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**Research Article**

# Essential oil profiles of major populations *Zingiber officinale* Rosc. utilized in Malaysia for traditional medicine

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***ABSTRACT.*** Zingiberaceae is one of the largest ginger families of the plant kingdom and are often regarded as an important herbaceous species. Domestic gingers, *Zingiber officinale* Rosc., of three major populations from China, Keningau and Bentong were analysed for their essential oil composition. A total of 80 volatile organic constituents from the essential oil were detected using Gas Chromatography- Mass Spectrometry (GCMS) and its volatiles hydrocarbons were determined to contain hydrocarbon monoterpene (7.14%-10.87%), oxygenated monoterpene (38.29%-39.29%), hydrocarbon sesquiterpene (19.56%-30.75%), oxygenated sesquiterpene (13.39%-15.79%) and oxygenated diterpene (2.25%-2.27%). Volatile hydrocarbon gernial (28.00%) was present as a major metabolite in the essential oil of *Z. officinale* from Keningau while neral (25.20%-28.33%) was detected as a major metabolite in essential oils of specimens from Bentong and China. Nine common volatile compounds (geraniol acetate, borneol, α-terpineol, β-linalool, ρ-cymen-8-ol, α-curcumene, caryophyllene oxide, nerolidol and α-elemol) were also found to be consistent in all the specimens studied.

*Keywords:Zingiber officinale* Rosc., essential oil, Geranial, Neral, chemotaxanomy, anti-bacterial activity.

## INTRODUCTION

*Zingiber officinale* Rosc., or the common ginger is a member of Zingiberaceae family that has been cultivated and used for thousands of years as a spice and condiment in food, beverages and traditional medicine. As a medicinal herb, the rhizome of this plant has been documented to be an essential component in traditional Chinese medicine and inAyurvedic and Tibb-Unani practices (Sukari *et al.*, 2008). The extract of ginger is extensively reported in the management of various ailments including headaches, colds, fever, nausea, rheumatic disorders, gingivitis and muscular discomfort. In recent years, secondary metabolites of ginger are shown to possess pharmacological potential in anti-oxidant, anti-inflammatory and anti-cancer activities (Chan *et al.*, 2007; Shukla & Singh, 2007; Singh *et al.*, 2008; Koch *et al.*, 2008). Diverse array of gingerols, shogaols, 3-dihydroshogaols, paradols, dihydroparadols, acetyl derivatives of gingerols, gingerdiols,

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mono- and di-acetyl derivatives of gingerdiols, 1-dehydrogingerdiones, diarylheptanoids, and methyl ether derivatives isolated from its rhizome have been identified as the main cause of pharmacological activities (Jolad *et al.*, 2004).

The fresh aromatic fragrance of this herb could be attributed to its rich highly volatile essential oil. To date, close to 50 volatile components of the oil have been identified using various techniques of gas chromatography (GC). A blend of monoterpenoids (β-phellandrene, cineole, curcumene, geraniol, citral and borneol) and sesquiterpenoids (zingiberol, β-sesquiphellandrene and α-zingiberene) were identified as major odour-defining compounds in the essential oil of *Z. officinale* (Smith & Robinson, 1981; MacLeod & Pieris, 1984). Based on extensive reports, it has been established that essential oil constituents vary by population due to variation in stock, locality and microclimate (Gardelli *et al.*, 2008). Since the differences in essential oil composition have an impact on the potency of its medicinal characteristics, it is imperative that sufficient information is acquired on the major populations that are widely used as traditional medicine in Malaysia. Since such information is not easily obtained for the major population of *Z. officinale* used in Malaysia, the present investigation delves upon the essential oil composition of three major populations of *Z. officinale* known for their medicinal importance in Malaysia.

## METHDOLOGY

### Plant materials and extraction of essential oil

Matured rhizomes of *Z. officinale* from Keningau (Sabah) and Bentong (Pahang) were collected from their respective farms in June 2011. The specimen from China was purchased from a traditional medicinal shop in Gaya Street, Kota Kinabalu. These specimens were identified based on their morphological appearances and were positively identified as *Z. officinale*. Ginger specimens were removed from mud and organic matter, and washed with distilled water three times. Cleaned specimens (300 g) were then chopped and subjected to hydro distillation using a Clevenger-type apparatus for eight hours. The distilled oil was collected in a pentane (Merck, Germany), dried over anhydrous sodium sulphate (Sigma, USA), concentrated *in vacuo*, stored in an air-tight glass vial flushed with nitrogen ($N_2$) gas and kept at -81°C prior to analysis.

### GC-MS analysis of the essential oil

Analysis of essential oil was performed using Shimadzu QP-2010 chromatography coupled with Shimadzu GCMSQP-2010 plus detector (Shimadzu Corp., Japan) using SGE BPX-5 (30.0m X 0.25 μmi.d., film thickness 0.25 μm) fused silica capillary column. High purity helium was used as the carrier gas at a constant flow rate of 0.8 ml/min. For analysis, a 1l sample was injected (split ratio 100:1) into GCMS using the AOC5000 autoinjector. The initial temperature was set at 50°C, heated at a rate of 3°C/min to 280°C and held isothermally for five minutes. The ion source temperature for this analysis was set at 200°C while interface temperature was set at 280°C. The mass spectrometer was set to operate in electron ionization mode with an ionizing energy of 70 eV as acquisition mass range from 40 a.m.u to 450 a.m.u. at 0.25 scan/s.

Resolved chromatography peaks were identified by comparing their mass fragment patterns against two standard libraries: 1) National Institute for Standard and Technology (NIST) 1998, 2) Flavours and Fragrance of Natural and Synthetic Compounds (FFNSC) V 1.2. In addition, retention indices of the respective peaks were determined based on a homologous series of *n*-alkanes ($C_8 - C_{40}$) (Custom Retention Time Index Standard, Restek Corp, United States) external standard analysed under the same operating conditions and calibrated based on Automatic Adjustment of Compound Retention Time (AART) function of the GCMS. Relative concentrations of the

essential oil components were calculated based on GC peak area with the AART correction factors.

## RESULTS AND DISCUSSION

The essential oils obtained from hydro-distillation were fragrant yellowish oils and their yields were calculated as 4.33%, 5.00% and 5.67% of the fresh rhizome of *Z. officinale* obtained from Keningau, Bentong and China, respectively. A total of 80 well resolved peaks were analysed and positively identified to be highly volatile organic constituents of the specimens investigated based on their retention indices (RI) and mass fragmentation pattern. The population from Keningau exhibited a total of 43 volatile constituents, compared to 44 and 55 constituents identified in specimens from Bentong and China, respectively. Detailed identification of volatile organic constituents of *Z. officinale* from the three populations studied is presented in Table 1. The chemical constituents are arranged according to increasing retention time.

**Table 1.** Essential oil composition (%) in *Z. officinale* from Keningau, Bentong, and China.

| RT (min) | RRI | Compounds | Concentration (%) | | | Identification mode |
|---|---|---|---|---|---|---|
| | | | K | B | C | |
| 8.49 | 933 | α-pinene | - | 0.64 | 0.15 | MS,FFNSC |
| 9.20 | 953 | Camphene | - | 3.75 | 0.86 | MS,FFNSC |
| 9.43 | 716 | Isobutylmethylcarbinol | - | - | 0.31 | MS, NIST |
| 10.66 | 938 | 5-hepten-2-one | - | 1.12 | - | MS, NIST |
| 11.48 | 1005 | Octanal | - | - | 0.07 | MS, NIST |
| 12.36 | 1042 | *o*-cymene | - | 0.67 | - | MS, NIST |
| 12.50 | 1030 | Limonene | - | 0.97 | 1.13 | MS,FFNSC |
| 12.69 | 1059 | Eucalyptol | - | 5.17 | 2.09 | MS, NIST |
| 14.40 | 1080 | *trans*-linalool oxide | - | 0.28 | - | MS,FFNSC |
| 15.16 | 1081 | *cis*-linalool oxide | - | 0.25 | - | MS,FFNSC |
| 15.38 | 1052 | 2-nonanone | - | 1.12 | 0.11 | MS,NIST |
| 15.44 | 1069 | Crypton | - | 0.77 | 0.12 | MS,NIST |
| 15.73 | 1082 | β-Linalool | 0.45 | 1.06 | 0.66 | MS,NIST |
| 18.28 | 1149 | Camphor | - | - | 0.41 | MS,FFNSC |
| 18.54 | 1121 | *L*-camphor | - | 0.48 | - | MS,NIST |
| 19.12 | 1125 | β-citronellal | 0.14 | - | - | MS,NIST |
| 19.38 | 1088 | Borneol | 3.40 | 6.29 | 10.06 | MS,NIST |
| 19.61 | 1089 | Camphene hydrate | - | 0.22 | 0.19 | MS,NIST |
| 19.88 | 1161 | α-pinene oxide | 0.10 | - | - | MS, NIST |
| 20.09 | 1137 | 4-terpineol | 0.14 | - | 0.40 | MS, NIST |
| 20.11 | 1197 | *p*-cymen-8-ol | 0.14 | 0.78 | 0.29 | MS, NIST |
| 20.43 | 1198 | α-terpineol | 1.38 | 2.05 | 4.56 | MS, FFNSC |
| 20.55 | 1197 | Myrtenal | - | - | 0.17 | MS, FFNSC |
| 20.80 | 1206 | Capraldehyde | - | - | 0.12 | MS, FFNSC |
| 21.54 | 1215 | Oxiranecarboxyaldehyde | - | - | 0.34 | MS, NIST |
| 21.49 | 1228 | Nerol | 0.67 | - | - | MS, NIST |
| 21.69 | 1232 | *L*-citronellol | 1.71 | 0.50 | 0.68 | MS, FFNSC |
| 22.79 | 1255 | Geraniol | 7.37 | 1.91 | 1.66 | MS, FFNSC |
| 22.81 | 1265 | Dec-2(*E*)-enal | 0.08 | - | - | MS, FFNSC |
| 22.90 | 1268 | Geranial | 28.00 | - | - | MS, FFSNC |
| 22.92 | 1275 | Isobornylformate | - | 0.80 | - | MS,NIST |
| 24.37 | 1285 | Bornyl acetate | 0.23 | - | 0.41 | MS, FFNSC |
| 24.72 | 1294 | Nonyl methyl ketone | 0.46 | 4.38 | 0.67 | MS, FFNSC |
| 27.15 | 1509 | Citronellyl 2-butenoate | - | - | 0.25 | MS, NIST |
| 27.46 | 1300 | Geranyl acetate | - | 0.66 | - | MS,FFNSC |
| 27.55 | 1328 | 2(1H)-Napthalenone | - | 0.58 | - | MS,NIST |
| 27.68 | 1476 | 2-tridecanol | - | 0.57 | - | MS,NIST |

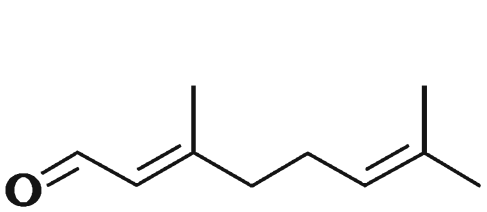
| | | | | | | |
|---|---|---|---|---|---|---|
| 27.89 | 1342 | Neric acid | - | 5.25 | - | MS,NIST |
| 28.00 | 1350 | Citronellyl acetate | 0.48 | - | - | MS, FFNSC |
| 28.05 | 1367 | Cyclosativene | 0.04 | 0.17 | 0.07 | MS, FFNSC |
| 28.12 | 1174 | Neral | - | 28.33 | 25.20 | MS,NIST |
| 28.34 | 1375 | α-copaene | 0.13 | 0.21 | 0.16 | MS, FFNSC |
| 28.47 | 1352 | Geraniol acetate | 2.96 | 0.31 | 0.31 | MS, FFNSC |
| 28.91 | 1386 | 1H-cycloprop[e]azulene | - | 0.13 | - | MS,NIST |
| 29.01 | 1390 | β-elemene | 0.20 | 0.31 | 0.17 | MS, FFNSC |
| 29.35 | 1496 | α-zingiberene | - | - | 0.08 | MS, FFNSC |
| 31.50 | 1452 | β-*E*-farnesene | 0.17 | - | 0.27 | MS, FFNSC |
| 31.67 | 1523 | β-sesquiphellandrene | 3.20 | - | 4.09 | MS, FFNSC |
| 32.02 | 1458 | Alloaromadendrane | 0.15 | - | 0.08 | MS, FFNSC |
| 32.85 | 1524 | α-curcumene | 8.38 | 11.77 | 9.28 | MS,NIST |
| 32.62 | 1480 | Germacrene D | - | 0.07 | 0.16 | MS,NIST |
| 33.66 | 1490 | Azulene | - | 1.14 | - | MS,NIST |
| 33.69 | 1435 | γ-muurolene | - | - | 1.46 | MS, NIST |
| 33.78 | 1432 | α-*trans*-bergamotene | 0.26 | - | 0.16 | MS, FFNSC |
| 33.83 | 1387 | 7-*epi*-sesquithujene | 4.79 | - | 1.18 | MS, FFNSC |
| 33.90 | 1508 | β-bisabolene | - | 1.54 | 2.83 | MS, FFNSC |
| 34.06 | 1504 | α-*E*,*E*-farnesene | 4.38 | - | - | MS, FFNSC |
| 34.20 | 1512 | γ-cadinene | 3.24 | - | 0.09 | MS, FFNSC |
| 34.23 | 1469 | δ-cadinene | 0.27 | - | - | MS,NIST |
| 34.33 | 1513 | β-cadinene | - | 3.01 | 2.24 | MS, FFNSC |
| 34.35 | 1523 | β-guaiene | - | 1.27 | 0.22 | MS,NIST |
| 34.56 | 1423 | β-cedrene | 4.23 | - | - | MS, FFNSC |
| 35.69 | 1546 | α-elemol | 0.67 | 0.57 | 0.58 | MS, FFNSC |
| 35.84 | 1507 | Caryophyllene oxide | 0.32 | 0.43 | 0.32 | MS,NIST |
| 36.09 | 1562 | Nerolidol | 1.05 | 0.53 | 0.93 | MS, FFNSC |
| 36.13 | 1557 | Germacrene B | 0.16 | - | 0.67 | MS, FFNSC |
| 37.08 | 1530 | Epiglobulol | 0.39 | - | 0.85 | MS,NIST |
| 37.88 | 1688 | α-bisabolol | 2.07 | - | 1.87 | MS, FFNSC |
| 40.56 | 1282 | *cis*-terpin hydrate | - | 0.32 | - | MS, FFNSC |
| 40.43 | 1593 | 2-Naphthalenemethanol | - | 1.48 | 0.34 | MS,NIST |
| 40.53 | 1656 | β-eudesmol | 2.06 | - | 1.14 | MS, FFNSC |
| 41.17 | 1594 | Viridiflorol | - | - | 0.25 | MS, FFNSC |
| 41.23 | 1696 | Juniper camphor | 0.17 | 0.23 | - | MS,FFNSC |
| 44.52 | 2046 | 1,6,10,14-hexadecatetraen-3-ol | 0.20 | - | 5.27 | MS, NIST |
| 44.60 | 1766 | *E*-nuciferol | 0.34 | 0.28 | 0.54 | MS, NIST |
| 44.86 | 1737 | Farnesal | 0.36 | - | 0.25 | MS, FFNSC |
| 45.52 | 1635 | (-)-Isolongifolol | 1.56 | - | 1.19 | MS, NIST |
| 54.46 | 1117 | Neopentylidenecyclohexane | - | - | 0.11 | MS, NIST |
| 56.08 | 1226 | Cyclopropanecarboxyaldehyde | - | 0.36 | - | MS, NIST |
| 68.32 | 2192 | Geranylgeraniol | 0.11 | - | 0.14 | MS, NIST |
| Composition of grouped volatile compounds (%) | | | | | | |
| *Monoterpenes (hydrocarbon)* | | | - | 10.87 | 7.14 | |
| *Monoterpenes (oxygenated)* | | | 38.64 | 50.00 | 39.29 | |
| *Sesquiterpenes (hydrocarbon)* | | | 30.75 | 19.56 | 26.14 | |
| *Sesquiterpenes (oxygenated)* | | | 14.95 | 15.79 | 13.39 | |
| *Diterpenes (oxygenated)* | | | 2.27 | - | 2.25 | |
| *Total (%)* | | | 86 61 | 92.73 | 88.21 | |

RRI, retention indices on BPX 5; Compounds are listed in order of elution based on BPX 5; Volatile compounds were identified based on their MS fragment pattern and retention index as compared with NIST and FFNSC data bases. Abbreviations: K~Keningau, B~Bentong and C~China.

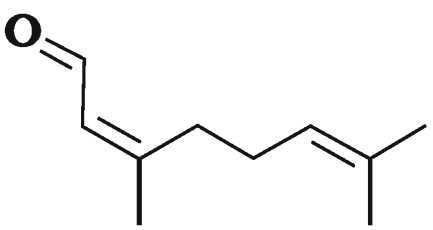
All three populations exhibited the presence of a major chemical constituent (25.00% - 29.00%). Geranial (28.00%) was present as a major metabolite in the oil of *Z. officinale* from Keningau while neral (25.20%-28.33%) was detected as a major metabolite in specimens from Bentong and China. The chemical structures of these two major metabolites are illustrated in Figure 1. These are stereoisomers of citral, alpha-citral (geranial) and beta-citral (neral). It is also interesting to note that geranial was only found in ginger from Keningau, while neral was only found in gingers from Bentong and China.

Geranial and neral have been reported as major components of essential oils of herbs like ginger and lemongrass. Both the isomers are usually found to be present together in the specimens studied (Tajidin *et al.,* 2012). Distribution trends observed in these ginger populations are unique. Selective presence of geranial and neral could be explained based on their biosynthesis pathway. Enzyme geraniol synthase converts geranyldiphosphate to geraniol, while geraniol is further converted to geranial by enzyme geraniol dehydrogenase (Wolken & Van der Werf, 2001). Upon conversion, usually spontaneous change occurs from geranial to neral. In the Keningau specimen, spontaneous change from geranial to neral is not detected. On the other hand, in the Bentong and China specimens, total conversion could have taken place. These observations could be due to dynamics of enzymes involved in the conversion between geranial and neral. It could also be due to some properties of growth conditions. This variation could be genetically driven as the conversion of chemical compounds is influenced by enzymes. Soil pH where the specimens were cultivated could be another important factor as acidity and alkaline of soil will influence the production of enzymes in plants. There is lack of investigation on this aspect and no certain conclusion can be drawn.

Detailed analysis also revealed the presence of additional nine common compounds (geraniol acetate, borneol, α-terpineol, β-linalool, ρ-cymen-8-ol, α-curcumene, caryophylleneoxide, nerolidol and α-elemol) that were found consistently in all the specimens studied. In addition, these volatiles could further be grouped into hydrocarbon monoterpene (7.14%-10.87%), oxygenated monoterpene (38.64%-39.29%), hydrocarbon sesquiterpene (19.56%-30.75%), oxygenated sesquiterpene (13.39%-15.79%) and oxygenated diterpene (2.25%-2.27%). Interestingly, the presence of hydrocarbon monoterpenes is only noted in specimens from China and Bentong. Presence of citral as a major volatile marker in the oil of *Z. officinale* is not alarming as it was also reported in gingers from India (Singh *et al.* in 2009). Although findings stated geranial is a common volatile metabolite found in plants, the differences in the chemical composition of the oil and oleoresin from the same plants could differ due




Geranial




Neral

**Figure 1.**The chemical structure of geranial and neral.

to environmental, genetic and microclimate factors (Smith & Robinson, 1981).

Majority of volatile chemotypes in this herbal plant were found to be in classes of oxygenated monoterpenes, hydrocarbon sesquiterpenes and oxygenated sesquiterpenes. Presence of oxygenated monoterpenes and oxygenated sesquiterpenes in the oil of *Z. officinale* has been associated to its bioactive potential in inhibiting the growth of pathogens and microbes. Volatile constituents such as α-terpineol, linalool and terpinen-4-ol were reported to show efficient activity against bacteria and fungi (Ali *et al.*, 2008). Moreover, ginger oil is reported to be 100% effective against fungi in food poison assay with no cyctotoxic effect (Singh *et al.*, 2008). This could explain the reason why ginger is incorporated into food to reduce spoilage.

Based on this investigation, it was apparent that *Z. officinale* from Keningau contains geranial as a major volatile chemical marker while *Z. officinale* from Bentong and China contained neral as their major chemical markers. Despite the above-mentioned difference, the composition of the primary volatile group of *Z. officinale* from the three different geographical regions is made up of oxygenated monoterpenes, hydrocarbon sesquiterpenes and oxygenated sesquiterpenes. In terms of biological potential, the chemical constituents analysis indicates that all three populations contain sufficient volatile essential oils to exhibit the efficacy expected in most traditional medicine practices.

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