Volatile Chemical Constituents of three *Ocimum* species (Lamiaceae) from Papua New Guinea

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ABSTRACT

Fresh aerial parts of three species of basil, Ocimum basilicum, O. tacilium *and* O. canum *were subjected to exhaustive hydrodistillation to afford pale yellow coloured oils in 1.0, 0.7 and 0.01 percent yields respectively. Detailed chemical evaluation by GC and GC/MS revealed* O. basilicum *to be composed of a total of eleven components representing 100 percent of the total oil composition. Neral (36.1 %) and geranial (44.5 %) were found to be the major components.* Ocimum tacilium *was found to be composed of five components representing 99.8 % of the total oil composition with estragole (96.6 %) being the major component. Five components were observed in* O. canum*, representing 72.3 percent of the total oil composition with eugenol (35.3 %) and linalool (27.2 %) as the major components. The high citral (neral + geranial) content in* O. basilicum *suggests that it belong to the citral chemotype while* O. tacilium *belong to the estragole chemotype and* O. canum *belong to the eugenol chemotype.*

1 INTRODUCTION

The genus *Ocimum* belongs to the family Lamiaceae and is comprised of more than 50 species of herbs and shrubs distributed in tropical and subtropical regions of Asia, Africa and the Americas. Most members of this family such as *Hyptis*, *Thymus*, *Origanum*, *Salvia* and *Mentha* species are considered economically useful because of their basic natural characteristics as essential oil producers. These essential oils are composed primarily of monoterpenes and sesquiterpenes (Lawrence, 1993) and have been the subject of extensive studies due to their economic importance.

The individual species within the genus *Ocimum* have been observed to show significant variation in the aromatic character as well as morphological features. Such observations have been attributed to the abundant cross-pollination that occurs within this genus resulting in considerable degrees of variation in the genotypes, hence diversity in growth characteristics, leaf size, flower colour, physical appearance and aroma (Lawrence 1988). Consequently, high diversity of species, subspecies, varieties and chemotypes are evident in this genus, each having distinct aromatic characters, morphological features and chemical composition in the essential oil distillates.

Such difference in the essential oil compositions in *O. basilicum* from different geographical localities led to the classification of basil into chemotypes on the basis of the prevalent chemical components (Lawrence, 1992) or components having composition greater than 20 percent (Grayer *et al*. 1996). Four main chemotypes and numerous other sub-chemotypes were established on the basis of the structural features of the main constituents as belonging to either the phenylpropanoid group (methyl chavicol, eugenol, methyleugenol and methyl cinnamate) or the terpenic group (linalool and geraniol), which are derived from the shikimic acid and the mevalonic acid biosynthetic pathways respectively. Other latter studies on the basils from other geographical regions have added new chemotypes to that list based on the established classification scheme (Lawrence, 1992; Grayer, 1996). Some of such chemotypic entries include terpenen-4-ol type from *O. canum* and thymol type from *O. gratissimum* (Sanda *et al*. 1998; Yusuf *et al*. 1998; Keita *et al*. 2000); geranyl acetate type from *O. minimum* (Ozcan and Chalchat, 2002); citral and camphor types from *O. americanum* (Mondello *et al*. 2002); and p-cymene type from *O. suave* (Keita *et al*. 2000). A report on the chemical constituents in *O. canum* from Rwanda indicated the oil to be composed of 60-90 percent linalool (Ntezurubanza *et al*. 1985).

There is a substantial wealth of literature on the chemical composition and biological activities of basil. The chemical compositions in the basils studied are composed mainly of monoterpenes or sesquiterpenes with predominant features representing the terpenic chemotype group such as linalool and geraniol or the phenylpropenic chemotype groups, while the observed biological activities are attributable to either the individual components within the matrix of the oil or due to a synergistic effect of the components (Lachowicz *et al*. 1998; Sinha and Gulani, 1990; Holm, 1999; Vasudaran *et al*. 1999; Carleton *et al*. 1992; Svoboda *et al*. 2003). The prospect of further developing and using essential oils exhibiting broad-spectrum biological activities holds promise in medicine and agriculture, owing to its low mammalian toxicity, biodegradability, non-persistence in the environment and affordability.

In spite of such wide-ranging studies on the essential oil composition in the *Ocimum* species, no data are available on the Papua New Guinean (PNG) cultivars of basil. As part of an ongoing research program to identify and document the chemical constituents in the essential oils from the diversity of aromatic flora of PNG, we report herein a complete analysis of the essential oils obtained from the aerial parts of *O. basilicum*, *O. tacilium* and *O. canum* collected respectively from Waigani in NCD, Isan (Kabwum District) in Morobe and Tabubil in the Western Provinces of PNG.

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2 MATERIALS AND METHODS

The fresh leaves of *Ocimum basilicum*, *O. tacilium* and *O. canum* were collected from different localities in PNG in 2004 and the voucher specimens deposited at the University of Papua New Guinea (UPNG) Herbarium in Port Moresby. The fresh leaves were cut into small pieces and subjected to exhaustive hydrodistillation over an 8-hour period in an all-glass standard distillation apparatus. The pure oil obtained was dried over anhydrous magnesium sulphate and analyzed by gas chromatography coupled to a mass spectrometer.

The oil sample was injected in hexane using the GC/MS on an Agilent 6890 gas chromatograph, equipped with a split/splitless injector and a 7963 Mass Selective Detector (MSD). Chromatography was performed on a BPX-5 capillary column (50m x 0.22mm and 1.0 µm film thickness – SGE, Melbourne) terminated at the MSD operating at: transfer temperature: 310^{o}C; ionization 70 eV, source temperature: 230^{o}C; quadrupole temperature 150^{o}C and scanning a mass range 35-550 m/z. The injector temperature was 250^{o}C and the carrier gas was helium at 23.10 psi and an average velocity of 28 cm/sec to the MSD. The column oven was programmed as follows: initial temperature: 50^{o}C; initial time 1.0 min; program rate 4^{o}C/min; final temperature 300^{o}C; final time 10 min.

The individual compounds in the oil were identified on the basis of their retention indices relative to known compounds, and further by comparison of their mass spectra with the authentic compounds or published spectral data (Adams, 1995).

3 RESULTS AND DISCUSSIONS

Hydrodistilled aerial parts of *O. basilicum*, *O. tacilium* and *O. canum* afforded pale yellow colored oils in 1.0, 0.4 and 0.01 percent yields respectively. GC/MS analysis of the oil indicated *O. basilicum* to be composed of 11 components; *O. tacilium* with 6 components and *O. canum* with 5 components as presented in Table 1. The major components of *O. basilicum* were geranial (44.5 %) and neral (36.1 %). The other important components identified were linalool (6.0 %), cis-α-bisabolene (3.8 %) and nerol (3.3 %) whilst other monoterpenes made up the remainder. Estragole (96.6 %) was found to be the major component of *O. tacilium* whilst the major components in *O. canum* were eugenol (35.3 %), linalool (27.2 %) and 1,8-cineole (5.6 %).

Table 1 Retention Index (RI) and percentage composition of the components of the *Ocimum basilicum*, *O. tacilium* and *O. canum*

		Percentage Composition (% Area)		
Chemical Constituents	**Retention Index (RI)**	***O. basilicum***	*O. tacilium*	*O. canum*
1,8-cineole	1058	-	-	5.6
linalool	1110	6.0	0.4	27.2
estragole (methyl chavicol)	1227	-	96.6	-
octyl acetate	1234	0.7	-	-
nerol	1242	3.3	-	-
neral	1261	36.1	-	-
cis-isocitral	1265	0.7	-	-
geranial	1288	44.5	0.4	-
trans-isocitral	1292	1.3	-	-
neryl acetate	1367	0.7	-	-
eugenol	1393	-	-	35.3
β-caryophyllene	1464	1.4	-	-
α-farnescene	1466	1.4	-	-
cis-α-bisabolene	1565	3.8	0.8	-
bicyclosesquiphellandrene	1555	-	-	2.6
cis-α-bisabolene	1565	3.8	0.8	-
γ-cadinene	1558	-	-	1.6
3-methoxy cinnamaldehyde	1629	-	1.6	-

Geranial and neral, the two co-occuring isomeric monoterpene aldehydes, collectively referred to as citral are commonly associated with the lemon grass oil (*Cymbopogon citratus*.). In this study, the total citral content in *O. basilicum* was found to be 80.6 %. Interestingly, this composition is comparable to that as reported from the lemon grasses *Cymbopogon citratus* (Poaceae) oil from PNG by Sino and coworkers (1992) and Wossa and co-workers (2004) containing 68 and 91 percent citral composition respectively. The citral chemotype in basil have been reported to occur in high proportion in a cultivar of *O. americanum* species, however none has been reported from *O. basilicum*. Furthermore, the major components reported in other cultivars of *O. basilicum* were not found in this cultivar except linalool, suggesting that this cultivar is of the citral chemotype in accordance with the proposed classification schemes (Lawrence, 1992; Grayer, 1996).

O. tacilium, on the other hand is an estragole rich cultivar with comparably higher estragole content, while *O. canum* is a eugenol-linalool rich cultivar. It was also noted that linalool was present in all the three species of basil while geranial and cis-α-bisabolene occurred in *O. basilicum* and *O. tacilium*. Eugenol and 1,8-cineole occurred only in *O. canum*. The other monoterpenes occurred in traces and in various proportions of composition.

On the basis of the chemical biogenesis as proposed earlier (Lawrence, 1992; Grayer *et al*. 1996), *O. basilicum* is composed predominantly of the terpenic group and is therefore derived from a single mevalonic acid biosynthetic pathway. Likewise, *O. tacilium* is composed predominantly of estragole, belonging to the phenylpropanoid group and is therefore derived through the shikimic acid pathway. *O. canum*, on the other hand, is composed of eugenol and linalool, which have been categorized as belonging to the phenylpropanoid and terpenic groups respectively. Eugenol and linalool in *O. canum* were found to be in quantities greater than 20 percent, which suggests that the biogenetic mechanisms that operate in the production of the components in *O. canum* are dual in nature.

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