SUPPLEMENTARY MATERIAL

Bioactivity and chemical characterization of *Lophostemon suaveolens* - an endemic Australian Aboriginal traditional medicinal plant

Tarannum Naz^a, Joanne Packer^{a,f}, Ping Yin^a, Joseph J. Brophy^b, Hans Wohlmuth^{c,d}, Dane E. Renshaw^c, Joshua Smith^c, Yaegl Community Elders^e, Subramanyam R. Vemulpad^a and Joanne F. Jamie^{a*}

^aIndigenous Bioresources Research Group, Faculty of Science and Engineering, Macquarie University, North Ryde, NSW 2109, Australia

^bSchool of Chemistry, University of New South Wales, Sydney, NSW 2052, Australia

^cSouthern Cross Plant Science, Southern Cross University, Lismore, NSW 2480, Australia

^dIntegria Healthcare, Ballina, NSW, 2478, Australia

^eYaegl Local Aboriginal Land Council, Maclean NSW, 2463, Australia

^fNational Institute of Complementary Medicine, University of Western Sydney, NSW, 2751

Abstract: *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* (MBC <63 µg/mL). The dichloromethane extract and chromatographic fractions therein inhibited nitric oxide in RAW264.7 murine macrophages (IC₅₀ 3.7-11.6 µg/mL) and also PGE₂ in 3T3 murine fibroblasts (IC₅₀ 2.8-19.7 µg/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, β-caryophyllene, α-humulene and α-pinene and the anti-inflammatory compounds β-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

* Correspondence to: Joanne F. Jamie, PhD, Indigenous Bioresources Research Group, Faculty of Science and Engineering, Macquarie University, North Ryde, NSW 2109, Australia. E-mail: joanne.jamie@mq.edu.au, Ph: 61298508283, Fax: 61298508313.

List of supporting information

Experimental

Figure S1. Dose response curves for *n*-hexane (LS-Hex), dichloromethane (LS-DCM) and water (LS-Water) extracts from *L. suaveolens* and the positive control dexamethasone in the NO inhibition assay.

Figure S2. GC-MS FID trace of the *n*-hexane fraction of *L. suaveolens* on a BP-20 column.

Figure S3. Eucalyptin (1), Betulinic acid (2).

Figure S4. Dose response curves for dichloromethane fractions from *L. suaveolens*: (a) NO inhibition assay; (b) PGE₂ inhibition assay.

Figure S5. Eucalyptin (1) high resolution MS.

Figure S6. Eucalyptin (1) ¹H NMR (CDCl₃, 600 MHz) spectrum.

Figure S7. Eucalyptin (1) ¹³C NMR (CDCl₃, 150 MHz) spectrum.

Figure S8. Betulinic acid (2) high resolution MS.

Figure S9. Betulinic acid (2) ¹H NMR (DMSO, 600 MHz) spectrum.

Figure S10. Betulinic acid (2) ¹³C NMR (DMSO, 150 MHz) spectrum.

Table S1. Antibacterial activity of *L. suaveolens* extracts and fractions in disc diffusion and MTT microdilution assays (Appendino et al. 2008).

Table S2. Nitric oxide (NO) and PGE₂ synthesis inhibitory activity of extracts and fractions from *L. suaveolens* (Shou et al. 2012).

Table S3. Antioxidant activity of extracts and fractions from *L. suaveolens* in ORAC assay (Shou et al. 2012).

Table S4. GC-MS analysis of *n*-hexane extract from *L. suaveolens* on BP-20 and DB-5 column.

References

Experimental

Ethics

This research was approved by the Human Research Ethics Committee at Macquarie University (HE27 JUL2007-R05356 and 5201200763). It was conducted under the framework of best ethical practice, working in partnership with Indigenous people (NHMRC 2003) and was governed by a cooperative research agreement with the Yaegl community (Brouwer et al. 2005).

Plant material

Fresh leaves of *Lophostemon suaveolens* were collected from Yamba, NSW (29°26'32.85"S, 153°21'06.23"E) in May 2011 and botanically identified by Mrs Alison Downing of Macquarie University Herbarium (voucher specimen #73009156). Fresh leaves (1 kg) of *L. suaveolens* were ground and extracted sequentially with *n*-hexane, dichloromethane, ethyl acetate and methanol (3 × w/v; 3 × 24 h) and dried under vacuum to afford the *n*-hexane (9.3 g), dichloromethane (24.1 g), ethyl acetate (16.5 g) and methanol (32.7 g) extracts. A water extract (2.0 g) was prepared separately by shaking 100 g of freshly ground leaves with deionised distilled water (3 × w/v; 3 × 24 h) and freeze drying.

Bacterial strains

Escherichia coli (β-lactamase negative, ATCC 25922), *Escherichia coli* (β-lactamase positive, ATCC 35218), *Streptococcus pyogenes* (clinical isolate), *Pseudomonas aeruginosa* (ATCC 27853), methicillin sensitive *Staphylococcus aureus* (MSSA, ATCC 29213), methicillin resistant *Staphylococcus aureus* (MRSA, ATCC BAA 1026), wild multidrug resistant *Staphylococcus aureus* (clinical isolate) and *Salmonella* ser. Typhimurium (clinical isolate) were used for the antibacterial studies. All bacteria cultures were provided by Dr John Merlino of the Department of Microbiology, Concord Hospital, Sydney. The research was approved by the Macquarie University Biosafety Committee (Approval Reference 08/06/LAB, TAN180512BHA).

Disc diffusion assay

20 µL of stock solutions of the dried plant extracts or antibiotic (vancomycin for Gram-positive bacteria and gentamycin for Gram-negative bacteria) in 20% aqueous DMSO were applied to Whatman filter paper (6 mm diameter) discs to give 2 mg of plant extract or 2 µg of antibiotic per disc. Discs with 20 µL of 20% aqueous DMSO served as negative controls. All discs were dried for 30 minutes prior to placing on agar plates. Overnight cultures of bacteria at $A_{600} = 0.08$ (10^7 - 10^8 CFU/mL) in Mueller-Hinton (MH) broth (Bacto Laboratories Pty Ltd) were swabbed over MH agar (for all tested bacteria except *Streptococcus pyogenes*) or horse blood agar plates (only for *S. pyogenes*) to produce a confluent lawn on the agar plate. Dried discs were placed on the inoculated

agar plates and the plates were incubated overnight at 37 °C. Antimicrobial activity was evaluated by measuring the diameter (including 6 mm disc) of the zone of inhibition.

MTT Microdilution assay

Minimum inhibitory concentrations (MICs) were determined using the MTT microdilution method (Appendino et al. 2008) with minor modifications. Briefly, plant extracts or fractions were prepared at 10 mg/mL in 20% aqueous DMSO and the antibiotic solution was prepared at 1 mg/mL in 20% aqueous DMSO. The microdilution assay was performed in MH broth. In 96-well clear bottom microtitre plates, samples (plant extract/fractions/antibiotic control) were serially diluted to give final concentrations of 2-1000 µg/mL (plant extract/fraction) and 0.02-10 µg/mL (antibiotic control). Samples (20 µL) were inoculated with 175 µL of microbial culture ($A_{600} = 0.08$ diluted 100-fold in MH broth). Plates were incubated at 37 °C for 18 h. 5 µl of methanolic solution (5 mg/mL) of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT from Sigma Aldrich) was added to each well and incubated at 37 °C for 60 min. The MIC values were defined as the lowest concentration of the test samples that inhibited visible growth of the microorganisms (the last well showing no yellow-blue change of colour of MTT).

Plant extracts having antimicrobial activity as determined by the MTT assay were assessed for their bacteriostatic and bactericidal properties by subculturing onto fresh agar after the MTT assay. Aliquots of 5 µL from the MTT assay plates were subcultured onto MHII agar plates. Agar plates were inverted and incubated overnight at 37 °C. Subcultures that did not produce colonies were deemed to be indicative of bactericidal activity by the plant extract. The minimum bactericidal concentrations (MBC) were defined as the lowest concentration of the test samples that showed no growth of the subcultures made from the MTT assay plates.

Inhibition of nitric oxide production in LPS stimulated RAW264.7 macrophages

Nitric oxide (NO) inhibitory activity was performed using RAW264.7 murine leukemic monocyte macrophages (ATCC, Manassas, VA, USA) stimulated with bacterial lipopolysaccharide (LPS) (Shou et al. 2012). Before performing NO inhibitory activity, the samples were tested in replicate experiments on the RAW264.7 cell line to assess cytotoxicity. Samples were added to cultures and incubated under the same conditions as those employed in tests for NO/TNF- α inhibitory activity (incubation at 37 °C in 5% CO₂ atmosphere for 24 h). Cell viability was assessed using the ATP-lite assay kit (PerkinElmer, Glen Waverly, Australia) (Shou et al. 2012). Half-maximum inhibitory concentration (IC₅₀) values were calculated using GraphPad prism version 4 (La Jolla, CA, USA).

Tumor necrosis factor-alpha (TNF- α) assay

The assay was performed with the same cell supernatant as the nitric oxide inhibition assay (Shou et

al. 2012). Supernatants were collected and frozen (-80° C) until the measurement of TNF- α concentration was performed. TNF- α was quantified using a Quantikine Mouse TNF- α immunoassay kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

Prostaglandin E₂ (PGE₂) inhibitory activity

3T3 Swiss albino mouse embryonic fibroblast cells (ATCC, Manassas, VA, USA) were used for the PGE₂ inhibitory activity assay. Cells were grown and seeded into 96-well cell culture plates and stimulated for PGE₂ production using calcium ionophore (Shou et al. 2012). An EIA kit-monoclonal (Cayman chemical company, Ann Arbor, MI, USA) was used as per the manufacturer's protocol for the determination of PGE₂ in culture supernatants. The percentage of inhibition of PGE₂ production by each sample, assayed in triplicate, was calculated relative to solvent only controls (0.5% v/v DMSO).

Selectivity index (SI)

The selectivity index was calculated as the ratio between EC₅₀ value for cytotoxic activity and the IC₅₀ value for the assay of interest (*e.g.* NO, TNF- α or PGE₂ inhibitory activity) (Cos et al. 2001).

Antioxidant assay

The antioxidant assay was performed in black 96-well fluorescence assay plates (Interpath, Heidelberg, Vic, Australia) using the ORAC assay (Shou et al. 2012). Epicatechin (Sigma-Aldrich, St Louis, MO, USA) was used as a positive control. The ORAC values were determined using a regression for Trolox concentration *versus* net area under the fluorescein decay curve (AUC). Antioxidant activity was expressed in micromoles of Trolox equivalents per gram of sample.

GC-MS analysis

The *n*-hexane extract of *L. suaveolens* leaves was analysed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). Analytical GC was carried out on a Shimadzu GC17A gas chromatograph with a BP-20 column (60 m $\times$ 0.25 mm $\times$ 0.25 μ m) programmed from 50 °C to 220 °C at 3 °C/min with helium as the carrier gas. The injector and detector were both at 220 °C. GC integrations were performed on a SMAD electronic integrator. GC-MS was carried out on a Shimadzu GCMS-QP5000 mass spectrometer operating at 70 eV ionisation energy. Two columns were used for GC-MS (a) a BP-20 column (30 m $\times$ 0.25 mm $\times$ 0.25 μ m) programmed from 35 °C to 220 °C at 3 °C/min and (b) a BP-5 column (30 m $\times$ 0.25 mm $\times$ 0.25 μ m), both with helium as carrier gas and injector temperature 220°C. Mass spectra were recorded in electron impact (EI) mode at 70 eV, scanning the 41-450 *m/z* range. Compounds were identified by their identical GC retention times and retention indices relative to *n*-alkanes and by comparison of their mass spectra

with either pure standards or published spectra in the NIST GC-MS library and those in the literature (Adams 2007; Joulain & Konig 1998; Stenhagen et al. 1974; Pherobase 2014; Flavornet 2014).

Isolation of bioactive compounds

The dichloromethane extract (8.0 g) was subjected to silica gel (Merck silica gel F₂₅₄) column chromatography and gradient eluted using *n*-hexane/ethyl acetate (100:0 to 0:100) and finally with ethyl acetate/methanol (100:0 to 0:100) to get twelve major fractions (LSL-1 to LSL-12 based on increasing polarity). Each of these fractions was tested for antibacterial activity. LSL-5, 7, 10, 11 and 12 were also tested for anti-inflammatory and antioxidant activities. Repeated column chromatography of LSL-5 using Sephadex LH-20 (Sigma Aldrich) with methanol followed by recrystallisation with methanol yielded eucalyptin (compound **1**, 6 mg). Preparative TLC (Uniplate) of LSL-7 (180 mg) with *n*-hexane/ethyl acetate (3:1) and recrystallisation with methanol afforded betulinic acid (compound **2**, 110 mg). Silica gel column chromatography of the *n*-hexane extract (2.3 g) eluting with petroleum ether (bp 40-60 °C) and recrystallisation with methanol yielded further eucalyptin (37 mg). Eucalyptin and betulinic acid were confirmed by IR, 1D and 2D NMR spectroscopy (Bruker DRX600K 600 MHz) and mass spectrometry (Bruker Apex 3). All data were in agreement with the literature (Huq & Misra 1997; Shu et al. 2012).

Figure S1. Dose response curves for *n*-hexane (LS-Hex), dichloromethane (LS-DCM) and water (LS-Water) extracts from *L. suaveolens* and the positive control dexamethasone in the NO inhibition assay.

Figure S2. GC-MS FID trace of the *n*-hexane fraction of *L. suaveolens* on a BP-20 column.

Figure S3. Eucalyptin (1), Betulinic acid (2).

Figure S4. Dose response curves for dichloromethane fractions from *L. suaveolens*: (a) NO inhibition assay; (b) PGE₂ inhibition assay.

a

b

Figure S5: Eucalyptin (**1**) high resolution MS.

Elemental Composition Report

Page 1

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 20.0

Selected filters: None

Monoisotopic Mass, Odd and Even Electron Ions

42 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)

Elements Used:

C: 0-50 H: 0-50 O: 0-10

RAB-LS22/AJ

ID:24473

HR0094AFAMM 81 (3.228)

AutoSpec Premier

26-Feb-2013

08:36:56

Magnet EI+

3.04e4

Minimum:

Maximum:

5.0 5.0 -1.5

20.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
326.1154	326.1154	0.0	0.0	11.0	3.5	C19 H18 O5

Figure S6: Eucalyptin (**1**) ^1H NMR (CDCl_3 , 600 MHz) spectrum.

Figure S7: Eucalyptin (**1**) ^{13}C NMR (CDCl_3 , 150 MHz) spectrum.

Figure S8. Betulinic acid (**2**) high resolution MS.

Elemental Composition Report

Page 1

Single Mass Analysis

Tolerance = 3.0 PPM / DBE: min = -1.5, max = 20.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

18 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

Elements Used:

C: 0-50 H: 0-50 O: 0-3

RAB-LS29/AJ

22572

1570 25 (1.121) Cm (25.29)

KE375

04-Dec-2012 12:29:16

1: TOF MS ES-
2.98e+003

Minimum:				-1.5		
Maximum:		5.0	3.0	20.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
455.3526	455.3525	0.1	0.2	7.5	3.5	C30 H47 O3

Figure S9. Betulinic acid (**2**) ^1H NMR (DMSO, 600 MHz) spectrum.

Figure S10. Betulinic acid (**2**) ^{13}C NMR (DMSO, 150 MHz) spectrum.

Table S1: Antibacterial activity of *L. suaveolens* extracts and fractions in disc diffusion and MTT microdilution assays (Appendino et al. 2008).

Extracts/ fractions	DD				MBC (µg/mL)*			
	Bacterial strains				Bacterial strains			
	MSSA	MRSA	MDRSA	Sp	MSSA	MRSA	MDRSA	Sp
<i>n</i> -Hexane	15	17	16	13	31.25	31.25	31.25	15.62
Dichloromethane	17	17	17	11	1.9	1.9	1.9	62.5
Ethyl acetate	13	14	13	na	na	na	na	na
Methanol	na	na	na	na	na	na	na	na
Water	10	10	10.5	na	1000	1000	1000	1000
LSL-4	–	–	–	–	500	500	500	125
LSL-5	–	–	–	–	1000	500	na	15.6
LSL-10	–	–	–	–	31.25	500	7.8	0.98
LSL-11	–	–	–	–	1.9	500	1.9	0.24
LSL-12	–	–	–	–	1.9	500	1.9	0.49
Vancomycin [#]	–	–	–	–	0.0012	0.0012	0.0012	0.0002

DD: disc diffusion assay. Samples were tested at 2 mg/disc. Activity measured as diameter of complete inhibition including 6 mm disc diameter; n = 3. *Initially the minimum inhibitory concentration (MIC, µg/mL) was determined as the well with the lowest concentration of sample that displayed no yellow-blue colour change in MTT assay. From the wells showing MIC, subcultures were made on to agar media to confirm the minimum bactericidal concentration (MBC, µg/mL). For the combinations of bacteria and test substances, the MIC and MBC values were identical. Activity based on median values (n = 4). MSSA: Methicillin sensitive *Staphylococcus aureus*; MRSA: Methicillin resistant *Staphylococcus aureus*; MDRSA: multidrug resistant *Staphylococcus aureus*; Sp: *Streptococcus pyogenes*. LSL fractions (LSL-4, 5, 10, 11 and 12) denote the column chromatography fractions of the dichloromethane extract. [#]Tested at 2 µg/disc for DD. na: not active at 2 mg/disc in DD assay. All extracts and fractions were also tested against *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella* ser. *Typhimurium* and no antibacterial activity was observed.

Table S2. Nitric oxide (NO) and PGE₂ synthesis inhibitory activity of extracts and fractions from *L. suaveolens* (Shou et al. 2012).

Extracts/ fractions	Cytotoxicity RAW264.7 ($\mu\text{g/mL}$)*	Inhibition of NO synthesis RAW264.7 ($\mu\text{g/mL}$)**	SI for the inhibition of NO synthesis	Cytotoxicity 3T3 ($\mu\text{g/mL}$)*	Inhibition of PGE ₂ synthesis 3T3 ($\mu\text{g/mL}$)**	SI for inhibition of PGE ₂ synthesis
<i>n</i> -Hexane	5.1 (3.9-6.7)	43.9 (23.1-83.6)	0.1	na	na	nr
Dichloromethane	9.7 (7.2-13.0)	4.6 (2.9-7.5)	2.1	na	na	nr
Water	>100	>71.4	na	na	na	nr
LSL-5	23.4 (13.0-42.0)	3.7 (2.4-5.7)	6.3	na	2.8 (2.1-3.6)	>36
LSL-7	6.0 (5.3-6.8)	7.1 (4.5-11.2)	0.8	na	na	nr
LSL-10	22.4 (9.06-56.0)	11.6 (8.1-16.6)	1.9	na	19.7 (13.9-28.0)	>5
LSL-11	81.6 (33.8-100)	7.0 (4.6-11.7)	11.7	na	11.5 (6.5-20.4)	>8
LSL-12	24.95 (21.0-29.5)	8.9 (5.2-15.2)	2.8	na	7.3 (5.6-9.5)	>13
Dexamethasone	—	6.2 (2.5-15.7) ^a	—	—	—	—
Curcumin	—	0.8 (0.7-1.0)	—	—	—	—
Indomethacine	—	—	—	na	78.1 (59.9-101.8) ^b	—

*Cytotoxicity was expressed as EC₅₀ (95% confidence interval); **Nitric oxide inhibitory activity and PGE₂ inhibitory activity were expressed as IC₅₀ (95% confidence interval); n = 3 for cytotoxicity and NO and PGE₂ inhibition activity. ^ang/mL; ^bnM; -: not tested; na: not active at 100 $\mu\text{g/mL}$; nr: not relevant. SI (selectivity index) represents the ratio between EC₅₀ value for cytotoxic activity and the IC₅₀ value for NO and PGE₂ inhibitory activity assays. LSL fractions (LSL-5, 7, 10, 11 and 12) denote the column chromatography fractions of the dichloromethane extract.

Table S3. Antioxidant activity of extracts and fractions from *L. suaveolens* in ORAC assay (Shou et al. 2012).

Extracts/ fractions	*Antioxidant activity mean $\pm$ SD μ M TE/g
<i>n</i> -Hexane	$9.83 \pm 1.61 \times 10^2$
Dichloromethane	$1.32 \pm 0.06 \times 10^3$
Water	$2.66 \pm 0.34 \times 10^3$
LSL-5	$2.08 \pm 0.46 \times 10^3$
LSL-7	na
LSL-10	$1.81 \pm 0.30 \times 10^2$
LSL-11	$1.58 \pm 0.03 \times 10^3$
LSL-12	$1.55 \pm 0.12 \times 10^3$
Epicatechin	$2.98 \pm 0.40 \times 10^4$

*TE = trolox equivalence. LSL-5, 7, 10, 11 and 12 fractions are fractions of LS-DCM. na: not active. LSL fractions (LSL-5, 7, 10, 11 and 12) denote the column chromatography fractions of the dichloromethane extract.

Table S4. GC-MS analysis of *n*-hexane extract from *L. suaveolens* on BP-20 and DB-5 column.

Compounds	Peak Position (BP-20 column)	LRI values (BP-20 column)	LRI values (BP-5 column)	Method of identification a = Published literature data* b = LRI index	% of identified compounds (BP-20 column)
α -pinene	2	1040	931	a,b	0.67
α -cubebene	10	1450	1351	a,b	0.49
α -copaene	14	1486	1377	a,b	0.46
β -caryophyllene	21	1590	1411	a,b	2.53
aromadendrene	25	1608	1442	a,b	15.47
<i>allo</i> -aromadendrene	30	1642	1460	a,b	7.04
α -humulene	34	1676	1453	a,b	1.52
bicyclogermacrene	42	1733	1499	a,b	0.90
cadina-1,4 diene	47	1779	1535	a,b	0.91
<i>trans</i> -calalmenene	49	1831	1524	a,b	3.15
benzyl alcohol	50	1846	1030	a,b	0.15
epiglobulol	68	2014	1568	a,b	2.69
ledol	69	2030	1594	a,b	1.22
globulol	75	2079	1585	a,b	4.47
spathulenol	80	2130	1579	a,b	12.46

LRI: linear retention indices. *(Adams 2007; Joulain & Konig 1998; Stenhagen et al., 1974; Pherobase 2014; Flavornet 2014).

References

- Adams RP. 2007. Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy (4th edn). Allured Pub. Corporation.
- Appendino G, Gibbons S, Giana A, Pagani A, Grassi G, Stavri M, Smith E, Rahman MM. 2008. Antibacterial cannabinoids from *Cannabis sativa*: A structure-activity study. J Nat Prod. 71:1427-1430.
- Brouwer N, Liu Q, Harrington D, Kohen J, Vemulpad S, Jamie J, Randall M, Randall D. 2005. An ethnopharmacological study of medicinal plants in New South Wales. Molecules. 10:1252-1262.
- Cos P, Calomme M, Sindambiwe J-B, De Bruyne T, Cimanga K, Pieters L, Vlietinck AJ, Berghe DV. 2001. Cytotoxicity and lipid peroxidation-inhibiting activity of flavonoids. Planta Med. 67:515-519.
- Flavornet [Internet]. 2014. Terry Acree & Heinrich America; [cited 2015 Jan 25]. Available from: http://www.flavornet.org/f_kovats.html.
- Huq F, Misra LN. 1997. An alkenol and C-methylated flavones from *Callistemon lanceolatus* leaves. Planta Med. 63:369-370.
- Joulain D, König WA. 1998. The Atlas of Spectral Data of Sesquiterpene Hydrocarbons. E B-Verlag, Hamburg.
- [NHMRC] National Health and Medical Research Council. 2003. Values and Ethics: Guidelines for Ethical Conduct in Aboriginal and Torres Strait Islander Health Research. Canberra.
- Pherobase [Internet]. 2014. The Pherobase: Database of Pheromones and Semiochemicals [cited 2015 Jan 25]. Available from: <http://www.pherobase.com/>.
- Shou Q, Banbury LK, Renshaw DE, Lambley EH, Mon H, Macfarlane GA, Griesser HJ, Heinrich MM, Wohlmuth H. 2012. Biologically active dibenzofurans from *Pilidiostigma glabrum*, an endemic Australian Myrtaceae. J Nat Prod. 75:1612-1617.
- Shu R-G, Hu H-W, Zhang P-Z, Ge F. 2012. Triterpenes and flavonoids from *Mosla chinensis*. Chem Nat Compd. 48:706-707.
- Stenhagen E, Abrahamsson S, McLafferty FW. 1974. Registry of Mass Spectral Data. Wiley, New York.