein inhibited nitric oxide in RAW264.7 murine macrophages (IC\(_{50}\) 3.7–11.6 \(\mu\)g/mL) and also PGE\(_2\) in 3T3 murine fibroblasts (IC\(_{50}\) 2.8–19.7 \(\mu\)g/mL). The crude *n*-hexane, dichloromethane and water extracts of the leaves and chromatographic fractions from the dichloromethane extract also showed modest antioxidant activity in the ORAC assay. GC–MS analysis of the *n*-hexane fraction showed the presence of the antibacterial compounds aromadendrene, spathulenol, \(\beta\)-caryophyllene, \(\alpha\)-humulene and \(\alpha\)-pinene and the anti-inflammatory compounds \(\beta\)-caryophyllene and spathulenol. Fractionation of the dichloromethane extract led to the isolation of eucalyptin and the known anti-inflammatory compound betulinic acid.

**Keywords:** apple gum; antimicrobial; *Staphylococcus aureus*; anti-inflammatory; antioxidant

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1. Introduction

*Lophostemon suaveolens* (Sol. ex Gaertn.) Peter G. Wilson & J.T. Waterh. (family Myrtaceae), also known as apple gum, has been used in northern New South Wales, Australia as a medicinal plant. According to first-hand reports of a respected Yaegl Elder of northern New South Wales (uncle Ron Heron), ash from the bark has been used as an antiseptic powder, and the milky sap has been used as an antiseptic face wash for acne and skin blemishes (Packer et al. 2012). Aligned with these traditional uses, we undertook chemical and biological investigations of *L. suaveolens*, with a focus on antibacterial, antioxidant and anti-inflammatory effects. Leaves for this study were selected on the advice of uncle Heron, as the milky sap was not readily accessible in quantities required for investigation.

2. Results and discussion

Fresh *L. suaveolens* leaves were extracted sequentially with *n*-hexane, dichloromethane, ethyl acetate and methanol, following standard natural products research methods (Houghton & Raman 1998). A water extract of the leaves was separately prepared to mimic common customary preparation methods of Australian Aboriginal people (Packer et al. 2012). Each extract was tested for antimicrobial activity, nitric oxide (NO), tumour necrosis factor-alpha (TNF-α) and prostaglandin E2 (PGE2) inhibitory activities and antioxidant activity. Antimicrobial screening was conducted against *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Salmonella* ser. *typhimurium* and methicillin sensitive and resistant strains of *Staphylococcus aureus* (Table S1) using disc diffusion and MTT-microdilution assays (Appendino et al. 2008). The *n*-hexane extract showed good bactericidal activity against all strains of *S. aureus* (31.25 μg/mL) and *S. pyogenes* (15.62 μg/mL) in the MTT assay and the dichloromethane extract showed potent bactericidal activity against all strains of *S. aureus* (1.9 μg/mL) and good bactericidal activity against *S. pyogenes* (62.5 μg/mL). The ethyl acetate extract showed moderate activity in only the disc diffusion assay against *S. aureus* and the methanol extract was inactive. The *n*-hexane and dichloromethane extracts also inhibited the synthesis of NO in LPS-stimulated RAW264.7 murine macrophages (Shou et al. 2012) in a dose dependent manner (Table S2, Figure S1), with the dichloromethane extract most promising (IC50 4.6 μg/mL) with a selectivity index of 2.1. None of the extracts showed any activity in the TNF-α or PGE2 inhibitory assays in stimulated 3T3 cells (Shou et al. 2012). Antioxidant activity of the extracts using the ORAC assay (Shou et al. 2012) was modest in comparison with the well-known antioxidant epicatechin (Table S3). Even the water extract, which showed the highest antioxidant activity, was an order of magnitude lower than pure epicatechin.

The *n*-hexane extract was analysed by GC–MS (Table S4 and Figure S2). The major constituents were aromadendrene (15.4%), spathulenol (12.46%), *allo*-aromadendrene (7.04%), globulol (4.47%), *trans*-calamene (3.15%), epiglobulol (2.69%), β-caryophyllene (2.53%), α-humulene (1.52%) and ledol (1.22%). Aromadendrene, spathulenol, globulol and β-caryophyllene have been previously identified in the essential oils of the leaves of *L. suaveolens* (Brophy et al. 2000). Aromadendrene (Dorman & Deans 2000), spathulenol (Bougatsos et al. 2004), β-caryophyllene (Ozturk et al. 2009), α-humulene (Dorman & Deans 2000) and α-pinene (Dorman & Deans 2000; Inouye et al. 2001) have been reported to have good antibacterial properties. β-Caryophyllene (Tung et al. 2008) and spathulenol (Ziae et al. 2011) have also been reported as having anti-inflammatory activity. Thus, the antibacterial and anti-inflammatory activities of the *n*-hexane extract are likely to be associated with the high content of these bioactive compounds.

Normal-phase silica gel column chromatography of the dichloromethane extract afforded 12 fractions (LSL-1–12, in order of elution, i.e. increasing polarity). Further chromatographic separation led to the isolation of eucalyptin (1) and betulinic acid (2) (Figure S3). All spectral
data were in agreement with the literature (Huq & Misra 1997; Shu et al. 2012). Betulinic acid has been reported to possess anti-inflammatory activity (Tsai et al. 2011; Viji et al. 2011). Fractions LSL-4, 5, 10, 11 and 12 were all bactericidal against *S. pyogenes* and the strains of *S. aureus* (Table S1), with LSL-10, LSL-11 and LSL-12 being most promising. Fractions LSL-5, 7, 10, 11 and 12 were tested for NO, TNF-α and PGE$_2$ inhibitory activity. All the fractions showed good inhibition of NO in LPS-stimulated RAW264.7 macrophages (Table S2 and Figure S4). The high selectivity index (11.7) of LSL-11 suggests that this fraction in particular might be a candidate for further work. LSL-5, 10, 11 and 12 also showed good inhibition (IC$_{50}$ 2.8–19.7 μg/mL) of PGE$_2$ production (Table S2 and Figure S4), with LSL-5 being the most promising fraction with IC$_{50}$ of 2.8 μg/mL and a very high selectivity index of >36. None of the extracts or fractions showed inhibition of TNF-α synthesis in LPS-stimulated RAW264.7 macrophages (results not shown). Modest antioxidant activity was observed with fractions (LSL-5, 10, 11 and 12) in the ORAC assay (Table S3).

3. Conclusion

The *n*-hexane and dichloromethane extracts obtained from the sequential extraction of fresh leaves of *L. suaveolens* exhibited antibacterial activity against *S. pyogenes* and methicillin sensitive and resistant strains of *S. aureus*. The *n*-hexane extract and in particular the dichloromethane extract and moderately polar chromatographic fractions therein (LSL-5, 7, 10, 11 and 12) showed NO inhibition activity. LSL-5, 10, 11 and 12 also had promising PGE$_2$ inhibitory activity. GC–MS analysis of the *n*-hexane extract identified known antibacterial (aromadendrene, spathulenol, β-caryophyllene, α-humulene and α-pinene) and anti-inflammatory (β-caryophyllene and spathulenol) compounds. Further investigation of the dichloromethane extract led to the isolation of eucalyptin and betulinic acid, which may be, at least in part, responsible for the observed inhibition of NO and PGE$_2$. This is the first report of bioactivity studies of *L. suaveolens* and thus extends the current knowledge of this relatively unexplored Australian plant. The finding of antibacterial and anti-inflammatory activities lends scientific support to the traditional medicinal uses of this plant and suggests that the leaves could provide a more accessible source of medicine from this plant, as compared with the sap.

**Supplementary material**

Supplementary material (experimental, figures and tables) relating to this article is available online.

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**Disclosure statement**

No potential conflict of interest was reported by the authors.

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