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Microdistillation and essential oil chemistry—a useful tool for detecting hybridisation in *Plectranthus* (Lamiaceae)

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Abstract

The essential oil composition is reported for *Plectranthus ciliatus*, *Plectranthus zuluensis* and their putative hybrid. The essential oil chemistry is in support of morphological data and pollination studies, which have indicated a natural hybrid between *P. ciliatus* and *P. zuluensis*. The hybrid plant contains terpenoids from both putative parents together with 'hybrid compounds,' which are not present in any of the two parents. The composition of the essential oil obtained through microdistillation is virtually identical to the analysis of the hydrodistilled essential oil.

Keywords: Plectranthus; Essential oil; Chemotaxonomy; Hybridisation

1. Introduction

In southern Africa, the Lamiaceae are most abundantly represented by the genus *Plectranthus* with 48 indigenous species (Hankey, 1999; Van Jaarsveld and Edwards, 1997; Edwards et al., 2000). This group of aromatic Labiates have been the subject of various taxonomic studies (Codd, 1975, 1985; Van Jaarsveld and Edwards, 1991, 1997; Edwards et al., 2000). The species in some complexes are notoriously difficult to identify. Pollination studies in the genus have revealed a number of natural hybrids amongst the medium-tubed species of *Plectranthus*, particularly at Oribi Gorge Nature Reserve (3030CA) in southern KwaZulu-Natal, South Africa. Potgieter et al. (2000) illustrated that the leaf and flower morphology of the putative *Plectranthus ciliatus* × *Plectranthus zuluensis* hybrid appear to be intermediate to that of the sympatric putative parents. *P. ciliatus* E. Mey. ex Benth is the only species in the study area that has nectar guides on both upper and lower corolla lips; thus, the presence of this character in the hybrid plant indicates putative parentage in *P. ciliatus* × *P. zuluensis*. Hybrids of *P. ciliatus* × *P. zuluensis* show a general habit and purple colouration on the lower leaf surface that is intermediate to that of the putative parent species. Corolla shape, size and colouration are also intermediate to that of the putative parents. Furthermore, two different dipteran species were seen to visit and move between the inflorescences of sympatric populations of *P. zuluensis* T. Cooke and *P. ciliatus* at Oribi Gorge; these are *Stenobasipteron wiedemanni*, a long-proboscid fly (family Nemestrinidae), and *Psilodera confusa*, a medium-proboscid fly (family Acroceridae) illustrated in Potgieter et al. (1999).

Hybrids are not unknown in this genus of horticulturally interesting plants and many crosses have been made by plant breeders (E. van Jaarsveld, personal communication). Natural hybridisation has a pronounced impact on the understanding of taxonomic relationships (reticulate vs. divergent evolution). As hybrids could often defy detection by morphology alone, additional information is required to detect and/or confirm possible hybridisation events. The aromatic character of *Plectranthus* leaves allows for the extraction and analysis of the essential oils. The aim of this investigation was to determine if essential oil chemistry could be used to confirm hybridisation in *Plectranthus*. As hybrid plants are often scarce and the essential oil yields for *Plectranthus* species is generally very low, microdistillation was investigated as an alternative method to extract the essential oils and compare it to the

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Table 1
Essential oil composition for (1) *P. ciliatus* (ex hort Witwatersrand Botanical Garden), (2) *P. ciliatus* (Ferncliff), (3A) *P. ciliatus* × *P.* zuluensis (ex Oribi Gorge), hydrodistilled oil, (3B) *P. ciliatus* × *P. zuluensis* (ex Oribi Gorge), microdistilled oil, (4) *P. zuluensis* (ex hort Witwatersrand Botanical Garden), (5) *P. zuluensis* (ex Oribi Gorge)

RRI : Relative Retention Indices

RRI	Compound	1	2	3A	3B	4	5
1032	α-Pinene	0.26	–	0.08	1.06	0.02	0.02
1035	α-Thujene	–	–	–	0.08	–	–
1076	Camphene	–	–	–	0.04	–	–
1118	β-Pinene	0.01	0.01	2.44	18.01	0.01	0.01
1132	Sabinene	0.01	0.03	0.08	0.45	0.01	–
1159	δ-3-Carene	0.12	–	0.47	2.49	–	–
1174	Myrcene	0.09	–	–	–	0.01	–
1176	α-Phellandrene	–	–	0.08	0.62	–	–
1187	*o*-Cymene	–	–	0.01	0.13	–	–
1188	α-Terpinene	–	–	0.01	0.48	–	–
1195	Dehydro-1,8-cineole	–	–	–	0.03	–	–
1203	Limonene	3.80	0.04	0.11	0.56	0.01	0.01
1205	Sylvestrene	0.02	–	–	–	–	–
1213	1,8-Cineole	–	0.01	–	–	0.01	0.01
1218	β-Phellandrene	–	–	0.07	0.66	–	–
1224	*o*-Mentha-1(7),5,8-triene	–	–	–	0.05	–	–
1225	(*Z*)-3-Hexenal	–	–	–	0.04	–	–
1232	(*E*)-2-Hexenal	–	–	–	–	–	0.02
1244	Amyl furan (=2-Pentyl furan)	–	–	–	0.02	–	–
1246	(*Z*)-β-Ocimene	1.27	–	0.02	0.09	–	–
1255	γ-Terpinene	0.02	–	0.13	3.58	–	–
1266	(*E*)-β-Ocimene	0.42	–	0.01	0.05	–	–
1278	*m*-Cymene	0.01	–	0.10	0.31	–	–
1280	*p*-Cymene	0.12	0.02	1.18	2.01	–	–
1286	Isoterpinolene	–	–	0.02	0.13	–	–
1290	Terpinolene	0.07	–	0.02	0.28	–	–
1319	(*E*)-2,6-Dimethyl-1,3,7-nonatriene	0.01	–	–	0.01	0.01	–
1345	3-Octyl acetate	–	0.07	0.02	–	–	0.04
1360	Hexanol	–	–	–	–	–	0.02
1382	*cis*-Alloocimene	0.02	–	–	–	–	–
1386	Octenyl acetate	0.76	4.25	0.06	0.09	–	–
1391	(*Z*)-3-Hexenol	–	–	–	–	–	0.02
1393	3-Octanol	–	0.03	0.02	0.01	0.05	0.05
1400	Nonanal				0.01	–	–
1406	α-Fenchone	–	–	–	–	0.01	–
1412	(*E*)-2-Hexenol	0.01	–	–	–	–	–
1415	Rose furan	0.02	–	–	–	–	–
1452	α,*p*-Dimethylstyrene	0.01	–	0.03	0.14	–	–
1452	1-Octen-3-ol	1.11	1.83	0.71	0.13	0.07	0.13
1466	α-Cubebene	0.15	0.10	0.05	0.04	0.04	0.11
1468	*trans*-1,2-Limonene epoxide	0.07	–	–	–	–	–
1474	*trans*-Sabinene hydrate	0.16	0.02	0.02	0.03	–	–
1479	δ-Elemene	–	0.09	–	–	–	–
1476	(*Z*)-β-Ocimene epoxide	0.01	–	–	–	–	–
1478	*cis*-Linalool oxide (*Furanoid*)	–	–	–	0.01	–	–
1495	Bicycloelemene	0.07	0.43	0.04	0.03	0.21	0.03
1492	Cyclosativene	0.90	–	0.08	0.04	–	–
1493	α-Ylangene	–	1.23	–	–	–	–
1497	α-Copaene	2.25	0.48	1.53	0.76	1.16	2.97
1528	α-Bourbonene	0.01	0.22	0.03	–	0.29	0.22
1532	Camphor	–	–	–	0.02	–	–
1535	β-Bourbonene	0.21	3.08	0.36	0.05	3.85	2.65
1544	α-Gurjunene	1.00	0.08	0.07	0.04	0.03	–
1545	*cis*-α-Bergamotene	–	0.08	0.06	–	–	–
1548	(*E*)-2-Nonenal	–	–	–	–	0.02	–
1549	β-Cubebene	0.26	0.28	0.44	0.18	0.60	1.25
1553	Linalool	0.21	0.30	0.98	2.14	1.24	6.21
1565	Linalyl acetate	–	–	–	–	–	0.06
1568	1-Methyl-4-acetylcyclohex-1-ene	–	0.04	0.03	–	–	–
1571	*trans*-*p*-Menth-2-en-1-ol	0.03	0.01	0.01	0.01	–	–

Table 1 (*continued*)

RRI : Relative Retention Indices

RRI	Compound	1	2	3A	3B	4	5
1572	β-Ylangene	0.06	0.51	0.09	0.03	–	0.39
1586	Pinocarvone	–	–	0.03	0.09	–	–
1594	*trans*-β-Bergamotene	–	0.34	0.21	0.09	–	–
1597	Bornyl acetate	0.10	5.48	0.02	0.09	–	–
1598	Thymol methyl ether (=Methyl thymol)	–	–	–	0.04	–	–
1599	(*E*,*Z*)-2,6-Nonadienal	–	–	–	–	0.01	–
1600	β-Elemene	0.81	1.16	0.56	0.25	0.38	0.36
1611	Terpinen-4-ol	–	–	–	0.01	–	–
1612	β-Caryophyllene	9.29	2.64	3.35	3.90	6.19	7.45
1614	Carvacrol methyl ether (=Methyl carvacrol)	–	–	–	0.06	–	–
1617	6,9-Guaiadiene	0.12	0.61	–	–	–	–
1638	*cis*-*p*-Menth-2-en-1-ol	–	–	0.02	0.04	–	–
1648	Myrtenal	–	–	0.04	0.11	–	–
1628	Aromadendrene	0.05	0.20	–	–	–	–
1639	*trans*-*p*-Mentha-2,8-dien-1-ol	0.06	–	–	–	–	–
1650	γ-Elemene	0.02	0.48	–	–	–	–
1658	Sabinyl acetate	–	–	1.44	2.51	–	–
1661	Alloaromadendrene	3.82	1.12	–	–	0.40	–
1664	*trans*-Pinocarveol	–	–	0.08	0.11	–	–
1668	(*Z*)-β-Farnesene	–	0.03	0.12	0.04	0.02	0.02
1674	γ-Gurjunene	–	0.20	–	–	0.54	0.23
1677	*epi*-Zonarene	0.16	–	–	–	–	–
1684	β-Guaiene	–	0.20	–	–	–	–
1687	α-Humulene	0.86	0.35	8.19	6.99	21.40	9.24
1698	Myrtenyl acetate	–	–	0.53	0.88	–	–
1700	*p*-Mentha-1,8-dien-4-ol (=Limonen-4-ol)	–	–	–	0.06	–	–
1704	γ-Muurolene	0.26	0.40	–	–	–	–
1706	α-Terpineol	0.17	–	0.08	0.51	–	0.11
1708	Ledene	2.09	–	–	–	–	–
1709	α-Terpinyl acetate	–	–	–	0.06	–	–
1726	Germacrene D	3.10	9.37	6.41	5.52	7.11	6.53
1740	α-Muurolene	0.38	–	–	–	–	–
1741	β-Bisabolene	–	1.32	0.74	0.29	–	–
1743	Eremophilene	–	–	–	–	0.06	–
1747	*trans*-Carvyl acetate	2.54	–	0.10	0.13	–	–
1755	Bicyclogermacrene	3.54	16.82	1.73	1.30	7.28	1.41
1773	δ-Cadinene	11.77	0.33	1.28	0.88	0.17	0.28
1776	γ-Cadinene	–	0.25	0.06	–	0.02	0.02
1782	*cis*-Carvyl acetate	0.34	–	–	2.00	–	–
1784	(*E*)-α-Bisabolene	–	5.97	8.10	2.56	–	–
1786	Kessane	–	–	–	0.13	–	–
1798	Methyl salicylate	–	–	–	0.16	–	–
1799	Cadina-1,4-diene (=Cubenene)	0.15	–	–	–	–	–
1804	Myrtenol	–	–	0.24	0.07	–	–
1808	Nerol	–	–	–	–	–	0.03
1810	3,7-Guaiadiene	0.08	–	–	–	–	
1830	2,6-Dimethyl-3(*E*),5(*E*),7-octatriene-2-ol	0.05	–	–	–	–	–
1838	β-Damascenone	–	–	–	0.03	–	–
1845	*trans*-Carveol	0.22	–	0.18	0.09	–	–
1853	*cis*-Calamenene	0.39	–	–	–	–	–
1853	Dehydrocostuslactone	–	–	10.61	6.86	–	–
1854	Germacrene-B	–	2.60	–	–	–	–
1857	Geraniol	–	–	–	–	0.01	0.08
1864	*p*-Cymen-8-ol	0.10	–	0.91	0.95	–	–
1871	*p*-Mentha-1,8-dien-10-yl acetate	0.01	–	–	–	–	–
1882	*cis*-Carveol	0.04	–	–	0.02	–	–
1900	*epi*-Cubebol	6.19	0.10	0.05	–	0.02	0.05
1941	α-Calacorene	0.26	0.03	–	–	–	–
1945	1,5-Epoxy-salvial-4-14-ene	–	0.16	–	–	0.06	0.02
1953	Palustrol	1.07	0.04	–	0.01	–	–
1957	Cubebol	1.95	0.27	0.07	–	–	0.05

(continued on next page)

Table 1 (*continued*)

RRI : Relative Retention Indices

RRI	Compound	1	2	3A	3B	4	5
1958	β-Ionone	–	–	–	0.02	–	–
1981	(*Z*)-Methyl cinnamate	–	–	–	–	0.01	–
1984	γ-Calacorene	0.27	–	–	–	–	–
2001	Isocaryophyllene oxide	–	0.14	0.10	–	0.05	0.03
2008	Caryophyllene oxide	1.50	1.13	0.55	0.37	0.19	0.17
2030	Methyl eugenol	–	–	0.13	–	8.56	2.93
2033	Epiglobulol	–	0.80	–	–	–	–
2037	Salvial-4(14)-en-1-one	–	0.12	0.04	–	–	–
2045	Humulene epoxide-I	–	–	–	–	0.10	0.02
2050	(*E*)-Nerolidol	–	–	0.32	0.14	0.16	0.06
2057	Ledol	7.42	0.11	0.21	0.12	–	–
2069	1,6-Germacradien-5β-ol (=Germacrene *D*-4β-ol), (=1(10),5-Germacradien-4β-ol)	2.24	0.46	–	–	–	–
2071	Humulene epoxide-II	–	–	0.79	0.42	0.71	0.16
2080	Cubenol	0.93	–	–	–	–	0.02
2081	Humulene epoxide-III	–	–	–	–	0.05	0.01
2088	1-*epi*-Cubenol	1.18	–	–	–	–	–
2096	Elemol	–	1.52	2.37	1.23	–	–
2096	(*E*)-Methyl cinnamate	–	–	–	–	0.02	–
2103	Guaiol	–	–	0.23	0.17	–	–
2104	Viridiflorol	0.55	0.27	–	–	0.03	–
2109	*cis*-Methylisoeugenol	–	–	–	–	0.51	0.78
2127	10-*epi*-γ-Eudesmol	–	–	9.00	6.50	–	–
2144	Spathulenol	2.23	15.52	1.11	0.36	0.60	0.09
2185	γ-Eudesmol	–	–	0.15	0.51	–	–
2187	T-Cadinol	3.38	–	–	–	–	–
2200	*trans*-Methylisoeugenol	–	–	–	–	0.62	2.79
2202	1,6-Germacradien-5α-ol (=Germacrene *D*-4α-ol), (=1(10),5-Germacradien-4α-ol)	0.15	–	–	–	–	–
2219	δ-Cadinol	0.68	–	–	–	–	–
2209	T-Muurolol	1.26	0.22	–	–	–	–
2232	α-Bisabolol	–	1.33	1.33	0.46	–	–
2245	Elemicine	–	–	4.30	2.16	10.56	0.63
2247	*trans*-α-Bergamotol	0.16	0.86	–	–	–	–
2255	α-Cadinol	2.99	0.66	–	–	–	0.04
2257	β-Eudesmol	–	–	0.40	0.38	–	–
2282	γ-Asarone	–	–	0.74	0.20	14.93	35.76
2361	(*Z*)-Asarone	–	–	–	–	–	2.48
2403	*trans*-Isoelemicine	–	–	–	0.02	2.79	1.54
2478	(*E*)-Asarone	–	–	–	–	0.15	3.66
2324	Caryophylla-2(12),6(13)-dien-5α-ol (=Caryophylladienol-II)	0.06	–	–	–	–	–
2438	Kaur-16-ene	0.07	–	–	–	–	–
2607	14-Hydroxy-δ-cadinene	0.02	–	–	–	–	–
2676	*epi*-13-Manool	0.37	–	1.32	0.03	–	–
	Total (%)	89.00	86.85	77.70	85.72	91.36	91.27

composition of hydrodistilled oils. Although the essential oil composition has been reported for some species of *Plectranthus* (Ascensão et al., 1998; Buchbauer et al., 1993; Smith et al., 1996), research on the South African representatives of this genus has been neglected. This paper forms part of a broader investigation on the composition, chemotaxonomy and biological activity of the essential oil of South African *Plectranthus* species.

2. Materials and methods

Leaf material of *P. ciliatus*, *P. zuluensis* and their putative hybrid were collected from various localities (Table 1). Only one clone of the putative hybrid was present; hence, only one collection could be made. Voucher specimens of *P. ciliatus* (Ferncliff) C. Potgieter 745, *P. ciliatus* × *P. zuluensis* (Oribi Gorge) C. Potgieter 556 and *P. zuluensis* (Oribi Gorge) C. Potgieter 64 are deposited at the University of KwaZulu–Natal Herbarium (UN). Vouchers of *P. ciliatus* (WBG) AV 78 and *P. zuluensis* (WBG) AV 23 are housed in the Department of Pharmacy and Pharmacology, University of the Witwatersrand. The essential oils were obtained through hydrodistillation (3 h) using a Clevenger apparatus. In addition, leaves collected from the hybrid plant (*P. ciliatus* × *P. zuluensis*) were air dried and subjected to microdistillation using the following method; crushed leaves (~500 mg) were placed in a sample vial together with 10 ml of water. NaCl (2.5 g) and water (0.5 ml) were placed in the collecting vial. *n*-Hexane (300 μl) was added into the collecting vial to trap volatile components. Sample vials were heated to 108 °C at a rate of 20 °C/min and then kept at 108 °C for 90 min, heated to 112 °C at a rate of 20 °C/min and kept at this temperature for 30 min. Finally the samples were subjected to post-run for 6 min under the same conditions. Collecting vials were cooled to −1 °C during distillation. After distillation was completed, the organic layer in the collection vial was analyzed by GC/MS.

The essential oils were analysed using a Hewlett-Packard G1800A GCD system. Innowax FSC column (60 m × 0.25 mm Ø, with 0.25 μm film thickness). Helium (0.8 ml/min) was used as carrier gas. GC oven temperature was kept at 60 °C for 10 min and programmed to 220 °C at a rate of 4 °C/min and then kept constant at 220 °C for 10 min to 240 °C at a rate of 1 °C/min. Mass range was recorded from *m*/*z* 35 to 425. Split ratio was 50:1 and the splitless mode was used for essential oil samples obtained from micro-distillation. Injection port temperature was at 250 °C. MS were taken at 70 eV. Relative percentage amounts of the separated compounds were calculated automatically from peak areas of the total ion chromatogram. Library searches were carried out using the Wiley GC/MS Library and the Başer Library of Essential Oil Constituents. Cluster analysis was carried out with the NTSYS-PC package version 2.00 (Rohlf, 1997). The entire data set as presented in Table 1 was converted to qualitative data (presence/absence) and a hierarchical cluster analysis was performed using the UPGMA clustering algorithm.

3. Results and discussion

The compounds identified in the essential oil for all the taxa analyzed are summarized in Table 1. Eighty-three compounds were identified in the essential oil of *P. ciliatus* (89%) from the Witwatersrand Botanical Garden and sixty-two compounds (86.9%) were identified in the essential oil obtained from *P. ciliatus* from Ferncliff. Although quantitative variations are apparent, the essential oil composition from the two plants growing some ca. 600 km apart is similar as indicated in the dendrogram presented (Fig. 1). Both samples contained 6,9-guaiadiene, aromadendrene, γ-elemene, γ-muurolene, α-calacorene, 1,6-germacradien-5β-ol, T-muurolol, *trans*-α-bergamotol and α-cadinol. These compounds are absent in both *P. zuluensis* and the putative *P. ciliatus* × *P. zuluensis* hybrid. The high correlation coefficient (Fig. 1) suggests that the oil composition of the two samples obtained for *P. zuluensis* are congruent. In both instances, α-humulene, germacrene D and γ-asarone are the major compounds.

The morphological data and field observations presented in the Introduction prompted us to investigate the essential oil as an independent test to confirm hybridisation events in *Plectranthus*. Demarne and Van der Walt (1989) and others (Emboden and Lewis, 1967; Kokkini, 1992; Viljoen and Van Wyk, 2001) have illustrated the value of using phytochemical data to confirm the origin of natural hybrids. Certain compounds found only in *P. ciliatus* are present in the hybrid (e.g., ledol) while some terpenoids found only in *P. zuluensis* are present in the hybrid (e.g., γ-asarone). A chemotaxonomic survey of the essential oils of South African species of *Plectranthus* shows that γ-asarone is restricted to *P. zuluensis* (Maistry, 2001). Compounds found in both parents are present in the hybrid (e.g., linalool). 'Hybrid compounds' are absent in both parents and only found in the hybrid, e.g., 10-epi-γ-eudesmol. It could be hypothesised that new compounds form when enzymes which were previously mutually exclusive in the two parents combine in the hybrid forming 'new hybrid phytochemicals'. Recently Viljoen and Van Wyk (2001) and Viljoen (1999) demonstrated very similar chemical patterns for phenolic compounds in artificial and natural hybrids of the genus *Aloe*. The cluster analysis (Fig. 1) shows the hybrid plant nested between the putative parents which is indicative of chemical 'characters' shared with both *P. ciliatus* and *P. zuluensis*. Fig. 1 also clearly indicates that the composition of the hydrodistilled oils and the oil obtained through micro-distillation are virtually identical. Microdistillation is a very quick and accurate method to extract the oils from small amounts of low-yielding leaf material such as *Plectranthus* hybrids.

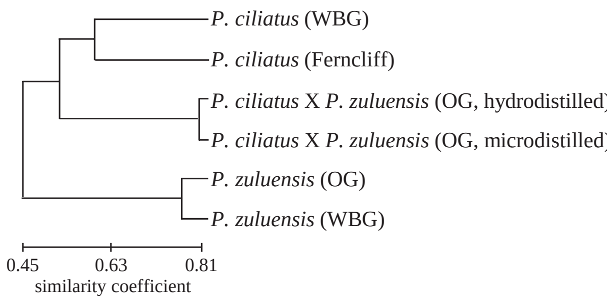

Fig. 1. Dendrogram constructed on qualitative data (absence/presence) using all compounds in Table 1. WBG = Witwatersrand Botanical Garden, Ferncliff = Ferncliff Nature Reserve, Pietermaritzburg, OG = Oribi Gorge Nature Reserve.

The importance of hybridisation was noticed by Linnaeus who proposed a model of speciation through hybridisation. Lotsy (1916, 1931) identified hybridisation as the most important factor in evolutionary change. Hybrids do, however, pose a problem to systematics as it is often believed that 'good species' do not hybridise. Many species concepts consider the process of natural hybridisation at best to be nonexistent and that they make taxonomic treatments difficult (Arnold, 1997). Since the cladistic method is based on the assumption of divergence, it is imperative to determine the role of hybridisation in any taxonomic study as this could lead to reticulate phylogenies. The example presented here provides evidence that natural hybridisation occurs in *Plectranthus*, which is instrumental in unraveling the evolutionary history and taxonomy of this genus.

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