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# Comparative GC-MS analysis of capsaicinoids and non-capsaicinoid compounds in the ethanolic extract of four different Capsicum annuum L. varieties

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## ABSTRACT

*India, known as the world's largest producer of fresh peppers and chillies, exhibits significant regional variations in the morpho-physiochemical characteristics of its chilli fruits. In this study, we conducted gas chromatography and mass spectrometry (GC-MS) analysis to compare the phytochemical constituents in four distinct Capsicum annuum L. varieties viz. Byadgi chilli, Mundu chilli, Bullet chilli, and Jwala chilli, were collected from four different agroecological regions across India. Our GC-MS analysis revealed consistent peaks corresponding to various capsaicinoids and non-capsaicinoid compounds in the ethanolic extracts of all four varieties of C. annuum, albeit with varying proportions. These compounds include capsaicin, dihydrocapsaicin, n-hexadecanoic acid, hexadecanoic acid ethyl ester, and Z, Z-9,12-octadecadienoic acid. Additionally, unique spectrum profiles of several specific compounds were detected, distinguishing particular varieties from each other. Our study offers preliminary insights into the active phytocompounds of various C. annuum varieties cultivated in diverse agroecological regions. Moreover, this research highlights the influence of environmental factors on phytochemical profiles, emphasizing the need for more detailed exploration in this area.*

***Keywords:*** *Agroecological regions, capsaicin, Capsicum annuum varieties, capsaicinoids, dihydrocapsaicin, ethanolic extracts, GC-MS, non-capsaicinoids*

## 1. INTRODUCTION

Plants can produce diverse low-molecular-weight organic compounds identified as secondary metabolites, often characterized by their unique and intricate chemical structures. These compounds, renowned for their fascinating biological properties, find applications in pharmaceuticals, insecticides, dyes, flavours, and fragrances [1]. Also, in recent years, the use of plants in managing insect pests has gained considerable importance. The growing significance of plant-based solutions in insect pest management underscores the pivotal role of plants as rich sources of biologically active compounds. Harnessing their potent biological activity, these compounds emerge as promising alternatives for effective insect pest control [2]. Chilli pepper is an annual herb in the Solanaceae family and is one of the most widely used plants in diverse regions worldwide. Chilli is known for its pungency, primarily due to capsaicinoid compounds, which are exclusively synthesized and stored in vesicles along the fruit's placenta epidermis [3]. Throughout various cultures, chilli has a rich historical tradition of serving as a food preservative. Its efficacy in repelling insect pests has been acknowledged and tactically integrated to protect grains and food items from insect infestations [4] and the effectiveness of chilli lies in its bio-active compounds [5]. In total, over 20 capsaicinoid compounds, such as capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homodihydrocapsaicin, homocapsaicin, norcapsaicin, and nornorcapsaicin etc. along with several other non- capsaicinoid compounds have been identified from various *Capsicum* Spp. [6, 7]. Diverse varieties and cultivars of chilli peppers exhibit variations in fruit morphology, phytoconstituents, pungency, bearing habits, essential oil contents etc. throughout different regions of India. These variances are thought to be influenced by a range of factors, such as genetic traits, growing conditions, geographic origin, chemo-types, and disparities in plant nutritional status [8]. However, to date, our understanding is limited by a dearth of accessible data regarding the comparative assessment of the chemical profiles of active phytocompounds from various chilli varieties cultivated under diverse agroecological conditions in India. Investigating this aspect could potentially elucidate the variability of their chemical profiles under differing growing conditions and would further facilitate the discovery of diverse phytoconstituents from the chilli varieties. With this background, the present study aimed to identify the capsaicinoids and non-capsaicinoid compounds present in the ethanolic extract of four different *C. annuum* varieties which are cultivated in different ecological regions of India using GC-MS analysis.

## 2. MATERIALS AND METHODS

### 2.1. Collection of Capsicum annuum samples

*C. annuum* samples (Fig. 1) for this study were collected from different zones (Table 1) in India between August and October 2022.

### 2.2. Preparation of Capsicum annuum samples

After collection, the samples were shade-dried for one week at

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ambient temperatures ranging from 28°C to 34°C during daylight hours. Subsequently, they were desiccated in a hot air oven for 24 h at temperatures between 60°C and 70°C before processing. Once dried, the samples were mechanically pulverised using a laboratory hammer mill (WKS-20B, 4 kW, China) and sifted through a 0.5 mm mesh size. The resulting fine powder was stored in tightly sealed, dry containers until the onset of the extraction process.

Powdered *C. annuum* samples were subjected to solid-liquid extraction using a Soxhlet extraction apparatus (Sigma-Aldrich in St. Louis, MO, USA). The extraction process involved placing 20 grams of powdered *C. annuum* samples into the thimble of the Soxhlet extractor, with a condenser connected to a round bottom flask containing ethanol (Sigma-Aldrich, HPLC/spectrophotometric grade) as the extracting solvent. Heat was applied, causing the ethanol to reflux at its boiling point of 78°C. This allowed the ethanol vapour to immerse the housed *C. annuum* samples, isolating the compounds of interest through evaporation and condensation, which continued for 6 hours until the ethanol solvent no longer showed any red colour. A constant temperature of 78°C was maintained throughout the extraction process until all the powdered *C. annuum* samples were fully exhausted. Subsequently, the extracts were cooled and adjusted to a total volume of 200 mL using ethanol. The resulting extracts were stored separately in amber bottles in a refrigerator maintained at 10 ± 2°C until they were ready for testing.

### 2.3. Identification of phytocomponents through gas chromatography and mass spectrometry (GC-MS) analysis

The ethanolic fraction of *C. annuum* sample extracts were analysed for active compounds using a Hewlett Packard 5890 gas chromatograph (QP2020, Shimadzu Corporation Kyoto, Japan) equipped with a mass detector, Turbo Mass Gold, and a column with Elite-1 Dimethyl silicone (DIMS) of dimensions 30 m x 0.25 mm ID x 1 mM df. The GC-MS analysis followed specific parameters, including auto injector-based injection, a column oven temperature of approximately 70°C, and an injection temperature of 240°C. The injection mode was set to split-less, with a flow control mode pressure of 61.3 kPa and a total flow rate of 14.00 mL $min^{-1}$. Helium (He) was used as the carrier gas with a flow rate of 1.00 mL $min^{-1}$ and a linear velocity of 36.7 cm $sec^{-1}$. The detection utilized a mass spectrometer (MS) operating in full scan mode with an interface temperature of around 280°C. The identification of the major constituents involved the utilisation of a computer-driven algorithm, which compared their retention time, retention index, and spectral data. Additionally, the mass spectrum of the analysed compounds was matched against the National Institute of Standards and Technology (NIST) library (Version 2.0, year 2005) for accurate characterisation. The software employed for gas chromatography-mass spectrometry analysis was Tubro Mass 5.1 [9 – 12].

## 3. RESULTS AND DISCUSSION

The identified capsaicinoid and non-capsaicinoid compounds of four different *C. annuum* varieties from different ecological regions of India along with their retention indices, percentage composition, and chemical formula are presented in Table 2. The results showed that the Byadgi chilli ethanol fraction (Fig. 2A) composed of capsaicinoid compounds such as capsaicin (6.58%), dihydrocapsaicin (3.69%), along with several non-capsaicinoid constituents n-hexadecanoic acid (16.87%), hexadecanoic acid, ethyl ester (7.53%), Z, Z-9,12-Octadecadienoic acid (28.50%), 9,12-Octadecadienoic acid, methyl ester (2.19%), 9,12-Octadecadienoic acid, ethyl ester (27.93), cyclo-nona siloxane (4.40%), and cyclo-octa siloxane (2.30%). The spectrum profile of GC-MS confirmed the presence of ten major capsaicinoid and non-capsaicinoid constituents such as n-hexadecanoic acid (6.76%), capsaicin (11.73%), dihydrocapsaicin (6.42%), hexadecanoic acid, ethyl ester (7.70%), Z, Z-9,12-Octadecadienoic acid (14.98%), 9,12-Octadecadienoic acid, methyl ester (3.20%), 9,12-Octadecadienoic acid, ethyl ester (21.95%), glycerine (10.91%), 5-Hydroxypipecolic acid (14.97%), and hydroxy-1-methyl proline (1.38%) in the Mundu chilli ethanolic extract (Fig. 2B). The Bullet chilli ethanol fraction revealed the presence of both capsaicinoid and non-capsaicinoid compounds (Fig. 2C) such as capsaicin (5.46%), dihydrocapsaicin (12.38%), n-hexadecanoic acid (13.72%), hexadecanoic acid, ethyl ester (9.16%), Z, Z-9,12-Octadecadienoic acid (46.28%), octadecanoic acid, ethyl ester (2.81%), melezitose (7.79%), and 6-O-Acetyl-beta-D-mannopyranose (2.40%). Similarly, the analysis detected the presence of 22 capsaicinoid and non-capsaicinoid compounds in the Jwala chilli ethanol extract (Fig. 2D) such as capsaicin (1.07%), dihydrocapsaicin (4.03%), nonivamide (1.00%), n-hexadecanoic acid (13.09%), n-pentadecylacetamide (30.51%), ethyl 14-methyl-hexadecanoate (1.01%), 1-(2,4-Dihydroxyphenyl)-2-(4-methoxy-3-nitrophenyl) ethanone (0.25%), hexadecanoic acid, ethyl ester (14.74%), Z, Z-9,12-Octadecadienoic acid (15.62%), hexadecanoic acid, methyl ester (0.26%), 9,12-Octadecadienoic acid, methyl ester (1.04%), silane, bis (fluoromethyl) dimethyl (0.11%), d-Mannose (4.97%), erythritol (5.41%), 2,3-dimethylfumaric acid (1.51%), sulfurous acid, dodecyl 2-propyl ester (0.46%), tetradecanoic acid (0.05%), pentadecanoic acid (1.26%), benzoic acid, 2-(2-chlorophenoxy) ethyl ester (2.65%), heptadecanoic acid (0.26%), n-decyl acetamide (0.24%), n-hexadecyl acetamide (0.38%), accounting for 100%. *Capsicum* spp. extracts are known to contain active compounds like capsaicin and other capsaicinoids along with several non-capsaicinoids, which can elicit irritation and respiratory effects in various organisms, including insects [13]. Among the capsaicinoid compounds, capsaicin and dihydrocapsaicin were consistently detected in all four *C. annuum* extracts, albeit in varying proportions. Notably, the Jwala chilli ethanol extract contained an additional capsaicinoid compound, nonivamide. Moreover, several non-capsaicinoid compounds such as n-hexadecanoic acid, hexadecanoic acid ethyl ester, and Z, Z-9,12-Octadecadienoic acid were identified as common constituents across the ethanolic fractions of the four *C. annuum* varieties. The individual chromatogram of commonly identified capsaicinoids and non-capsaicinoid compounds from the four *C. annuum* varieties are illustrated in Figures 3A-3C and 4A-4C. Conversely, specific compounds were found to be unique to a particular variety. There is a growing recognition of the correlation between phytochemical components and their respective biological activities [1]. Chilli fruits cultivated across various regions of India display a diverse range of variations in their physiochemical properties [6]. The biochemical components contributing to chilli pepper fruit quality are influenced by both cultivar selection and the variability of environmental conditions (E) across different geographic locations [14]. Our study highlights the presence of important phytocomponents from different *C. annuum* varieties as elucidated by GC-MS analysis.

Thus, this approach serves as an initial step towards comprehending the variability nature of active compounds from different chilli varieties cultivated under diverse agroecological conditions, facilitating further in-depth investigations into their respective biological activities.

## 4. CONCLUSION

In conclusion, the present study presents the preliminary findings of analysing phytocompounds from ethanolic extracts of different *C. annuum* varieties using GC-MS analysis, revealing the dynamic nature of active compounds influenced by the agroecological conditions of *C. annuum* cultivation. These results underscore the need for comprehensive investigations into the biological activities of the identified phytocompounds. Moreover, the findings should inspire further research into the potential applications of these plant extracts, particularly in the development of pharmaceuticals, insecticides, and other innovative products. This research highlights the significant impact of environmental factors on phytochemical profiles, offering valuable insights for future studies.


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**Conflicts of Interest:** The authors declare no conflicts of interest.

***Figure 1 Collected chilli varieties from four different agroecological zones of India that have been used in this study***


*$V_1$ – Byadgi chilli*
*Southern Zone*


*$V_2$ – Mundu chilli*
*Southern Zone*


*$V_3$ – Bullet chilli*
*Eastern Zone*


*$V_4$ – Jwala chilli*
*Western Zone*

***Fig. 2 Chemical constituents (%) of the four different chilli varieties under GC-MS analysis (Dotted pattern and grey shaded boxes represent the capsaicinoids and non-capsaicinoid compounds respectively)***

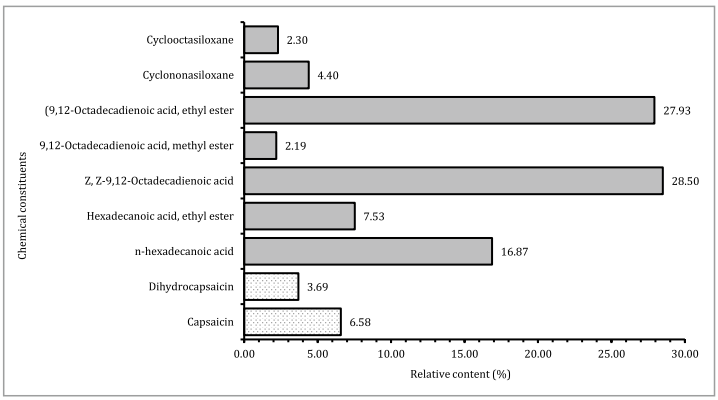



***Fig. 2A Byadgi chilli variety***

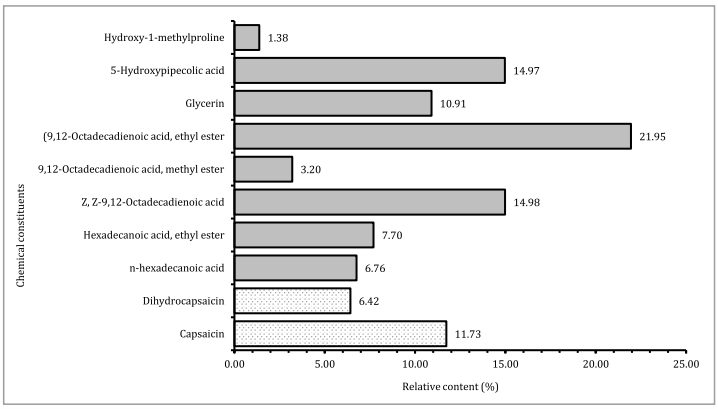



***Fig. 2B Mundu chilli variety***

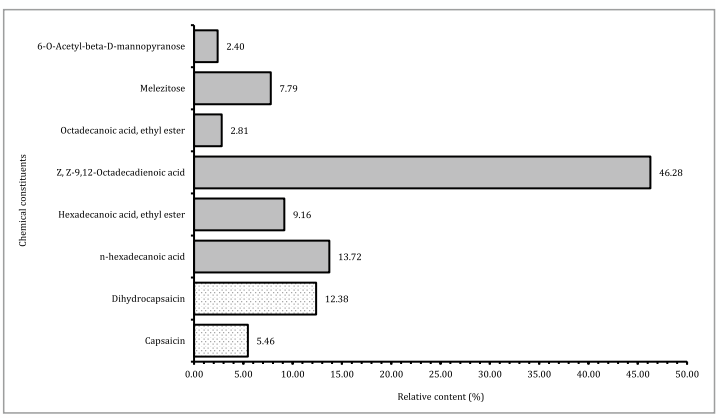



***Fig. 2C Bullet chilli variety***

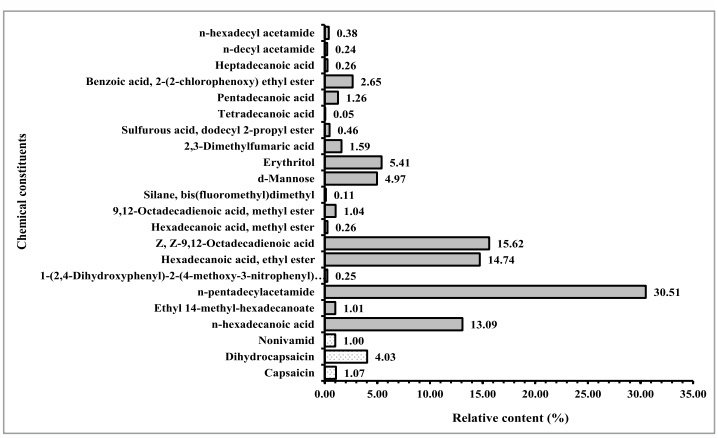



***Fig. 2D Jwala chilli variety***

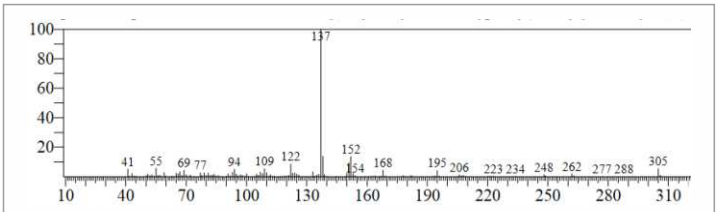
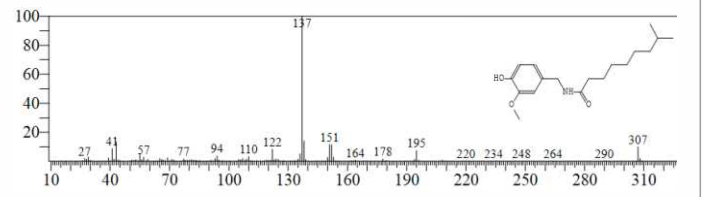


Fig. 3A Capsaicin Fig.



3B Dihydrocapsaicin

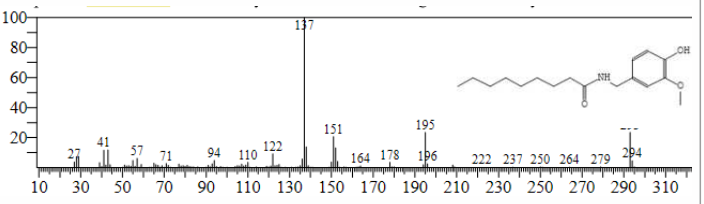


Fig. 3C Nonivamide

*Fig. 3 The relevant mass spectra of capsaicinoid compounds identified from the ethanolic extract of four different Capsicum annuum varieties*

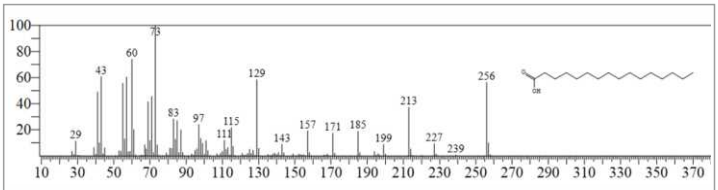


Fig. 4A n-hexadecanoic acid

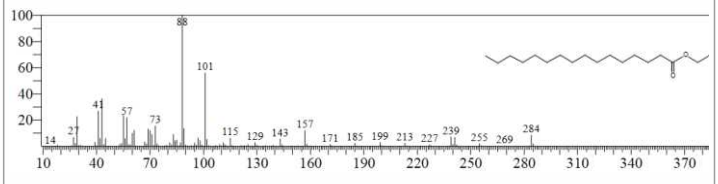


Fig. 4B Hexadecanoic acid, ethyl ester

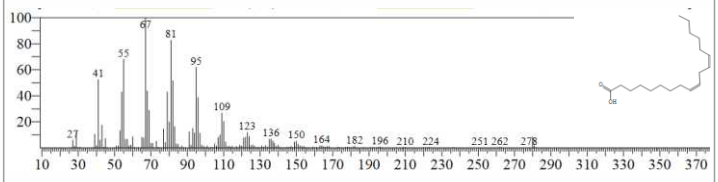


Fig. 4C Z, Z-9,12-Octadecadienoic acid

*Fig. 4 The relevant mass spectra of commonly identified non-capsaicinoid compounds from the ethanolic extract of four different Capsicum annuum varieties*

*Table 1. Sample information of four different chilli varieties collected from different zones of India for the study.*

| Sl. No. | Collected chilli varieties | | Collected Zone | Collected zone-specific agro-climatic regions ** | Collected Co-ordinates |
|---|---|---|---|---|---|
| | Common name | Scientific name | | | |
| 1 | Byadgi chilli (BYC) | *Capsicum annuum* L. var. *acuminatum* | Southern India (RS*: Karnataka) | Southern plateau and hills region | N 13° 04′ 41.1564″ E 77° 34′ 45.2676″ |
| 2 | Mundu chilli (MC) | *Capsicum annuum* L. | Southern India (RS*: Tamil Nadu) | West Coast plains and Ghat region | N 11° 00′ 54.6696″ E 76° 55′ 57.4212″ |
| 3 | Bullet chilli (BC) | *Capsicum annuum* L. var. *annuum* | Eastern India (RS*: West Bengal) | Lower Gangetic Plain region | N 22° 56′ 42. 882″ E 88° 32′ 00.8556″ |
| 4 | Jwala chilli (JC) | *Capsicum annuum* L. | Western India (RS*: Maharashtra) | Eastern plateau and hills region | N 19° 20′ 56.9688″ E 74° 38′ 46.0068″ |

*RS*: Representative state from which the chilli samples have been collected for the repellency study*
*** Source: https://iasri.icar.gov.in/agridata/23data/chapter1/db2020tb1_2.pdf*

*Table 2. Chemical constituents (%) of the four tested Capsicum annuum varieties under GC-MS analysis*

| Compound | Chemical formula | Retention index* | Retention time (min) | Relative content (%) ** | | | |
|---|---|---|---|---|---|---|---|
| | | | | BYC | MC | BC | JC |
| Ethyl 14-methyl-hexadecanoate | $C_{19}H_{38}O_2$ | 2013 | 5.00 | - | - | - | 1.01 |
| n-hexadecanoic acid | $C_{16}H_{32}O_2$ | 1968 | 5.34 | 16.87 | 6.76 | 13.72 | 13.09 |
| n-pentadecylacetamide | $C_{17}H_{35}NO$ | 1714 | 6.74 | - | - | - | 30.51 |
| Capsaicin | $C_{18}H_{27}NO_3$ | 2541 | 10.73 | 6.58 | 11.73 | 5.46 | 1.07 |
| 1-(2,4-Dihydroxyphenyl)-2-(4-methoxy-3-nitrophenyl) ethanone | $C_{15}H_{13}NO_6$ | 2728 | 10.93 | - | - | - | 0.25 |
| Dihydrocapsaicin | $C_{18}H_{29}NO_3$ | 2533 | 10.98 | 3.69 | 6.42 | 12.38 | 4.03 |
| Hexadecanoic acid, ethyl ester | $C_{18}H_{36}O_2$ | 1978 | 5.69 | 7.53 | 7.70 | 9.16 | 14.74 |
| Z, Z-9,12-Octadecadienoic acid | $C_{18}H_{32}O_2$ | 2183 | 6.60 | 28.50 | 14.98 | 46.28 | 15.62 |
| Hexadecanoic acid, methyl ester | $C_{17}H_{34}O_2$ | 1878 | 5.34 | - | - | - | 0.26 |
| 9,12-Octadecadienoic acid, methyl ester | $C_{19}H_{34}O_2$ | 2093 | 6.33 | 2.19 | 3.20 | - | 1.04 |
| 9,12-Octadecadienoic acid, ethyl ester | $C_{20}H_{36}O_2$ | 2193 | 6.77 | 27.93 | 21.95 | - | - |
| Octadecanoic acid, ethyl ester | $C_{20}H_{40}O_2$ | 2177 | 6.93 | - | - | 2.81 | - |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Cyclononasiloxane | $C_{18}H_{54}O_9Si_9$ | 1860 | 4.78 | 4.40 | - | - | - |
| Cyclooctasiloxane | $C_{16}H_{48}O_8Si_8$ | 1654 | 6.45 | 2.30 | - | - | - |
| Glycerin | $C_3H_8O_3$ | 967 | 2.56 | - | 10.91 | - | - |
| 5-Hydroxypipecolic acid | $C_6H_{11}NO_3$ | 1471 | 2.68 | - | 14.97 | - | - |
| Hydroxy-1-methylproline | $C_6H_{11}NO_3$ | 1318 | 3.00 | - | 1.38 | - | - |
| Melezitose | $C_{18}H_{32}O_{16}$ | 4506 | 2.49 | - | - | 7.79 | - |
| 6-O-Acetyl-beta-D-mannopyranose | $C_8H_{14}O_7$ | 1888 | 2.52 | - | - | 2.40 | - |
| Silane, bis(fluoromethyl)dimethyl | $C_4H_{10}F_2Si$ | 275 | 2.02 | - | - | - | 0.11 |
| d-Mannose | $C_6H_{12}O_6$ | 1698 | 2.48 | - | - | - | 4.97 |
| Erythritol | $C_4H_{10}O_4$ | 1229 | 2.51 | - | - | - | 5.41 |
| 2,3-Dimethylfumaric acid | $C_6H_8O_4$ | 1293 | 2.66 | - | - | - | 1.59 |
| Sulfurous acid, dodecyl 2-propyl ester | $C_{15}H_{32}O_3S$ | 2071 | 4.28 | - | - | - | 0.46 |
| Tetradecanoic acid | $C_{14}H_{28}O_2$ | 1769 | 4.55 | - | - | - | 0.05 |
| Pentadecanoic acid | $C_{15}H_{30}O_2$ | 1869 | 4.84 | - | - | - | 1.26 |
| Benzoic acid, 2-(2-chlorophenoxy) ethyl ester | $C_{15}H_{13}ClO_3$ | 2089 | 5.65 | - | - | - | 2.65 |
| Heptadecanoic acid | $C_{17}H_{34}O_2$ | 2067 | 6.07 | - | - | - | 0.26 |
| n-decyl acetamide | $C_{12}H_{25}NO$ | 1709 | 7.31 | - | - | - | 0.24 |
| n-hexadecyl acetamide | $C_{18}H_{37}NO$ | | 7.46 | - | - | - | 0.38 |
| Nonivamid | $C_{17}H_{27}NO_3$ | 2498 | 9.68 | - | - | - | 1.00 |
| | | | Total | 99.99 | 100.00 | 100.00 | 100.00 |

**Retention index as determined on a dimethyl silicone (DIMS) column using the homologous series of n-alkanes [15].*
*** BYC- Byadgi chilli; MC- Mundu chilli; BC- Bullet chilli; JC- Jwala chilli.*

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