

Supplementary Material

Sexual dimorphism in the dioecious monocot *Lomandra leucocephala* ssp. *robusta* and its potential ecosystem and conservation significance

Jenna T. Draper^{A,D}, John G. Conran^A, Nicholas Crouch^B, Philip Weinstein^A and Bradley S. Simpson^C

^ASchool of Biological Sciences, Molecular Life Sciences Building, The University of Adelaide, North Terrace, Adelaide, SA 5005, Australia.

^BConservation and Horticulture, Technical and Further Education SA, Urrbrae Campus, 505 Fullarton Road, Netherby, SA 5062, Australia.

^CUniversity of South Australia, Clinical and Health Sciences, Health and Biomedical Innovation South Australia, GPO Box 2471, Adelaide, SA 5001, Australia.

^DCorresponding author. Email: jenna.draper@adelaide.edu.au

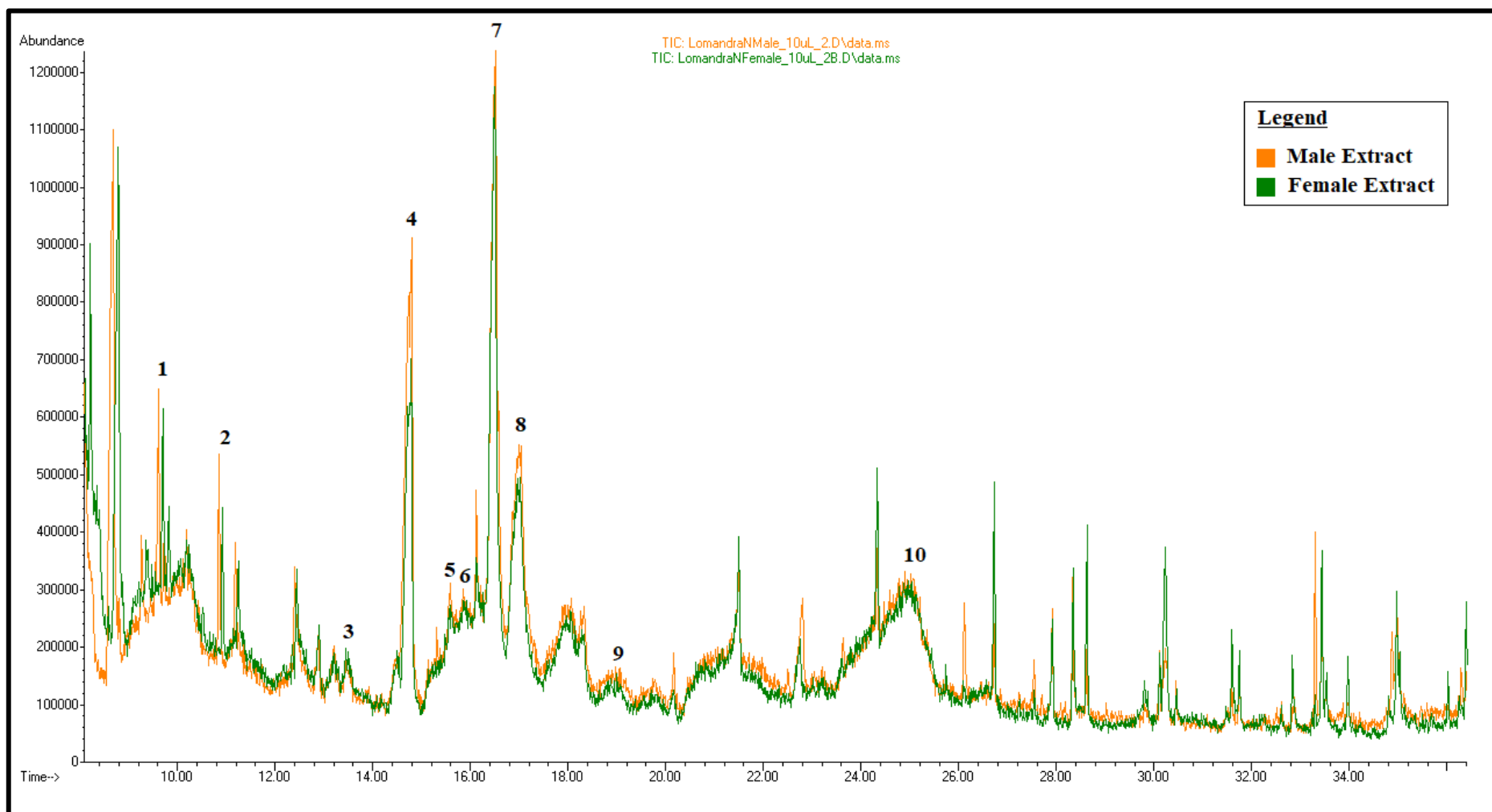


Fig. S1. Overlaid GC-MS chromatogram of male (yellow) and female (green) floral methanol extracts. Numbered peaks include those with the greatest difference in area % between sexes.

Table S1. Table of peak retention time, peak area, identification and quality match of methanolic floral extracts analysed by GC-MS, numbered according to SM 1, with compounds in common underlined

Peak number	Sex	Retention time (min)	Peak area (%)	Top three compound matches	Match quality
1	Male	9.620	1.73	<u>4,5-Dihydro-2-methylimidazole-4-one</u> 2,3-2H-4-Methyl-imidazole-2-one <u>1,3-Dihydro-4-methyl-2H-imidazol-2-one</u>	83 74 74
	Female	9.710	3.09	<u>1,3-Dihydro-4-methyl-2H-imidazol-2-one</u> <u>4,5-Dihydro-2-methylimidazole-4-one</u> 2-Methyl-3,4,5,6-tetrahydropyrazine	78 50 25
2	Male	10.195	2.57	<u>1,3-Dihydroxy-2-propanone</u> <u>dl-Glyceraldehyde dimer</u> 1-Propanol	74 64 59
	Female	10.189	4.67	<u>1,3-Dihydroxy-2-propanone</u> <u>Glycoaldehyde dimer</u> 1,3-Dihydroxyacetone dimer	56 53 40
3	Male	13.458	0.70	Butanedial 1,2,15-Pentadecanetriol Ethoxymethyl-oxirane	49 42 40
	Female	13.447	1.85	2-Nitro-1-butanol Hydrazinecarboxylic acid, ethyl ester Butoxymethyl-oxirane	33 33 25
4	Male	14.800	8.50	<u>2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one</u> <u>2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one</u> 2,4,5-Trimethyl-1,3-dioxolane	87 62 35
	Female	14.79	4.79	<u>2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one</u> <u>2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one</u>	74 43

				4-Methyl-1,3-dioxane	25
5	Male	15.590	2.26	Ethoxymethyl-oxirane 1,1,1-Triethoxy-propane N-Aminomorpholine, Glyoxal imine	42 40 35
	Female	15.597	3.09	2,3-Dihydroxy-propanal 7-n-Pentadecylaminomethyl-6-hydroxy-5,8-quinolinedione DL-Arabinose	56 40 39
6	Male	15.860	2.28	<u>DL-Arabinose</u> DL-Xylose 2,3-Dihydroxy-propanal	50 32 25
	Female	15.854	1.15	<u>DL-Arabinose</u> 2,3-Dihydroxy-propanal d-Glycero-d-ido-heptose	50 43 45
7	Male	16.513	11.47	Trifluoro-acetic acid, ethyl ester <u>1-Ethoxy-2,2,2-trifluoro-ethanol</u> 1,1,1-Trifluoro-2-butanone	9 9 7
	Female	16.495	9.98	<u>1-Ethoxy-2,2,2-trifluoro-ethanol</u> Ethanesulfonyl chloride Pentyl hydroperoxide	10 4 2
8	Male	16.986	8.64	<u>1,2,3-Propanetriol, monoacetate</u> 3-Hydroxy-4-methyl-pentanoic acid, methyl ester 1,2,3-propanetriol, 1-acetate	64 50 50
	Female	17.028	6.53	<u>1,2,3-Propanetriol, monoacetate</u> 2,3-Butanedione, monooxime 2,3-Pentanedione	39 17 17

9	Male	18.980	1.75	Carbonic acid, diethyl ester d-Glycero-d-ido-heptose Carbamic acid, (2-chloroethylidene)bis-, diethyl ester	50 45 45
	Female	18.998	1.09	Ethylene glycol diglycidyl ether Vitamin d3 2-Deoxy-D-galactose	53 42 37
	Male	24.903	10.43	Acetic acid, hydroxy-, ethyl ester Methyl 3,6-anhydro- α -D-glucopyranoside Ethyl ether	72 50 50
	Female	29.975	8.32	Carbamic acid, (2-chloroethylidene)bis-, diethyl ester d-Glycero-d-ido-heptose sec-Butyl nitrite Ethylene glycol diglycidyl ether	40 37 37 37

Table S2. Table of compound activities for compounds identified to be in the top three quality matches in male and/or female methanolic floral extracts analysed by GC-MS (yellow shading = in male extract only, green = in female extract only, grey = in male and female extract)

<u>Peak number</u>	<u>Compound</u>	<u>Information</u>	<u>Reference</u>
1	4,5-Dihydro-2-methylimidazole-4-one	Anticancer activity	Sunil KS, Akki S, Ashika BD, Chitralli LR, Naresh S, Balasubramanian S (2018) GCMS and FTIR analysis on the methanolic extract of <i>Coriandrum sativum</i> leaves. <i>European Journal of Pharmaceutical and Medical Research</i> 5 , 454-460.
1	2,3-2H-4-Methylimidazole-2-one	-	None found
	2H-Imidazol-2-one, 1,3-dihydro-4-methyl-	-	None found
1	2-Methyl-3,4,5,6-tetrahydropyrazine	-	None found
2	1,3-Dihydroxy-2-propanone	1. Three carbon sugar, is used in lots of metabolic pathways, is phosphorylated and made into a hexose for carbon synthesis, carrier of phosphate for moving between the cytosol and chloroplast, oxidised to pyruvate. 2. Has a 'sweet', 'cool' taste.	1. Gee, RW, Byerrum, RU, Gerber, DW, Tolbert, NE (1988) Dihydroxyacetone phosphate reductase in plants. <i>Plant Physiology</i> 86 , 98-103. 2. Birch, G (1976) Structural relationships of sugars to taste. <i>CRC Critical Reviews in Food Science and Nutrition</i> 8 , 57 - 95.
2	1-Propanol	Fermentation product	None found

2	dl-Glyceraldehyde dimer	<ol style="list-style-type: none"> 1. Triose sugar, is used in glycolysis as a carrier of phosphate as it is added to make G3P. 2. Is sweet tasting to humans. 	<ol style="list-style-type: none"> 1. Allaby, M (2012) Glyceraldehyde. In 'A Dictionary of Plant Sciences.' (Ed. M Allaby.) (Oxford University Press: England) 2. Oertly, E, Myers, R (1919) A new theory relating constitution to taste. Simple relations between the constitution of aliphatic compounds and their sweet taste. <i>Journal of the American Chemical Society</i> 41, 855 - 876.
2	1,3-Dihydroxyacetone dimer	Dimer of 1,3-dihydroxy-2-propanone,	(see references on 1,3-dihydroxy-2-propanone)
3	1,2,15-Pentadecanetriol	Also found in <i>Coriandrum</i> flowers	Dharmalingam R, Nazni P (2013) Phytochemical evaluation of <i>Coriandrum</i> L flowers. <i>International Journal of Food and Nutritional Sciences</i> 2 , 34-39.
3	Butanedial	<ol style="list-style-type: none"> 1. Also found in medicinal hibiscus flowers 2. Hibiscus flowers have a wide variety of medicinal uses 	<ol style="list-style-type: none"> 1. Rassem HHA, Nour AH, Yunus RM (2017) GC-MS analysis of bioactive constituents of <i>Hibiscus</i> flower. <i>Australian Journal of Basic and Applied Sciences</i> 11, 91-97. 2. Missoum A (2018) An update review on <i>Hibiscus rosa sinensis</i> phytochemistry and medicinal uses. <i>Journal of Ayurvedic and Herbal Medicine</i> 4, 135-146.
3	Ethoxymethyl-oxirane	Also called ethylene oxide, it can be derived from ethylene (which is a plant growth regulator) after use in cellular processes and in the presence of oxygen	K.D. Golden and O.J. Williams, 2014. Ethylene Oxide in Plant Biological Systems: A Review. <i>Asian Journal of Biological Sciences</i> 7 , 144-150.
3	2-Nitro-1-butanol	-	None found
3	Hydrazinecarboxylic acid, ethyl ester	Potentially a natural insecticide	Attia S, Grissa KL, Lognay G, Bitume E, Hance T, Mailleux AC (2013) A review of the major biological approaches to control the worldwide pest <i>Tetranychus urticae</i> (Acari: Tetranychidae) with special reference to natural pesticides. <i>Journal of Pest Sciences</i> 86 , 361-386.
3	Butoxymethyl-oxirane	-	(see references on ethoxymethyl oxirane)

4	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	<ol style="list-style-type: none"> 1. Antioxidant 2. Makes up a considerable quantity of date palm fruit extract, which has good antioxidant activity 3. Also found in garden mint 	<ol style="list-style-type: none"> 1. Yu, X, Zhao, M, Liu, F, Zeng, S, Hu, J (2013) Identification of 2,3 dihydro-3,5 dihydroxy-6-methyl-4H pyran-4-one as a strong antioxidant in glucose–histidine Maillard reaction products. <i>Food Research International</i> 51, 397-403. 2. Siddeeg, A, Zeng, X-A, Ammar, AF, Han, Z (2019) Sugar profile, volatile compounds, composition and antioxidant activity of Sukkari date palm fruit. <i>Journal of Food Science and Technology</i> 56, 754-762. 3. Imad, HH, Hussein, JH, Muhanned, AK, Nidaa, SH (2015) Identification of five newly described bioactive chemical compounds in methanolic extract of <i>Mentha viridis</i> by using gas chromatography - mass spectrometry (GC-MS). <i>Journal of Pharmacognosy and Phytotherapy</i> 7, 107-125.
4	2,4,5-Trimethyl-1,3-dioxolane	<p>Has been identified as a main constituent in crude extracts of walnut tree bark which also exhibited antimicrobial properties</p> <p>Also a common component in wine, sherry and brandy</p>	Ara I, Shinwari MMA, Rashed SA, Bakir MA (2013) Evaluation of antimicrobial properties of two different extracts of <i>Juglans regia</i> tree bark and search for their compounds using gas chromatography-mass spectrum. <i>International Journal of Biology</i> 5 , 92 – 102.
4	4-Methyl-1,3-dioxane-	-	None found
5	1,1,1-Triethoxy-propane	-	None found
5	N-Aminomorpholine, Glyoxal imine	Found in the butanol extracts of <i>Merrimia borneensis</i> at 1% of total extract,	Hossain MA, Shah MD, Sakari M (2011) Gas chromatography-mass spectrometry analysis of various organic extracts of <i>Merremia borneensis</i> from Sabah. <i>Asian Pacific Journal of Tropical Medicine</i> , 637 – 641.
5	2,3-Dihydroxy-propanal	<ol style="list-style-type: none"> 1. Found in hibiscus extract and is antimicrobial and an antioxidant 2. Also found in some Saudi honeys depending on the 	<ol style="list-style-type: none"> 1. Imad, HH, Hussein, JH, Muhanned, AK, Nidaa, SH (2015) Identification of five newly described bioactive chemical compounds in methanolic extract of <i>Mentha viridis</i> by using gas chromatography - mass spectrometry (GC-MS). <i>Journal of Pharmacognosy and Phytotherapy</i> 7, 107-125. 2. Alotibi IA, Harakeh SM, Al-Mamary M, Mariod AA, Al-Jaouni SK, Al-

		<p>cultivar which show varying degrees of antimicrobial activity</p> <p>3. Also found in the reasonably high quantities in floral volatiles of a few tree peony cultivars</p>	<p>Masaud S, Alharbi MG, Al-Hindi RR (2018) Floral markers and biological activity of Saudi Honey. <i>Saudi Journal of Biological Sciences</i> 25, 1369 – 1374.</p> <p>3. Zhao J, Hu Zeng-hui H, Leng P, Zhang H, Cheng F (2012) Fragrance composition in six peony cultivars. <i>Korean Journal of Horticultural Science and Technology</i> 30, 617 – 625.</p>
5	7-n-Pentadecylaminomethyl-6-hydroxy-5,8-quinolinedione	-	None found
6	DL-Arabinose	<p>1. Part of cell wall polysaccharides</p> <p>2. Helps to maintain cell wall integrity and therefore assists with cell wall tolerance under high salt conditions</p> <p>3. Arabinose concentrations fall as flowers mature and senesce as the cell walls degrade</p>	<p>1. Burget EG, Verma R, Molhoj M, Reiter W (2003) The biosynthesis of l-arabinose in plants: Molecular cloning and characterization of a golgi-localised UDP-D-xylose 4-epimerase encoded by the <i>MUR4</i> gene of Arabidopsis. <i>The Plant Cell</i> 15, 523 – 531.</p> <p>2. Zhao C, Zayed O, Zeng F, Liu C, Zhang L, Zhu P, Hsu C, Tuncil YE, Tao WA, Carpita NC, Zhu J (2019) <i>New Phytologist</i> 224, 274 – 290.</p> <p>3. O'Donoghue EM (2006) Flower petal cell walls: Changes associated with flower opening and senescence. <i>New Zealand Journal of Forestry Science</i> 36, 130 – 144.</p>
6	DL-Xylose	<p>1. A nectar sugar but is not proven to be able to be digested by any birds or insects that consume it mixed with other sugars the nectar</p> <p>2. Also might be toxic to bees at too high a quantity</p>	<p>1. Allsopp MH, Jackson S (1998) Xylose as a nectar sugar: The response of cape honeybees, <i>Apis mellifera capensis</i> Eschscholtz (Hymenoptera: Apidae). <i>Comparative Biochemistry and Physiology Part B</i> 131, 613 – 620.</p> <p>2. Crane E (1978) On the scientific front. <i>Bee World</i> 59, 37 – 38.</p>

6	d-Glycero-d-ido-heptose	Found in Ayurvedic medicines but is metabolised in the fermentation production into some other products that may contribute to their antioxidant effect	Vinothkanna A, Soundarapandian S (2018) Influence of intrinsic microbes on phytochemical changes and antioxidant activity of the Ayurvedic fermented medicines: <i>Balarishta</i> and <i>Chandanasava</i> . <i>Ayu</i> 39 , 169 – 181.
9	Carbonic acid, diethyl ester	-	None found
9	Acetic acid, trifluoro-, ethyl ester	-	None found
7	1-Ethoxy-2,2,2-trifluoro-ethanol	-	None found
7	1,1,1-Trifluoro-2-butanone	Also found in medicinal plant <i>Gigantochloa ligulata</i>	Peng, W, Wu, Y-Q, Song, Y (2009) 'Evaluation on Application Potential of <i>Gigantochloa ligulata</i> for Biomedicine, 2009 Third International Conference on Bioinformatics and Biomedical Engineering.' Beijing, China. (IEEE. Available at http://ieeexplore.ieee.org.proxy.library.adelaide.edu.au/stamp/stamp.jsp?tp=&arnumber=5162805&isnumber=5162128)
7	Ethanesulfonyl chloride	-	None found
7	Pentyl hydroperoxide	Also found in flowers of <i>Pogostemon quadriflorus</i>	Jisha M, Zeinul NHH, Leena P (2016) GC-MS analysis of leaves and flowers of <i>Pogostemon quadriflorus</i> (Benth.) F.Muell.(Lamiaceae). <i>World Journal of Pharmaceutical Research</i> 5 , 667 – 681.
8	1,2,3-Propanetriol, monoacetate	1. Precursor to an antifungal and has antimicrobial, anti-inflammatory and anticancer effects from <i>Broussonetia luzonica</i> extracts	1. Casuga FP, Castillo AL, Corpuz MJT (2016) GC-MS analysis of bioactive compounds present in different extracts of an endemic plant <i>Broussonetia luzonica</i> (Blanco) (Moraceae) leaves. <i>Asian Pacific Journal of Tropical Medicine</i> 6 , 957 – 961. 2. El-Sharkawy HHA, Rashad YM, Ibrahim SA (2018) Biocontrol of stem rust disease of wheat using arbuscular mycorrhizal fungi and <i>Trichoderma</i> spp.

		<p>2. Antifungal activity in test study of <i>Trichoderma</i> spp.</p> <p>3. Insecticidal from <i>Calotropis gigantea</i> extracts</p> <p>4. Found also in saffron honey</p>	<p><i>Physiological and Molecular Plant Pathology</i> 103, 84 – 91.</p> <p>3. Habib R, Karim MR (2016) Chemical characterization and insecticidal activity of <i>Calotropis gigantea</i> L. flower extract against <i>Tribolium castaneum</i> (Herbst). <i>Asian Pacific Journal of Tropical Disease</i> 6, 996 – 999.</p> <p>4. Nayik GA, Nanda V (2015) Characterisation of the volatile profile of unifloral honey from Kashmir Valley of India by using solid-phase microextraction and gas chromatography-mass spectrometry. <i>European Food Research and Technology</i> 240, 1091 – 1100.</p>
8	3-Hydroxy-4-methyl-pentanoic acid, methyl ester	-	None found
8	1,2,3-Propanetriol, 1-acetate	<p>1. Also found in pomegranate peel extracts</p> <p>2. Is also found in extracts of <i>Mucuna pruriens linn</i> seeds and is antibacterial</p> <p>3. Also found in extracts of <i>Wedelia biflora</i></p>	<p>1. Harini K, Mohan CC, Karthikeyan RS, Sukumar M (2018) Effect of <i>Punica granatum</i> peel extracts on antimicrobial properties in Walnut shell cellulose reinforced bio-thermoplastic starch films from cashew nut shells. <i>Carbohydrate Polymers</i> 184, 231 – 242.</p> <p>2. Jhariya S, Kakkar A (2016) Analysis of bioactive components from ethyl acetate and ethanol extracts of <i>Mucuna pruriens linn</i> seeds by GC-MS technique. <i>Journal of Chemical and Pharmaceutical Research</i> 8, 403 – 409.</p> <p>3. Arockia SP, Amaladasan M, Gowri J, Dharmalingam V, Prabha A, Rajendran R (2015) Gas chromatography-mass spectrometry analysis of different solvent crude extracts from the coastal region of <i>Wedelia biflora</i>.L. <i>International Research Journal of Biological Sciences</i> 4, 1 – 5.</p>
8	2,3-Butanedione, monooxime	Is a non-muscular myosin inhibitor	Radford FE, White RG (2011) Inhibitors of myosin, but not actin, alter transport through <i>Tradescantia</i> plasmodesmata. <i>Protoplasma</i> 248 , 205-216.
8	2,3-Pentanedione	Found in <i>Viola tianshanica</i> essential oil, which has antioxidant activity	Yan J, Qu Z, Xiao Y, Qiu G, Zhang T, Wu Z, He X, Hu X (2010) Chemical composition and antioxidant activity of the essential oil of endemic <i>Viola tianshanica</i> . <i>Natural Product Research</i> 25 , 1635 – 1640.
9	Vitamin d3	Is responsible in plants as well as animals for calcium	Boland RL (1986) Plants as a source of vitamin D ₃ metabolites. <i>Nutrition Reviews</i> 44 , 1 – 8.

		intake, which can effect plant growth	
9	Carbamic acid, (2-chloroethylidene)bis-, diethyl ester	-	None found
9	2-Deoxy-D-galactose	Toxic to termites in a delayed response	Veillon LJ (2003) The biological activity of rare carbohydrates and cyclitols in <i>Coptotermes formosanus</i> . Liuisiana State University Digital Commons, USA.
10	Acetic acid, hydroxy-, ethyl ester	-	None found
10	.alpha.-D-Glucopyranoside, methyl 3,6-anhydro-	-	None found
10	Ethyl ether	-	None found
10	sec-Butyl nitrite	-	None found
10	Ethylene glycol diglycidyl ether	-	None found

Table S3. Tables of T-test assuming equal variance results testing for differences in peak area between northern and southern male, and northern and southern female *L. leucocephala* ssp. *robusta* ($\alpha = 0.05$)

Peak	North male average peak area ratio	South male average peak area ratio	P-value	T-statistic
1	0.47678	0.39968	0.4870	0.76479
2	0.25255	0.17344	0.2434	1.3669
3	0.51413	0.65761	0.3531	-1.0498
4	4.7419	4.0262	0.5310	0.68496

Peak	North female average peak area ratio	South female average peak area ratio	P-value	T-statistic
1	0.74840	0.58671	0.4854	0.76791
2	0.50589	0.31758	0.2268	1.4269
3	0.92089	0.70540	0.4052	0.92956
4	7.7838	5.9406	0.3899	0.96332