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*Full Length Research Paper*

# Characterization of ginger (*Zingiber officinale* Rosc.) germplasm based on volatile and non-volatile components

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**A sample of world germplasm of ginger consisting of 46 accessions was profiled for its volatile oil constituents, using gas chromatography-mass spectrophotometry (GC/MS) and grouped with three similarity coefficients, besides analyzing its pungent principles using high performance liquid chromatography (HPLC). A total of 60 compounds were identified in the essential oil. Two of the three similarity coefficients (Jaccard's and Sorensen - Dice similarity coefficients) placed the accessions in 14 groups. The dendrograms constructed using this two similarity coefficients were similar in form and content. However, the grouping pattern of the accessions in the third dendrogram (Simple Matching similarity coefficient) was slightly different. Three accessions namely, 'Kintoki', 'Brazil' and 'Pink Ginger' were unique. HPLC analysis of the pungent principles showed 6-gingerol as the predominant pungent principle in all the ginger accessions except the exotic ginger, 'Oman', in which 8-shogaol was the predominant one.**

**Key words:** Essential oil, gingerol, shogaol, *Zingiber officinale*.

## INTRODUCTION

Ginger (*Zingiber officinale* Rosc.), a monocotyledon belonging to family Zingiberaceae, is an important spice and medicinal plant originated in South-East Asia and introduced to many parts of the globe (Park and Pizutto, 2002; Burkill, 1996). Rhizome of ginger has been used as a medicine in Chinese, Indian and Arabic herbal traditions since ancient times as carminative or antiflatulent, diaphoretic, antispasmodic, expectorant, peripheral circulatory stimulant, astringent, appetite stimulant, anti-inflammatory agent, diuretic and digestive aid, etc. (Kizhakkayil and Sasikumar, 2011). Moreover it also imparts flavour and pungency to food and beverages and is mainly consumed as fresh paste, dried powder, slices preserved in syrup, candy (crystallized ginger) or flavouring tea.

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About 50 cultivars in addition to seven improved varieties have been reported in India (Sasikumar et al., 1999). Essential oil and pungent principles are the deciding factors for the qualities of ginger cultivars. Essential oil compositions of ginger from different geographical area have already been reported (Wohlmuth et al., 2006; van Beek et al., 1987; MacLeod and Pieris, 1984). Although many cultivars are prevalent in the country, only minimum attention has been paid to the systematic evaluation and characterization of this clonally propagated crop. Chemical investigation carried out in the past showed that ginger essential oil is mainly composed of zingiberene, α-curcumene, β-sesquiphellandrene, citral and camphene, etc; and these compounds are characteristic for geographical and varietal properties of ginger (Singh et al., 2008; Lawrence, 1997; Lawrence, 2000).

The present study is an attempt to characterize a global collection of ginger germplasm based on the gas chromatography-mass spectrophotometry (GC/MS) profile of volatile oil and the high performance liquid

chromatography (HPLC) analysis of pungent principles.

## MATERIALS AND METHODS

### Plant materials

Fully matured rhizomes of ginger accessions were collected from the Germplasm Conservatory of Indian Institute of Spices Research, Peruvannamuzhi, Kozhikode, Kerala, India. 46 ginger (*Z. officinale*) accessions, which include released varieties, exotic and primitive types were used in the study (Table 1). Fresh rhizomes were used for the HPLC analyses of ginger whereas sun dried rhizomes were used for GC/MS analysis.

### GC/MS analysis of essential oil

### Chemical analysis

The steam volatile oil from ginger was extracted and the oil was analyzed using a Shimadzu GC-2010 gas chromatograph equipped with QP 2010 mass spectrometer and RTX–5 column (30 m × 0.25 mm, film thickness 0.25 µm). Helium was used as the carrier gas at a flow rate of 1.67 ml $min^{-1}$. The injection port was maintained at 250°C; the detector temperature was 220°C; oven temperature was programmed as follows: 60°C for 5 min and then increased to 110°C at the rate of 5°C $min^{-1}$, then up to 170°C at the rate of 3°C $min^{-1}$, again up to 220°C at the rate of 5°C $min^{-1}$, at which the column was maintained for 3 min. The split ratio was 1:40 and ionization energy 70 eV.

### Statistical analysis

GC/MS data were analyzed based on the Jaccard's similarity coefficient, Sorensen - Dice similarity coefficient and Simple Matching similarity coefficients for binary data via SIMQUAL of the NTSYSpc ver. 2.02i Package. For the dendrogram data, the presence of compounds with ≤ 1% concentration indicates '1', ≤ 5% indicates '1,1', ≤ 10% indicates '1,1,1',≤ 15% indicates '1,1,1,1' , ≤ 20 indicates '1,1,1,1,1', ≤ 25% indicates '1,1,1,1,1,1', ≤ 30 indicates '1,1,1,1,1,1,1' and the absence of the particular compound indicates '0'. The dendrograms were constructed based on these data.

### HPLC analysis of ginger for gingerol and related compounds

Gingerol and its related compounds were analyzed in a Waters HPLC system and quantitatively detected using a Diode array detector. The stationary phase was C-18, particle size 5 µm, pre – packed in a 250 × 4.6 mm I.D column. The mobile phase used was water and acetonitrile with a flow rate of 1 ml $min^{-1}$. Pump A carried water and pump B carried acetonitrile. A rheodyne injector with a 20 µl loop was used for injecting the sample. The analyses were carried out at ambient temperature. Diode array detector with 282 nm was used for the analysis. The programme for HPLC analysis is shown in the Table 2.

## RESULTS

## GC/MS analysis

The GC/MS analysis of essential oil from the sun-dried ginger cultivars identified a total of 60 compounds constituting 71.15 to 94.57% of the total volatile oil constituents (Table 3). The highest percentage of essential oil constituents was identified in Acc. No. 50 while the lowest was identified in the exotic ginger 'Kintoki', respectively.

Zingiberene was the major component present in the essential oils of all the 46 ginger accessions except the exotic ginger 'Kintoki', in which α curcumene was the major component. The highest percentage of zingiberene was observed in the cultivar, 'Angamali' (29.6%) and the lowest percentage in 'Kintoki' (6.79%). Z-citral (neral) and E-citral (geranial) were the important monoterpene aldehydes found in all the ginger genotypes except the exotic ginger 'Brazil'. One of the major sesquiterpenes, farnasene, was absent in the essential oil of the ginger 'Accession 50' and present in all other accessions studied. The highest percentage of farnasene was observed in the exotic ginger 'Brazil' (15.1%) and the lowest percentage in the cultivar 'Ambalawayalan' (4.1%). High amount of α curcumene and β sesquiphellandrene were detected in all the ginger accessions. Alpha curcumene ranged from 1.9 ('Angamali' and 'Naval parasi') to 6.66% (Suruchi') and β sesquiphellandrene ranged from 6.39 (Rio-de-Janeiro) to 12.17% (Brazil). 1, 8-cineole was found in all the ginger genotypes except (Kintoki). The content ranged from 1.08 (Bhaise) to 6.96% (Jolpaiguri). Alpha pinene, another important monoterpene observed in all the accessions, ranged from 0.46 (Mananthodi) to 1.9% (Kintoki). Beta phellandrene was absent in both (Kakakkalan) and (Jolpaiguri).

Among the released varieties studied, the percentage of zingiberene was highest in 'Suprabha' (24.56%) followed by 'Mahima' (23.95%), 'Suruchi' (22.96%), 'Varada' (21.64%) and 'Rejatha' (20.93%). Other major compounds identified were z-citral, citral, farnesene, beta-sesquiphellandrene, α- curcumene, camphene, beta phellandrene, 1, 8-cineol, endo borenol and nerolidol.

Among the primitive type gingers, the highest content of zingiberene (25.86%) and lowest content of farnesene (4.29%) were observed in the collection, 'Sabarimala'. Citral content was high in 'Kakkakkalan' when compared to the other primitive type gingers. The amount of β-sesquiphellandrene was found to be almost equal among the primitive type gingers, 'Sabarimala', 'Kozhikkalan', 'Kakakkallan' and 'Ellakkallan'.

## Cluster analysis

Similarity was calculated using Jaccard's, Sorensen - Dice and Simple Matching coefficients. In all the similarity matrices, 'Ambalawayalan' and 'Thodupuzha collection-2' showed maximum similarity (0.94, 0.94 and 0.96 in Jaccard's, Sorensen - Dice and Simple Matching, respectively) and the least similarity between 'Kintoki' and 'Jamaica' (0.46, 0.63 and 0.59 in Jaccard's, Sorensen-Dice and Simple Matching, respectively). The dendrograms constructed UPGMA method from Jaccard's, Sorensen- Dice and Simple Matching coefficients based

**Table 1.** Ginger accessions studied.

| S/N | Name | Acc No. | Remark |
|---|---|---|---|
| 1 | 'Varada' | 64 | Released variety from Indian Institute of Spices Research, Kozhikode, Kerala, India. |
| 2 | 'Mahima' | 117 | Released variety from Indian Institute of Spices Research, Kozhikode, Kerala, India. |
| 3 | 'Rejatha' | 35 | Released variety from Indian Institute of Spices Research, Kozhikode Kerala, India. |
| 4 | 'Suruchi' | 714 | Released variety from Orissa University of Agriculture and Technology, High Altitude Research Station, Pottangi, Koraput, Orissa, India. |
| 5 | 'Suprabha' | 293 | Released variety from Orissa University of Agriculture and Technology, High Altitude Research Station, Pottangi, Koraput, Orissa, India. |
| 6 | 'Himachal' | 294 | Land race from Himachal Pradesh, India. |
| 7 | 'Maran' | 295 | Land racefrom Assam, India. |
| 8 | 'Nadia' | 27 | Land race from West Bengal, India. |
| 9 | 'Karakkal' | 20 | Land race from Pondicherry, India. |
| 10 | 'Mananthody' | 244 | Land race from Wayanadu, Kerala, India. |
| 11 | 'Sabarimala' | 246 | Primitive type collected from Sabarimala forests, Western Ghats, Kerala, (slender rhizome), India. |
| 12 | 'Kozhikkalan' | 537 | Primitive type collected from Nedumangad, Kerala (slender rhizome), India. |
| 13 | 'Ellakallan' | 463 | Primitive type collected from Idukki, Kerala (slender rhizome), India. |
| 14 | 'Kakakkalan' | 558 | Primitive type collected from Nedumangad, Kerala (slender rhizome), India. |
| 15 | 'Pakistan' | 733 | From Pakistan. |
| 16 | 'Oman' | 734 | From Oman. |
| 17 | 'Brazil' | 736 | From Brazil. |
| 18 | 'Jamaica' | 17 | From Jamaica originally. |
| 19 | 'Rio-de-Janeiro' | 59 | From Brazil originally. |
| 20 | 'Pink ginger' | 731 | Collected from Meghalaya State, India. |
| 21 | 'Bakthapur' | 563 | From Nepal. |
| 22 | 'Kintoki' | 648 | From Japan. |
| 23 | 'Nepal' | 575 | Collected from Nepal. |
| 24 | 'China' | 9 | Originally from China. |
| 25 | 'Juggigan' | 18 | Originally from Nigeria. |
| 26 | 'Acc. No. 50' | 50 | Kerala, India. |
| 27 | 'Pulpally' | 56 | Collected from Pulpally,Kerala, India. |
| 28 | 'Acc. No.95' | 95 | From Kerala, India. |
| 29 | 'Ambalawayalan' | 109 | Collected from Wynad, Kerala, India. |
| 30 | 'Kozhikkode' | 162 | Collected from Kozhikkode, Kerala ,India. |
| 31 | 'Thodupuzha-1' | 204 | Collected from Thodupuzha, Kerala, India. |
| 32 | 'Konni local' | 206 | Collected from Konni, Kerala, India. |
| 33 | 'Angamali' | 214 | Collected from Angamali, Kerala, India. |
| 34 | 'Thodupuzha -2' | 217 | Collected from Thodupuzha, Kerala, India. |
| 35 | 'Kottayam' | 225 | Collected from Kottayam, Kerala, India. |
| 36 | 'Palai' | 228 | Collected from Palai market, Kerala, India. |
| 37 | 'Silent valley' | 240 | Collected from Silent valley forests of Western Ghats, India. |
| 38 | 'Waynadu local' | 251 | Collected from Wynad, Kerala, India. |
| 39 | 'Vizagapatnam-1' | 411 | Collected from Vizagapatnam, Andrapradesh, India. |
| 40 | 'Vizagapatnam-2' | 420 | Collected from Vizagapatnam, Andrapradesh, India. |
| 41 | 'Fiji' | 430 | From Queensland. |
| 42 | 'Gorubathani' | 515 | Collected from Sikkim, India. |
| 43 | 'Bhaise' | 552 | Collected from Kalimpong, West Bengal, India. |
| 44 | 'Naval parasi' | 569 | Collected from Nepal. |
| 45 | 'Neyyar ' | 650 | Collected from Neyyar, Kerala, India. |
| 46 | 'Jolpaiguri' | 654 | Collected from Jolpaiguri, West Bengal, India. |

**Table 2.** Chromatographic programme time (min).

| Time (min) | Water (%) | Acetonitrile (%) |
|---|---|---|
| 00 | 55 | 45 |
| 08 | 50 | 50 |
| 15 | 45 | 55 |
| 40 | 10 | 90 |
| 45 | 55 | 45 |
| 55 | 55 | 45 |

**Table 3.** Essential oil profile of 46 ginger accessions.

| | | Accession | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Compound** | **RI** | **9** | **18** | **50** | **56** | **95** | **109** | **162** | **204** | **206** | **214** | **217** | **225** | **228** | **240** | **251** | **411** | **420** | **430** | **511** | **552** | **569** | **650** | **654** |
| 2- Heptanol | 902 | - | 0.22 | 0.14 | 0.13 | 0.29 | 0.15 | 0.18 | 0.12 | - | 0.10 | 0.21 | 0.27 | 0.24 | 0.40 | - | 0.22 | 0.14 | 0.20 | 0.87 | - | 0.14 | 0.43 | - |
| Alpha pinene | 935 | 1.20 | 1.07 | 1.50 | 1.53 | 1.39 | 1.63 | 1.35 | 1.64 | 1.37 | 1.29 | 1.31 | 1.47 | 0.61 | 1.43 | 1.11 | 1.32 | 1.22 | 1.30 | 1.33 | 1.05 | 0.80 | 1.73 | 1.71 |
| Camphene | 950 | 3.05 | 3.65 | 5.84 | 5.45 | 4.86 | 5.82 | 5.18 | 5.60 | 5.52 | 4.38 | 4.66 | 4.80 | 2.50 | 4.87 | 3.31 | 4.68 | 4.31 | 4.69 | 4.22 | 3.93 | 2.26 | 6.26 | 6.34 |
| 2-Beta pinene | 977 | 0.21 | 0.13 | 0.21 | 0.12 | 0.17 | 0.22 | 0.19 | 0.19 | - | 0.18 | 0.20 | 0.19 | 0.10 | 0.15 | 0.21 | 0.19 | 0.15 | 0.17 | 0.30 | - | 0.17 | 0.19 | 0.23 |
| 6-Methyl-5-hepten-2-one | 989 | 0.54 | 0.33 | 0.27 | 0.15 | 0.43 | 0.18 | 0.22 | 0.46 | - | 0.23 | 0.21 | 0.40 | 0.48 | 0.29 | 0.13 | 0.33 | 0.38 | 0.34 | 0.36 | 0.15 | 0.27 | 0.45 | 0.34 |
| Beta-myrcene | 993 | 1.02 | 1.02 | 1.12 | 1.11 | 1.24 | 1.00 | 1.00 | 1.36 | 1.10 | 0.88 | 0.94 | 1.26 | 0.85 | 1.18 | 1.03 | 1.12 | 1.11 | 1.2 | 1.17 | 1.27 | 0.96 | 1.39 | 1.19 |
| Alpha phellandrene | 1006 | 0.23 | 0.19 | 0.21 | 0.12 | 0.22 | 0.22 | - | 0.23 | - | 0.23 | 0.18 | 0.23 | 0.11 | 0.22 | 0.21 | 0.16 | 0.17 | 0.21 | - | 0.10 | 0.24 | 0.25 | - |
| Beta phellandrene | 1030 | 4.57 | 3.03 | 3.96 | 4.38 | 4.10 | 3.42 | 3.44 | 3.86 | 2.22 | 4.28 | 2.65 | 4.21 | 3.33 | 3.93 | 3.54 | 2.55 | 3.26 | 3.71 | 4.08 | 1.89 | 3.97 | 4.31 | - |
| 1,8-ceneole | 1032 | 3.27 | 2.30 | 4.17 | 3.03 | 2.72 | 3.85 | 3.26 | 3.00 | 5.69 | 2.77 | 3.32 | 3.25 | 2.01 | 2.29 | 2.50 | 3.28 | 2.50 | 2.75 | 5.26 | 1.08 | 2.91 | 3.12 | 6.96 |
| Cis-ocimene | 1051 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 2.67 | - | - | - |
| Alpha terpinolene | 1090 | 0.30 | 0.21 | 0.23 | 0.23 | 0.26 | 0.23 | 0.21 | 0.26 | 0.28 | 0.21 | 0.21 | 0.24 | 0.22 | 0.23 | 0.21 | 0.24 | 0.22 | 0.25 | 0.22 | 0.20 | 0.19 | 0.34 | 0.25 |
| 2-nonanone | 1092 | - | 0.14 | - | - | 0.18 | - | - | - | 0.10 | - | - | 0.16 | 0.21 | 0.22 | - | 0.13 | - | 0.11 | 0.26 | - | - | 0.32 | 0.12 |
| Verbenone | 1099 | 0.55 | 0.42 | 0.37 | 0.47 | 0.63 | 0.33 | 0.33 | 0.70 | 0.44 | 0.44 | 0.41 | 0.63 | 0.71 | 0.49 | 0.46 | 0.62 | 0.58 | 0.60 | 0.51 | 0.24 | 0.38 | 0.68 | 0.39 |
| Linalool | 1103 | 0.95 | 1.20 | 2.84 | 2.43 | 1.36 | 2.61 | 2.42 | 1.37 | 2.94 | 0.76 | 2.07 | 1.44 | 1.84 | 1.51 | 0.61 | 1.06 | 1.34 | 1.26 | 1.29 | 1.45 | 1.06 | 1.77 | 1.04 |
| Camphor | 1148 | 0.12 | - | 0.17 | 0.15 | 0.11 | 0.16 | 0.15 | 0.12 | 0.21 | 0.10 | 0.16 | 0.13 | 0.16 | 0.11 | - | 0.13 | 0.12 | 0.11 | 0.12 | - | 0.13 | 0.13 | 0.17 |
| Citronella | 1156 | 0.10 | 0.20 | 0.27 | 0.28 | 0.22 | 0.33 | 0.36 | 0.32 | 0.30 | 0.11 | 0.26 | 0.16 | 0.18 | 0.33 | 0.15 | 0.16 | 0.22 | 0.23 | 0.17 | 0.19 | 0.17 | 0.24 | 0.23 |
| Endo borneol | 1069 | 0.96 | 1.63 | 1.87 | 1.53 | 2.19 | 1.79 | 1.67 | 2.37 | 2.11 | 1.54 | 1.49 | 1.97 | 2.18 | 2.00 | 0.33 | 1.56 | 2.13 | 2.23 | 1.21 | 1.73 | 0.87 | 2.7 | 2.04 |
| Terpinene-4-ol | 1179 | - | - | 0.12 | - | - | 0.13 | - | - | - | - | 0.10 | - | - | 0.10 | - | - | 0.10 | 0.12 | - | - | - | - | - |
| Alpha terpineol | 1194 | 0.89 | 0.61 | 0.91 | 0.84 | 0.66 | 0.90 | 0.81 | 0.78 | 1.08 | 0.72 | 0.82 | 0.83 | 0.85 | 0.65 | 0.25 | 0.77 | 0.72 | 0.76 | 1.27 | 0.59 | 0.75 | 0.86 | 0.94 |
| Myrtenal | 1200 | - | - | - | - | 0.11 | - | - | 0.11 | - | - | - | - | 0.14 | 0.10 | - | - | 0.10 | 0.10 | - | - | - | - | - |
| Beta citronellol | 1232 | 0.72 | 0.83 | 0.60 | 0.62 | 1.18 | 0.60 | 0.79 | 1.34 | 0.66 | 0.53 | 0.48 | 0.93 | 1.71 | 1.20 | 0.58 | 0.88 | 0.99 | 0.96 | 1.03 | 0.97 | 0.94 | 1.25 | 0.88 |
| Z-citral (Neral) | 1247 | 4.64 | 6.64 | 4.05 | 3.57 | 4.40 | 4.46 | 5.55 | 6.05 | 5.69 | 2.28 | 3.54 | 6.85 | 3.22 | 7.66 | 2.60 | 4.38 | 6.32 | 5.89 | 7.73 | 6.71 | 8.07 | 4.26 | 7.01 |
| trans-2-caren-4-ol | 1255 | - | - | - | - | - | - | - | 0.15 | - | 0.20 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Nerol | 1257 | - | - | - | - | - | - | - | 0.39 | - | - | - | - | - | - | - | - | - | 0.41 | - | - | - | - | - |
| Trans -geraniol | 1259 | 2.11 | 1.18 | - | 0.12 | 0.58 | - | 0.19 | - | 0.12 | - | - | 2.23 | 0.87 | 0.73 | 0.28 | 0.62 | 0.83 | - | 1.61 | 1.25 | 1.79 | 0.41 | 0.56 |
| 1-decanol | 1263 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Citral (Geranial) | 1279 | 7.43 | 10.00 | 6.41 | 5.62 | 6.88 | 6.89 | 8.76 | 9.26 | 8.74 | 3.58 | 5.43 | 10.70 | 5.28 | 11.80 | 4.13 | 6.78 | 9.76 | 9.20 | 12.10 | 10.50 | 12.50 | 6.69 | 10.80 |

**Table 3.** Contd.

| | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Endo bornyl acetate | 1289 | - | 0.25 | - | - | 0.31 | - | - | 0.24 | - | 0.15 | - | 0.27 | 0.30 | 0.28 | - | 0.25 | 0.28 | 0.30 | 0.10 | 0.24 | - | 0.35 | 0.28 |
| 2-Undecanone | 1295 | - | 0.32 | 0.12 | 0.20 | 0.35 | 0.14 | 0.20 | 0.17 | 0.23 | 0.16 | 0.20 | 0.32 | 0.46 | 0.50 | - | 0.27 | 0.20 | 0.26 | 0.58 | 0.15 | 0.15 | 0.61 | 0.22 |
| Citronellyl acetate | 1354 | - | 0.12 | - | - | 0.17 | - | - | 0.11 | - | - | - | 0.11 | 0.15 | 0.15 | - | 0.20 | 0.11 | 0.11 | 0.14 | 0.11 | 0.10 | 0.16 | 0.17 |
| Cyclosativen | 1366 | 0.10 | 0.11 | - | - | - | - | - | - | - | - | 0.11 | - | 0.15 | 0.12 | 0.12 | 0.11 | 0.11 | 0.11 | - | 0.11 | - | 0.10 | - |
| Alpha copaene | 1378 | 0.31 | 0.28 | 0.27 | 0.30 | 0.29 | 0.31 | 0.28 | 0.26 | 0.30 | 0.32 | 0.35 | 0.23 | 0.31 | 0.30 | 0.35 | 0.34 | 0.29 | 0.30 | 0.28 | 0.29 | 0.29 | 0.28 | 0.27 |
| Geranyl acetate | 1385 | 0.66 | 0.53 | - | - | 0.28 | - | - | 0.13 | - | 0.12 | - | 0.81 | 0.44 | 0.28 | 0.21 | 0.54 | 0.30 | 0.17 | 0.55 | 0.36 | 0.57 | 0.22 | 0.41 |
| Beta elemene | 1394 | 0.59 | 0.56 | 0.64 | 0.66 | 0.48 | 0.62 | 0.53 | 0.50 | 0.56 | 0.54 | 0.60 | 0.46 | 0.61 | 0.56 | 0.63 | 0.62 | 0.52 | 0.49 | 0.46 | 0.53 | 0.48 | 0.57 | 0.53 |
| Gama elemene | 1435 | 0.18 | - | - | 0.16 | - | - | - | - | - | 0.13 | - | - | 0.14 | - | - | 0.12 | - | - | - | - | - | - | - |
| Beta farnasene | 1458 | - | - | 0.55 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Allo aroma dendrene | 1464 | 0.42 | 0.27 | 0.17 | 0.36 | 0.17 | 0.34 | 0.23 | 0.13 | 0.21 | 0.28 | 0.45 | 0.16 | 0.20 | 0.14 | 0.51 | 0.27 | 0.15 | 0.14 | 0.27 | 0.29 | 0.39 | 0.13 | 0.20 |
| Alpha guaen | 1478 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.27 |
| Beta cubebene | 1485 | 3.16 | 1.89 | - | 2.48 | - | 2.27 | 2.42 | - | 2.15 | 3.24 | 2.74 | 1.78 | 1.91 | 1.72 | 3.59 | 2.52 | - | 1.74 | 1.97 | - | 2.84 | 1.81 | 2.10 |
| Alpha curcumene | 1486 | 2.49 | 3.81 | 6.14 | 4.10 | 6.15 | 3.99 | 3.31 | 5.25 | 3.87 | 1.90 | 4.06 | 2.85 | 4.84 | 3.31 | 2.64 | 3.70 | 5.98 | 3.53 | 2.38 | 5.31 | 1.90 | 3.08 | 3.12 |
| Beta selinene | 1490 | 0.16 | - | - | - | 0.13 | - | - | - | - | - | - | - | - | 0.11 | - | - | 0.12 | 0.12 | - | - | - | - | - |
| Gama cadinene | 1505 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Zingiberene | 1508 | 19.50 | 22.20 | 28.80 | 27.10 | 22.00 | 26.40 | 25.60 | 19.20 | 24.30 | 29.60 | 28.00 | 18.50 | 19.20 | 19.00 | 26.10 | 24.30 | 19.60 | 21.30 | 17.60 | 21.60 | 20.60 | 21.30 | 21.70 |
| Alpha muurolene | 1510 | - | - | - | - | - | 0.10 | - | - | - | 0.10 | 0.11 | - | - | - | 0.12 | 0.10 | 0.10 | - | - | - | - | - | - |
| Beta besabolene | 1512 | - | - | 7.34 | 2.86 | - | 2.85 | 2.41 | - | 2.68 | - | 2.85 | - | - | - | - | - | - | - | - | - | - | - | - |
| Epibicyclo sesquiphellandrene | 1517 | 0.53 | - | 0.29 | 0.37 | 0.26 | 0.36 | 0.37 | 0.21 | 0.24 | 0.45 | 0.42 | 0.25 | 0.23 | 0.22 | 0.67 | 0.39 | 0.22 | 0.21 | 0.39 | 0.24 | 0.55 | 0.20 | 0.20 |
| Farnesene | 1519 | 12.20 | 12.70 | - | 4.56 | 12.00 | 4.10 | 4.55 | 11.30 | 4.21 | 13.40 | 4.88 | 10.30 | 11.90 | 11.20 | 14.70 | 11.40 | 12.20 | 12.00 | 9.56 | 12.90 | 11.90 | 11.30 | 9.41 |
| Torreyol | 1521 | 0.32 | - | - | - | - | - | 0.17 | - | - | 0.21 | 0.18 | - | - | - | 0.32 | 0.20 | - | - | 0.18 | - | 0.23 | 0.12 | - |
| Calarene | 1527 | 0.23 | 0.21 | - | - | 0.23 | 0.26 | 0.23 | 0.20 | 0.17 | - | 0.28 | 0.18 | 0.23 | 0.20 | 0.30 | 0.24 | 0.21 | 0.22 | 0.18 | 0.22 | 0.20 | 0.20 | 0.13 |
| □--sesquiphellandrene | 1535 | 8.20 | 9.54 | 10.60 | 11.30 | 9.55 | 10.70 | 10.30 | 8.47 | 10.50 | 12.10 | 11.90 | 8.04 | 9.42 | 8.33 | 10.70 | 10.50 | 8.98 | 9.36 | 7.06 | 9.20 | 7.99 | 8.73 | 9.13 |
| Alpha bergamotene | 1539 | 0.25 | 0.27 | 0.24 | 0.28 | 0.23 | 0.27 | 0.27 | 0.24 | 0.27 | - | 0.34 | 0.22 | 0.32 | 0.25 | 0.32 | - | 0.26 | 0.26 | 0.19 | 0.25 | 0.20 | 0.24 | 0.26 |
| Elemol | 1555 | 0.97 | 0.60 | 0.77 | 0.77 | 0.58 | 0.81 | 0.71 | 0.74 | 0.73 | 0.73 | 0.65 | 0.71 | 0.99 | 0.65 | 0.79 | 0.76 | 0.61 | 0.63 | 0.65 | 0.74 | 0.66 | 0.81 | 0.75 |
| Ledol | 1558 | - | - | - | - | - | 0.24 | - | - | - | - | - | - | 0.39 | - | - | - | - | - | - | - | 0.20 | - | - |
| Germacrene B | 1562 | 0.48 | 0.41 | 0.28 | 0.34 | 0.43 | 0.48 | 0.38 | 0.40 | 0.36 | 0.43 | 0.50 | 0.36 | 0.44 | 0.41 | 0.61 | 0.45 | 0.40 | 0.45 | 0.35 | 0.53 | 0.53 | 0.41 | 0.37 |
| Nerolidol | 1568 | 1.56 | 1.26 | 1.13 | 1.32 | 1.20 | 1.22 | 1.10 | 1.18 | 1.17 | 0.96 | 1.33 | 1.21 | 1.79 | 1.11 | 1.63 | 0.84 | 1.34 | 1.23 | 0.71 | 1.31 | 1.22 | 1.13 | 0.74 |
| (-) epiglobulol | 1592 | 0.91 | 1.10 | 0.52 | 0.59 | 0.90 | 0.58 | 0.61 | 1.28 | 0.85 | 0.96 | 0.94 | 0.82 | 0.84 | 1.00 | 0.95 | 1.25 | 0.85 | 1.14 | 0.46 | 0.79 | 0.46 | 0.85 | 0.82 |
| Viridiflorol | 1617 | 1.05 | 1.01 | 0.99 | 1.39 | 1.14 | 1.09 | 1.04 | 1.13 | - | 1.16 | 1.16 | 1.01 | 1.52 | 0.91 | 1.16 | 1.10 | 1.13 | 1.01 | 0.82 | 1.01 | 0.82 | 0.72 | 0.72 |
| Juniper camphor | 1624 | 0.63 | - | - | 0.36 | - | 0.34 | 0.36 | - | 0.37 | 0.39 | 0.40 | - | 0.54 | - | 0.60 | - | 0.32 | - | 0.35 | 0.34 | 0.45 | - | - |
| (-) Farnesol | | 0.79 | 0.70 | - | 0.82 | 0.79 | 0.77 | 0.69 | 0.77 | - | 0.80 | 0.82 | - | 1.06 | 0.65 | 0.92 | - | - | - | 0.61 | 0.69 | 0.59 | - | - |
| Beta eudesmol | 1655 | 0.92 | 0.51 | 0.46 | 0.6 | 0.61 | 0.57 | 0.53 | 0.49 | 0.43 | 0.61 | 0.62 | 0.56 | 0.82 | 0.44 | 1.01 | 0.48 | 0.51 | 0.47 | 0.60 | 0.54 | 0.70 | 0.47 | 0.35 |
| | | 89.47 | 93.65 | 94.57 | 92.8 | 92.43 | 93.73 | 92.35 | 92.08 | 92.2 | 93.55 | 92.54 | 91.5 | 87 | 93.73 | 90.59 | 90.88 | 91.5 | 92.35 | 91.6 | 93.72 | 92.59 | 91.6 | 93.35 |

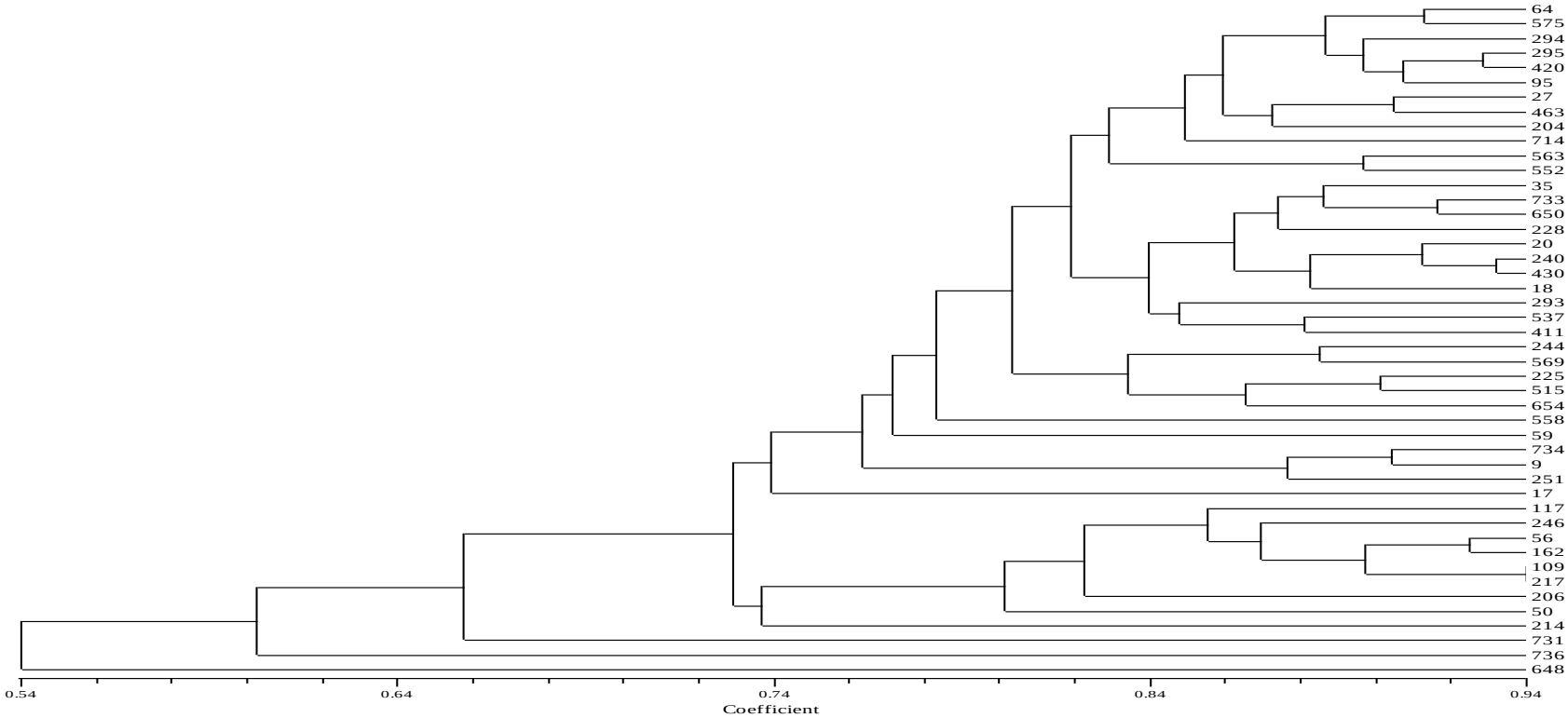


**Figure 1.** Dendrogram of genetic similarity among 46 ginger varieties/cultivars based on GC/MS data using Jaccard's similarity coefficients.

on GC/MS data are given in Figures 1 to 3. The dendrograms split at coefficients 0.82, 0.82 and 0.87, respectively and placed the accessions in 14 different groups in the Jaccard's similarity and Sorensen - Dice similarity and 15 groups in the Simple Matching similarity. The grouping pattern of the Jaccard's similarity and the Sorensen patterns - Dice similarity were similar (Figures 1 and 2) whereas in case of Simple Matching similarity dendrogram (Figure 3), some alterations in the arrangement of the groups were observed when compared to the other two dendrograms.

### HPLC analysis of ginger for gingerol and shogaol

The levels of gingerols and shogaols in the 46 ginger accessions were determined using HPLC. The percentages of gingerols and shogaols are given in Table 4. Among the gingerols and shogaols identified, 6- gingerol was the predominant one in all the ginger accessions except the exotic ginger, 'Oman', in which 8-shogaol was the predominat one . Highest level of 6-gingerol was recorded in the cultivar, 'Angamali' (3.11%) and the least in the exotic ginger, 'Oman' (0.36%). Even though 6-shogaol was present in all the samples, its concentration was relatively low when compared with 6-gingerol. 8-gingerol, 10-gingerol, 10-shogaol were also present in many of the ginger accessions.

## DISCUSSION

### GC/MS analysis of essential oil

Essential oil content gives a pleasant aroma to

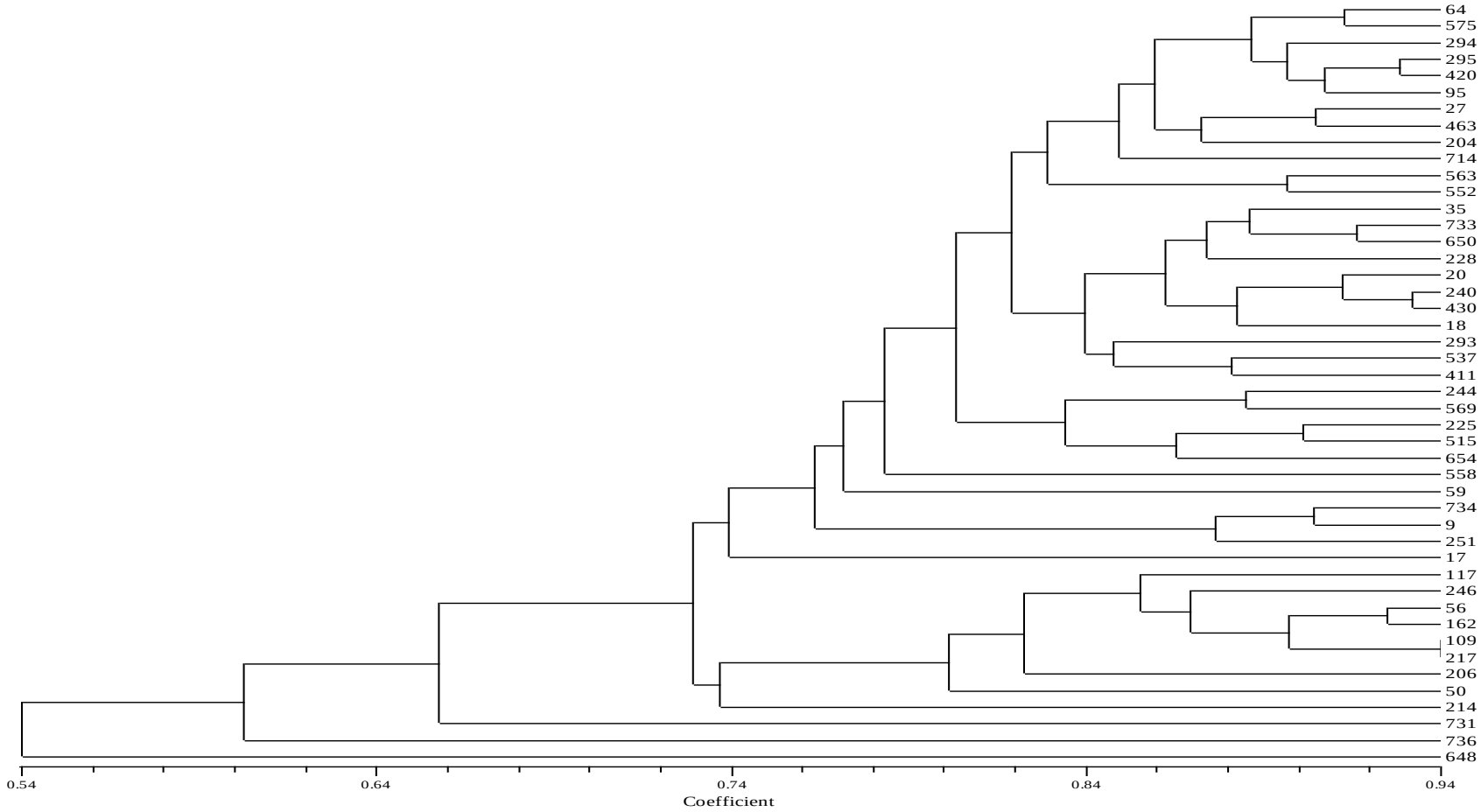


**Figure 2.** Dendrogram of genetic similarity among 46 ginger varieties/cultivars based on GC/MS data using Sorenson - Dice similarity coefficient.

ginger and to the end products. Thus the essential oil composition is indispensable in determining the various grades and prices of the produce. Mono and sesqui terpenoids are present in the volatiles, but sesqui-terpenoids are quantitatively the major constituents (Tonnessen and Karlsen, 1983). The essential oil profile of the 46 accessions revealed that zingiberene is the major compound followed by beta-sesquiphellandrene, α-curcumene, farnasene and sesquiterpene alcohols. In the exotic ginger, 'Kintoki', α-curcumene was the major compound. Sankarikutty et al. (1982) reported that α-curcumene is a secondary product formed from zingiberene and beta-phellandrene. Secondary product usually forms in the dried samples of ginger. Ginger oil isolated from the fresh ginger rhizome and Kintoki contained highest percentage of α-curcumene which indicates the source of the essential oil also determine the percentage of the α-curcumene level. 'Kintoki', an exotic type from Japan, *Z. officinale* var. *rubens* whereas other accessions are *Z. officinale* R. Moderate citral content (lemony aroma) was reported in the essential oils of ginger from different parts of

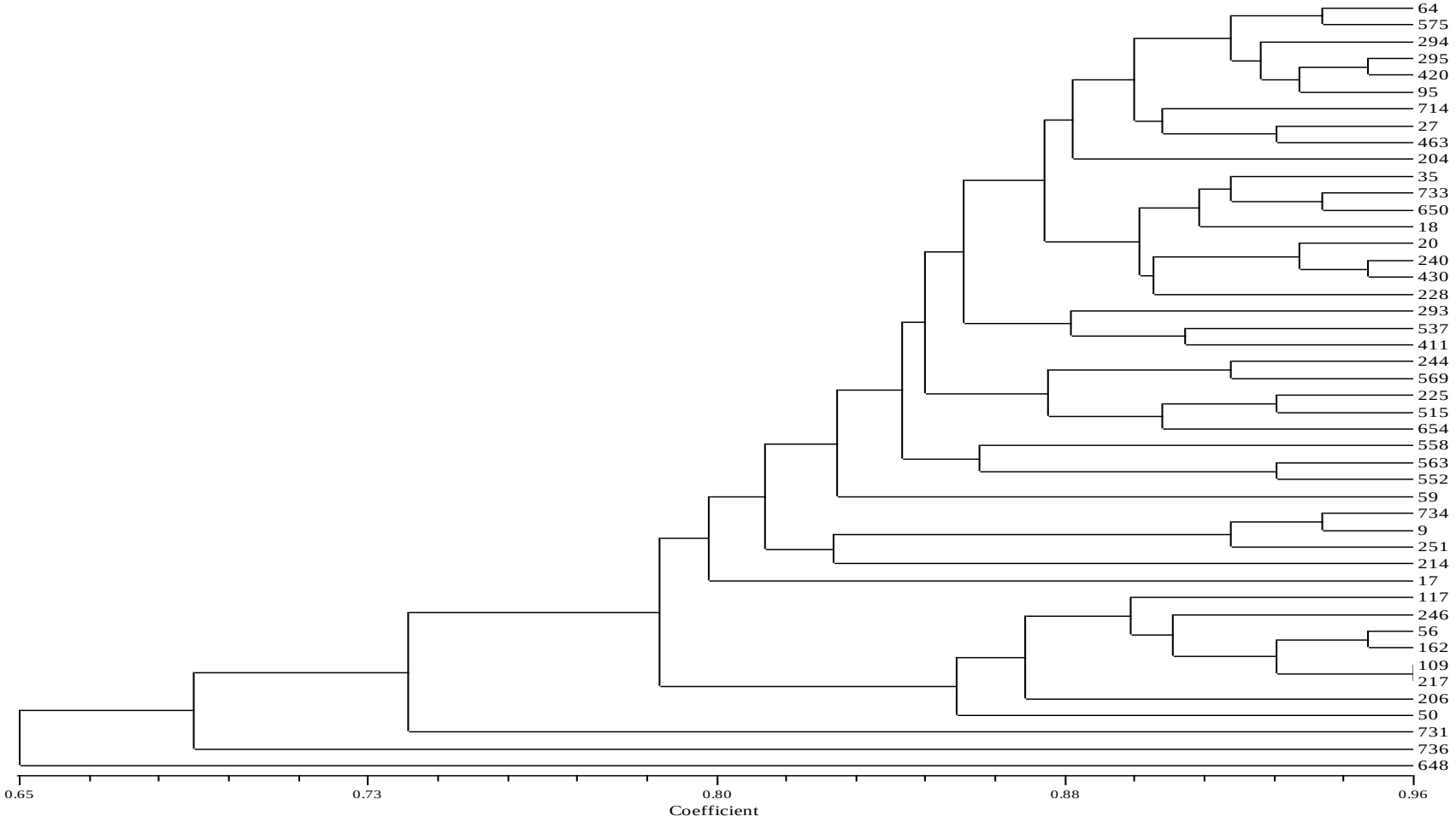


**Figure 3.** Dendrogram of genetic similarity among 46 ginger varieties/cultivars based on GC/MS data using Simple Matching similarity coefficient.

India (Raina et al., 2005). Australian gingers are known to be a good source of citral (50 to 70%) while the Jamaican ginger is low in citral content (Wohlmuth et al., 2006). In this study, the accession, 'Jamiaca' yielded less citral content. Sun drying is known to have an adverse effect on citral content in ginger oil (Menon et al., 2007). However, in the present study citral was present in all the accessions except Pink ginger, although the rhizomes were sundried.

Cluster analysis of the 'Pink ginger' accessions based on the similarity coefficients using Jaccard's, Sorensen- Dice and Simple Matching revealed close similarity among the ginger accessions. Group 1 included 10 accessions; among these two were released varieties ('Varada' and 'Suruchi'). A collection from Nepal and some important cultivars like 'Maran', 'Nadia'

**Table 4.** Gingerol and shogaol content in ginger accessions.

| S/N | Name of the cultivar/variety | 6-gingerol (%) | 8-gingerol (%) | 6-shogaol (%) | 10-gingerol (%) | 8-shogaol (%) | 10-shogaol (%) |
|---|---|---|---|---|---|---|---|
| 1 | 'Varada' | 0.65 | 0.03 | 0.08 | 0.09 | 0.09 | 0.05 |
| 2 | 'Mahima' | 1.61 | - | 0.21 | 0.13 | 0.24 | 0.24 |
| 3 | 'Rejatha' | 1.07 | 0.06 | 0.05 | 0.07 | 0.09 | 0.05 |
| 4 | 'Suruchi' | 1.74 | - | 0.2 | 0.07 | 0.15 | 0.28 |
| 5 | 'Suprabha' | 0.43 | - | 0.08 | - | 0.1 | 0.28 |
| 6 | 'Himachal' | 1.22 | - | 0.17 | 0.15 | 0.17 | 0.14 |
| 7 | 'Maran' | 0.73 | - | 0.09 | 0.05 | 0.14 | 0.56 |
| 8 | 'Nadia' | 0.72 | 0.06 | 0.07 | 0.13 | 0.12 | 0.18 |
| 9 | 'Karakkal' | 1.43 | - | 0.08 | 0.11 | - | 0.14 |
| 10 | 'Mananthody' | 1.54 | 0.07 | 0.18 | 0.07 | 0.18 | 0.16 |
| 11 | 'Sabarimala' | 1.8 | 0.07 | 0.24 | 0.08 | 0.31 | 0.1 |
| 12 | 'Kozhikkalan' | 1.78 | - | 0.22 | 0.07 | 0.2 | 0.08 |
| 13 | 'Ellakallan' | 1.32 | 0.07 | 0.17 | 0.06 | 0.2 | 0.15 |
| 14 | 'Kakakkalan' | 1.25 | 0.06 | 0.15 | 0.05 | 0.15 | 0.21 |
| 15 | 'Pakistan' | 0.45 | 0.06 | 0.07 | 0.09 | 0.09 | 0.34 |
| 16 | 'Oman' | 0.36 | 0.02 | 0.05 | 0.02 | 0.67 | 0.28 |
| 17 | 'Brazil' | 1.2 | 0.05 | 0.17 | 0.18 | 0.11 | 0.13 |
| 18 | 'Jamaica' | 1.26 | 0.05 | 0.13 | 0.05 | 0.17 | 0.13 |
| 19 | 'Rio-de-Janeiro' | 0.88 | - | 0.13 | 0.06 | 0.17 | 0.15 |
| 20 | 'Pink ginger' | 0.69 | 0.07 | 0.08 | 0.12 | 0.12 | 0.16 |
| 21 | 'Bakthapur' | 1.78 | 0.03 | 0.11 | 0.15 | 0.12 | 0.38 |
| 22 | 'Kintoki' | 1.2 | - | 0.10 | - | 0.14 | 0.07 |
| 23 | 'Nepal' | 0.8 | 0.06 | 0.08 | 0.1 | 0.1 | 0.13 |
| 24 | 'China' | 1.22 | 0.04 | 0.23 | 0.16 | 0.13 | 0.1 |
| 25 | 'Juggigan' | 0.43 | 0.04 | 0.1 | 0.18 | 0.12 | 0.06 |
| 26 | 'Acc. No. 50' | 2.39 | 0.06 | 0.25 | 0.14 | 0.17 | 0.17 |
| 27 | 'Pulpally' | 1.13 | 0.06 | 0.12 | 0.1 | 0.15 | 0.12 |
| 28 | 'Acc. No. 95' | 0.46 | - | 0.1 | 0.14 | 0.07 | 0.21 |
| 29 | 'Ambalawayalan' | 1.81 | 0.08 | 0.15 | - | 0.2 | 0.23 |
| 30 | 'Kozhikode' | 1.0 | - | 0.15 | - | 0.22 | 0.3 |
| 31 | 'Thodupuzha -1' | 1.39 | 0.05 | 0.18 | 0.14 | 0.2 | 0.16 |
| 32 | 'Konni local' | 1.25 | 0.05 | 0.14 | 0.06 | 0.18 | 0.04 |
| 33 | 'Angamali' | 3. 11 | 0.06 | 0.29 | 0.08 | 0.21 | 0.1 |
| 34 | 'Thodupuzha -2' | 1.7 | - | 0.12 | - | 0.15 | 0.14 |
| 35 | 'Kottayam' | 0.93 | 0.05 | 0.14 | 0.07 | 0.13 | 0.16 |
| 36 | 'Palai' | 0.83 | 0.05 | 0.05 | - | 0.11 | 0.07 |
| 37 | 'Silent valley' | 0.66 | 0.05 | 0.12 | 0.16 | 0.08 | 0.03 |
| 38 | 'Wayanadu local' | 1.0 | 0.08 | 0.07 | 0.1 | 0.12 | 0.07 |
| 39 | 'Vizagapatnam -1' | 2.71 | 0.08 | 0.41 | 0.23 | 0.31 | 0.24 |
| 40 | 'Vizagapatnam -2' | 0.49 | 0.04 | 0.08 | 0.04 | 0.01 | 0.04 |
| 41 | 'Fiji' | 1.11 | 0.08 | 0.13 | 0.09 | 0.14 | 0.13 |
| 42 | 'Gorubathani' | 0.98 | 0.08 | 0.09 | 0.16 | 0.17 | 0.23 |
| 43 | 'Bhaise' | 0.87 | 0.08 | 0.09 | 0.05 | 0.14 | 0.19 |
| 44 | 'Naval parasi' | 0.87 | - | 0.1 | 0.1 | 0.11 | 0.09 |
| 45 | 'Neyyar' | 0.49 | - | 0.5 | - | 0.1 | 0.12 |
| 46 | 'Jolpaiguri' | 1.45 | - | 0.18 | - | 0.23 | 0.1 |

and 'Thodupuzha' were also included in the same group with almost same chemical composition in the essential oil. In group 2, Bhaise and 'Bakthapur' were included, which were originated from nearby geographical locations of Sikkim and Nepal, respectively. In group 3, two released varieties, 'Rejatha' and 'Suprabha' were included along with the two exotic collections namely, 'Pakistan' and 'Fiji'; and some other local cultivars namely, 'Kakakkalan', 'Rio-de-Janeiro', 'Angamali', 'Jamaica', 'Konni Local', Acc. No. 50, 'Pink ginger', 'Brazil' and 'Kintoki', indicating their distinctiveness. Two exotic collections 'Oman' and 'China' formed group 7 in the dendrogram. Group 10 was formed with a released variety Mahima and with few cultivars/primitive types namely, 'Pulpally', 'Ambalawayalan', 'Kozhikkode', 'Sabarimala' and 'Thodupuzha'. The dendrogram showed

that three ginger accessions namely, ‘Kintoki’, ‘Pink ginger’ and ‘Brazil’ showed maximum distance with other ginger accessions.

Alternatively, the variability for the essential oil components may be narrow in the primary genepool of the crop. Among the accessions studied the exotic ginger types ‘Kintoki’ and ‘Brazil’ as well as the collection from Meghalaya, India, ‘Pink ginger’ were unique. HPLC analysis of ginger accessions GC/MS method is not suitable for the analysis of pungency, because the high temperature will convert gingerol to shogaol (Harvey, 1981; Chen et al., 1986). Gingerols and shogaols are pungency stimulating non-volatile compounds found in ginger (Zachariah et al., 1993). In order to find out the variability in pungent components among germplasm collections of ginger, HPLC is a better option for the analysis. HPLC analysis results showed higher percentage of gingerol compared to shogaol in all the accessions except ‘Neyyar’. Jiang et al. (2006) also reported similar results. Ratio of 6-gingerol to 6-shogaol decides the quality of the ginger (Schwertner and Rios, 2007). Present study using HPLC observed meager levels of shogaol in all the accessions of ginger; supporting the finding of Jolad et al. (2005). Pungency of the ginger gradually decreases when the amount of gingerol decreeses and shoagaol increases (Zachariah et al., 1993). Wohlmuth et al. (2005) reported the absence of shogaol in the fresh ginger from Australia and supported the hypothesis that shogaols are not native constituents of fresh ginger rhizomes, but formed from gingerols by dehydration as a result of heat or acidic or alkaline condition, but in the present study shogaol was found with gingerol, as a naturally occurring constituent in most of the accessions.

Three pungent compounds of gingerols are reported and quantified in ginger (Chen et al., 1986). Among the gingerols and shogaols, 6-gingerol was the most abundant pungent compound reported (Chen et al., 1986; Bartley, 1995). In the present study, maximum 6-gingerol obtainned was in 3.11% in the cultivar 'Angamali'. Other cultivars like 'Accession 50' and 'Vizagapatnam-1' also gave above 2% gingerol content. Total gingerols concentration was found to be highest in the cultivar 'Angamali'. It was the most pungent of the accessions assayed. In the present study, highest level of 6-shogaol (0.41%) was found in the cultivar ‘Vizagapatnam-1'. 6-shogaol was more pungent than 6-gingerol (Suekawa et al., 1984).

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