

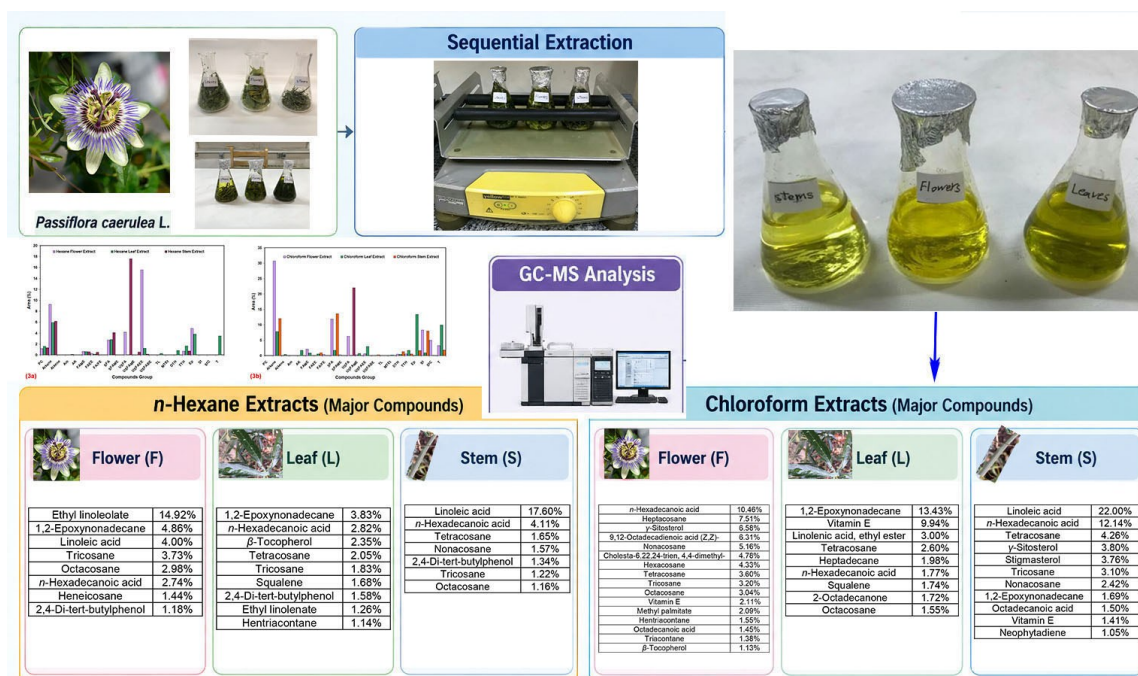
Solvent- and Organ-dependent Chemical Profiling of *Passiflora caerulea* L. Extracts by GC-MS

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GRAPHICAL ABSTRACT



Solvent- and Organ-dependent Chemical Profiling of *Passiflora caerulea* L. Extracts by GC-MS

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The chemical composition of sequential hexane and chloroform extracts obtained from the flowers (F), leaves (L), and thin stems (S) of *Passiflora caerulea* (PC) harvested during summer were investigated using gas chromatography-mass spectrometry (GC-MS). The total percentages of identified compounds in the hexane extracts were 39.4%, 22.5%, and 31.8% for the flower, leaf, and stem, respectively, whereas the corresponding values for the chloroform extracts were 70.3%, 43.7%, and 62.2%. In the hexane extracts, ethyl linoleolate was the predominant constituent in the flower extract (14.9%), while linoleic acid (17.6%) was the major constituent of the stem extract. 1,2-Epoxy-nonadecane was detected as a notable constituent of the flower and leaf hexane extracts (4.9% and 3.8%), respectively. In the chloroform extracts, linoleic acid was the predominant constituent in the stem extract (22.0%), whereas *n*-hexadecanoic acid was the major constituent in the flower and stem extracts (10.5% and 12.1%), respectively. The leaf chloroform extract contained a notable proportion of 1,2-epoxy-nonadecane (13.4%) and vitamin E (9.9%). Furthermore, heptacosane (7.5%), γ -sitosterol (6.6%), 9,12-octadecadienoic acid (Z,Z)- (6.3%), nonacosane (5.2%), were the major constituents in the flower chloroform extract. Overall, the extracts exhibited distinct chemical profiles depending on plant organ and extraction solvent, with fatty acids, fatty acid esters, terpenoids, and tocopherols constituting the major classes of identified compounds.

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Keywords: *Passiflora caerulea*; Flower; Leaf; Stem; Sequential extraction; Chemical composition; GC-MS

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INTRODUCTION

The genus *Passiflora* L. (Passifloraceae) comprises more than 500 species distributed mainly throughout tropical and subtropical regions, particularly in the neotropics. It is recognized for its remarkable botanical and phytochemical diversity (Ożarowski and Karpiński 2021; Guimarães *et al.* 2026). Species of *Passiflora* are predominantly climbing plants characterized by tendrils and distinctive flowers with a complex corona. Their chemical composition includes diverse classes of secondary metabolites, particularly flavonoids, phenolic compounds, β -carboline alkaloids, terpenoids, fatty acids, and sterol-related constituents. These major classes of metabolites contribute to the chemical diversity of the genus and have been reported across different *Passiflora* species (Costa *et al.* 2016; Wosch *et al.* 2017). Recent systematic analysis of 89 primary studies identified approximately 360 compounds and isomers across 114

Passiflora species but also demonstrated that phytochemical information remains concentrated in a relatively small number of commercially important species (Guimarães *et al.* 2026).

Passiflora caerulea L., commonly known as blue passionflower, is a perennial climbing species of Passifloraceae native to South America and widely cultivated outside its native distribution, including as an ornamental plant (Fig. 1).



Fig. 1. The flower, leaf, and stem of *Passiflora caerulea* L.

The species is morphologically characterized by its climbing habit, tendrils, palmately lobed leaves, and conspicuous blue-white flowers. Its occurrence and cultivation in Iran have also been documented in botanical and horticultural sources (Mozaffarian 2004). Correct species identification is particularly important in phytochemical studies of *Passiflora* because closely related species may differ considerably in their metabolite composition. Thus, chemical findings obtained from *P. incarnata*, *P. edulis*, or other *Passiflora* species should not automatically be attributed to *P. caerulea*. This distinction is relevant to the present study because previous phytochemical research has been disproportionately focused on a limited number of well-studied *Passiflora* species.

Although *P. caerulea* has been investigated for its biological and medicinal potential, its phytochemical profile remains less extensively characterized than those of several commercially important *Passiflora* species. Bioactivity-guided fractionation of *P. caerulea* leaves has demonstrated the occurrence of flavonoids and other phenolic constituents, while sequential fractionation has yielded chemically distinct fractions obtained with solvents of different polarity (El-Askary *et al.* 2017).

More recently, metabolomic analysis of *P. caerulea* leaves and pulp confirmed the presence of a diverse range of metabolites and further demonstrated the chemical and biological potential of this species (Gerasimova *et al.* 2024; Garmidolova *et al.* 2025). In addition, Ospina-Calvo *et al.* (2024) demonstrated the occurrence of several β -carboline alkaloids, including norharmaline, harmaline, harmine, harmol, harmaline, and harmalol, in *P. caerulea* flowers. Importantly, their concentrations differed markedly among floral organs and developmental stages, providing direct evidence that metabolite distribution in *P. caerulea* is organ-dependent.

The plant organ is therefore an important factor when assessing the chemical composition of *Passiflora*. Leaves, stems, and flowers have different physiological functions, anatomical characteristics, and metabolic activities and may consequently

accumulate different groups and concentrations of metabolites. Comparative chromatographic studies of *Passiflora* taxa have demonstrated differences in chemical profiles among species and plant materials, supporting the need for organ-specific phytochemical characterization (Costa *et al.* 2016; Wosch *et al.* 2017). The documented differences in the distribution of β -carboline alkaloids among *P. caerulea* floral organs further reinforces the importance of considering plant tissue when interpreting its chemical composition (Ospina-Calvo *et al.* 2024).

Extraction methodology represents another major determinant of the chemical profile obtained from plant material. Because plant metabolites differ in polarity and solubility, the use of a single solvent generally recovers only a portion of the extractable constituents. Sequential extraction with solvents of different polarity can therefore provide complementary chemical fractions and improve the coverage of lipophilic and moderately non-polar metabolites (Francischini *et al.* 2020). In particular, hexane is well suited to the extraction of highly lipophilic compounds, including hydrocarbons, fatty acids, fatty-acid derivatives, tocopherols, and sterol-related constituents (Hosseinihashemi *et al.* 2013, 2016, 2025), whereas chloroform can recover a somewhat broader range of non-polar and moderately lipophilic compounds (Hosseinihashemi *et al.* 2016). Hexane and chloroform have also previously been used in the fractionation of *P. caerulea* leaf extracts, providing additional support for their suitability for comparative extraction of non-polar constituents (El-Askary *et al.* 2017).

Gas chromatography-mass spectrometry (GC-MS) is a valuable technique for characterizing volatile, semi-volatile, and thermally stable constituents of complex plant extracts. The combination of mass-spectral matching with chromatographic retention data and experimental retention indices provides a useful approach for comparative profiling of non-polar plant constituents. GC-MS investigations of *Passiflora* species have reported fatty acids, hydrocarbons, sterols, terpenoid-related compounds, and other lipophilic metabolites, although the available data remain uneven among species and plant organs (Costa *et al.* 2016; Wosch *et al.* 2017). In interpreting such profiles, particular attention should also be given to compounds that may originate from laboratory or environmental contamination.

Despite the increasing interest in *P. caerulea*, comparatively limited information is available on the organ-specific distribution of non-polar constituents in its aerial tissues. In particular, comparative GC-MS characterization of flower, leaf, and stem extracts obtained sequentially using solvents of different polarity remains insufficiently documented. Addressing this gap is important because both plant organ and extraction solvent can substantially influence the resulting chemical fingerprint, while extrapolation of chemical characteristics from better-studied *Passiflora* species may lead to inappropriate species-level interpretation (Guimarães *et al.* 2026).

Therefore, the present study aimed to characterize and compare the chemical composition of sequential hexane and chloroform extracts prepared separately from the flower, leaf, and stem of *Passiflora caerulea* L. collected in northern Iran using GC-MS. The study specifically examines the extent to which plant organ and extraction solvent affect the composition and relative abundance of the detected constituents, thereby providing additional chemical information on the comparatively less-characterized species *P. caerulea*.

EXPERIMENTAL

Plant Material, Sample Preparation, and Sequential Solvent Extraction

Fresh flowers, leaves, and thin stems of *Passiflora caerulea* L. were collected in July 2019 from the Babol Plain, during the flowering stage. Babol are located in Mazandaran province, northern Iran, between 52° 35' - 52° 45' E and 36° 31' - 36° 37' N. The total surface and the mean altitude of the Babol wetlands are 1470 ha and 14.7 m, respectively. The rainiest month is October. The mean annual precipitation is 738.7 mm and the mean annual temperature is 16.3 °C. The maximum and minimum mean temperatures are 29.3 °C, and 4.5 °C, respectively (Ghahremaninejad *et al.* 2012). The plant material was identified as belonging to the genus *Passiflora* by Prof. Dr. HossinAshrafi, Assistant Professor, Department of Wood Science and Paper Technology, Ka.C., Islamic Azad University, Karaj, Iran. A voucher specimen was deposited under voucher number 5252.

Following collection, the flowers, leaves, and thin stems were manually separated and dried in the shade at room temperature (~25 °C) with estimated ambient humidity (~50%) for approximately two months. The dried plant materials were stored and transported in glass containers prior to extraction. No grinding or milling was performed before extraction. Each plant organ was processed separately throughout the extraction procedure (Fig. 2).

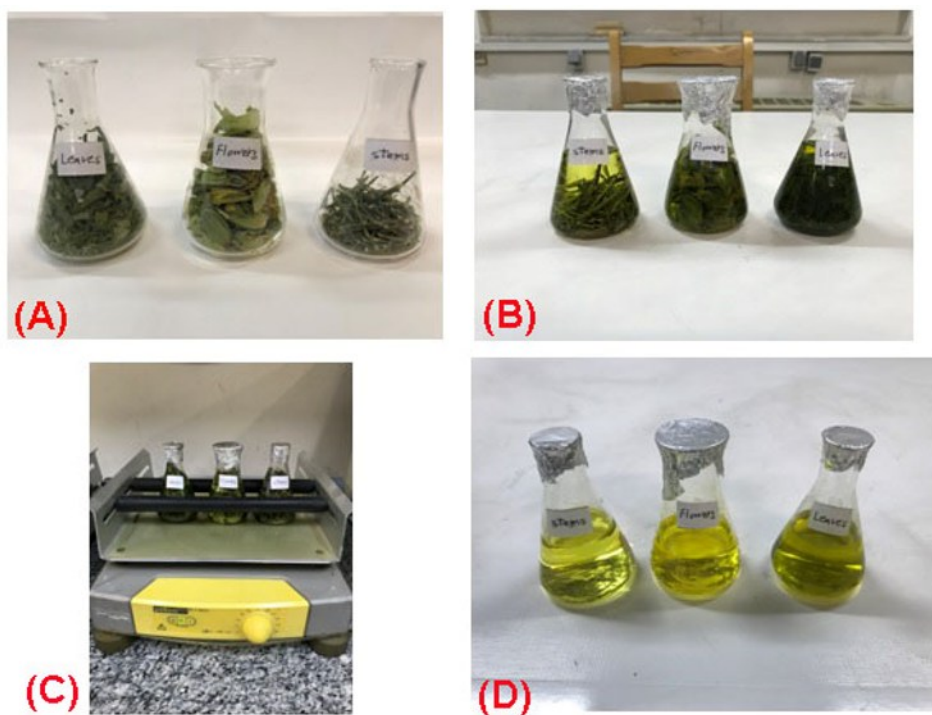


Fig. 2. Preparation of the *P. caerulea* extracts: Three 150-mL Erlenmeyer flasks containing the prepared flower, leaf, and thin-stem samples ready for extraction (A); three 150-mL Erlenmeyer flasks containing the prepared flower, leaf, and thin-stem samples immersed in hexane (B); an orbital shaker containing three 150-mL Erlenmeyer flasks with the flower, leaf, and thin-stem samples immersed in hexane (C); three 150-mL Erlenmeyer flasks containing the chloroform extracts of the flower, leaf, and thin-stem samples (D). No photographs were taken of the resulting hexane extracts of the flowers, leaves, and stems.

Sequential solvent extraction was performed using hexane followed by chloroform. For the initial extraction, 7 g of each dried plant material (flower, leaf, and stem) was weighed separately and transferred into a 150-mL Erlenmeyer flask. Approximately 100 mL of hexane was added to each flask. The flasks were covered with aluminum foil and sealed with adhesive tape to minimize solvent evaporation. The samples were extracted at laboratory temperature for 24 h using an orbital shaker (OS 2 basic, Yellow Line, IKA-Werke GmbH & Co. KG, Germany) operated at 700 rpm.

Following extraction, the mixtures were filtered through Whatman filter paper, and the resulting hexane extracts were collected separately according to plant organ. The residual plant materials were then left at room temperature for 6 days to allow residual hexane to evaporate before the subsequent extraction step (Fig. 2).

For the second extraction, approximately 100 mL of chloroform was added to each corresponding plant residue. The samples were extracted under the same conditions as described for the hexane extraction, *i.e.*, at room temperature for 24 h with continuous agitation at 700 rpm. The mixtures were subsequently filtered through Whatman filter paper, and the resulting chloroform extracts were collected separately according to plant organ.

The hexane and chloroform extracts were maintained separately and concentrated by solvent evaporation under a laminar flow hood. The resulting crude extracts were retained for subsequent chemical composition analysis.

Extracts Analysis and Identification of Chemical Constituents

The extracts obtained from *P. caerulea* flower, leaf, and stem were analyzed by GC-MS. Accordingly, 100 μ L of each extract was dried over anhydrous sodium sulfate and redissolved in 900 μ L of hexane. For consistency with the actual sample preparation procedure, this step was performed by taking 100 μ L of the concentrated extract residue and adding 900 μ L of hexane for dilution; the entire resulting 1-mL solution was not injected into the instrument, and only 1 μ L was injected for GC-MS analysis. The samples were analyzed using an Agilent 7890A gas chromatography coupled to an Agilent 5975C mass spectrometer (Agilent Technologies, Palo Alto, CA, USA). An HP-5MS cross-linked capillary column (30 m long and 0.25 mm internal diameter, 0.25 μ m film thickness) was used for the analyses.

The GC-MS operating conditions were as follows: helium was used as the carrier gas at a flow rate of 1 mL/min. The injector and transfer-line temperatures were maintained at 260 and 270 °C, respectively. The oven temperature was initially set at 60 °C and held for 2 min, followed by an increase at a rate of 7 °C/min to 280 °C, where it was held for 15 min. The mass spectrometer was operated with an electron ionization energy of 70 eV, and the injection was performed at a split ratio of 50:1. The total chromatographic run time recorded by the GC-MS system was 48.4 min (Sabzikar *et al.* 2020).

Compounds were identified by comparing their mass spectra with those in the Wiley 275 L (1998) and NIST-05 mass spectral libraries, matching and comparison of retention times with published data (Julian and König 1988; Adams 2001). Identification was further supported by comparison of the calculated retention indices with published retention-index data. Retention indices (R_I) were calculated using a homologous series of *n*-alkanes (C_9 to C_{23}) according to the method of Kováts (1958).

The relative percentages of all identified components were calculated from their corresponding peak areas, with the total chromatographic peak considered as 100%.

RESULTS AND DISCUSSION

GC-MS Profiles of *Passiflora caerulea* Extracts

Chemical composition of the hexane and chloroform extracts

The GC-MS analysis revealed considerable differences in the chemical profiles of the flower, leaf, and stem extracts of *P. caerulea* L., as well as between the sequential hexane and chloroform fractions (Tables 1 and 2).

Table 1. Chemical Composition of Hexane Extracts from the Flower, Leaf, and Thin Stem of *P. caerulea* L.

Compound	RT (min) (F-L-S)	Flower (%)	Leaf (%)	Stem (%)	KI ^{exp} (F-L-S)	KI ^{lit}
2,4-Di-tert-butylphenol	16.61-16.59-16.61	1.18	1.58	1.34	1520-1519-1520	1520
Hexadecane	17.99	0.27	0.53	0.30	1601	1600
2,2-Dimethyl-N-phenethylpropionamide	20.10	nd	nd	0.14	1734	1719
Octadecane	21.12-21.13	nd	0.13	0.15	1800	1800
Neophytadiene	21.72	nd	0.67	nd	1841	1842
2-Hexadecene, 2,6,10,14-tetramethyl-	21.81	nd	0.17	nd	1848	1847
Nonadecane	22.58-22.59	nd	0.09	0.12	1899-1900	1900
Methyl palmitate	22.97-22.96-22.97	0.65	0.62	0.57	1928-1927-1928	1927
n-Hexadecanoic acid	23.57-23.63-23.64	2.74	2.82	4.11	1971-1975-1976	1959
Ethyl palmitate	23.92-23.91-23.92	0.27	0.15	0.48	1995-1995-1996	1993
Methyl linoleate	25.28	nd	nd	0.53	2097	2098
Heneicosane	25.32-25.31	1.44	0.18	nd	2100-2099	2100
Linoleic acid	25.87-26.04	4.00	nd	17.60	2144-2157	2156
9,12-Octadecadienoic acid (Z,Z)-	26.08	0.18	nd	nd	2160	2130
Ethyl linoleolate	26.13	14.92	nd	nd	2164	2162
Ethyl linolenate	26.22-26.25-26.25	0.64	1.26	0.15	2171-2173-2173	2166
Docosane	26.59	0.83	nd	nd	2200	2200
Tricosane	27.83-27.82-27.83	3.73	1.83	1.22	2301-2301-2301	2300
4,8,12,16-Tetramethyl- heptadecan-4-olide	28.51	nd	0.29	nd	2359	2349
Tetracosane	29.01-32.28	nd	2.05	1.65	2401-2401	2400
Octacosane	33.29	2.98	nd	1.16	2801-2801	2800
Squalene	33.61-33.62-33.62	0.72	1.68	0.74	2833-2833-2833	2835
Nonacosane	34.34	nd	nd	1.57	2903	2900
δ-Tocopherol	35.00	nd	0.53	nd	2958	2960
1,2-Epoxy-nonadecane	36.15-36.17	4.86	3.83	nd	-	-
β-Tocopherol	36.23	nd	2.35	nd	3051	3043
γ-Tocopherol	36.43	nd	0.59	nd	3065	3074
Hentriacontane	37.01	nd	1.14	nd	3104	3100
-	Total Identified	39.41	22.49	31.83	-	-

Notes. KI^{exp}, experimental Kovats retention index; KI^{lit}, literature Kovats retention index; RT, retention time; nd, not detected

The hexane extracts contained 15, 20, and 16 distinct chromatographic constituents in the flower, leaf, and stem, respectively, whereas the corresponding chloroform extracts contained 30, 21, and 22 distinct constituents. The total identified peak areas were 39.4%, 22.5%, and 31.8% for the flower, leaf, and stem hexane extracts, respectively, and 70.3%,

43.7%, and 62.2% for the corresponding chloroform extracts (Tables 1 and 2). These differences indicate that the chemical profiles of *P. caerulea* extracts vary according to plant organ and extraction solvent (Costa *et al.* 2016; Wosch *et al.* 2017).

The marked variation observed between the two extraction fractions is also chemically meaningful. Solvent polarity and selectivity are important determinants of the classes and relative proportions of compounds recovered from plant tissues. The lower polarity of hexane favors the extraction of highly lipophilic constituents, whereas the relatively higher polarity of chloroform can facilitate the recovery of a somewhat broader range of lipophilic and moderately non-polar compounds, including fatty acids, sterol-related compounds, and oxygenated metabolites (Lefebvre *et al.* 2021). Because chloroform was applied sequentially to the residue remaining after hexane extraction, these differences reflect not only solvent selectivity but also the sequential extraction procedure.

The flower hexane extract was characterized by a predominance of lipid-related compounds and long-chain hydrocarbons. Ethyl linoleolate represented the largest identified constituent (14.92%), followed by 1,2-epoxynonadecane (4.86%), linoleic acid (4.00%), octacosane (2.98%), and *n*-hexadecanoic acid (2.74%). The occurrence of hydrocarbons and lipid-associated compounds is consistent with the low polarity of hexane and its affinity for highly lipophilic constituents (Lefebvre *et al.* 2021).

The leaf hexane fraction showed a distinct chemical profile compared with the flower fraction. 1,2-Epoxynonadecane was the most abundant identified constituent (3.83%), followed by *n*-hexadecanoic acid (2.82%), β -tocopherol (2.35%), tricosane (1.83%), squalene (1.68%), and 2,4-di-*tert*-butylphenol (1.58%). The occurrence of tocopherol, squalene, fatty acid, hydrocarbon, and other lipid-associated constituents indicates a chemically diverse lipophilic fraction. Although the species investigated in the present study differs from that examined by Aman *et al.* (2016), some of the compound classes identified here have also been reported in GC-MS profiles of *P. incarnata* extracts.

The stem hexane extract exhibited the most pronounced dominance of individual compounds. Linoleic acid represented 17.60% of the total identified peak area, followed by *n*-hexadecanoic acid (4.11%). Other identified constituents occurred at considerably lower proportions, including tricosane (1.22%), octacosane (1.16%), and squalene (0.74%). The high proportion of linoleic acid in the stem fraction indicates a substantial contribution of unsaturated fatty acids to its non-polar chemical profile. Fatty acids are recurrent constituents of *Passiflora* tissues, although their abundance can vary considerably among species and organs (della Cuna *et al.* 2018; 2021).

The chloroform extracts of *P. caerulea* displayed distinct chemical profiles characterized by fatty acids, long-chain hydrocarbons, sterol-related compounds, tocopherols, and other lipophilic constituents (Table 2). Similar classes of compounds have been reported in other *Passiflora* species, including *P. incarnata*, suggesting that these lipid-associated constituents may also occur in the aerial tissues of *P. caerulea* (Aman *et al.* 2016; Song *et al.* 2018). The flower chloroform extract was characterized by a diverse profile of fatty acids, hydrocarbons, sterol-related compounds, and other lipophilic constituents. *n*-hexadecanoic acid was the major identified constituent (10.46%), followed by heptacosane (7.51%), γ -sitosterol (6.58%), and 9,12-octadecadienoic acid (6.31%). Other notable constituents included nonacosane (5.16%), cholesta-6,22,24-trien-4,4-dimethyl- (4.78%), hexacosane (4.33%), and tetracosane (3.60%). The predominance of fatty acids and hydrocarbons in this fraction is consistent with previous reports describing palmitic acid, linoleic acid, sterols, and other lipophilic constituents in *Passiflora* tissues (Aman *et al.* 2016; Song *et al.* 2018).

Table 2. Chemical Composition of Chloroform Extracts from the Flower, Leaf, and Thin Stem of *P. caerulea* L.

Compound	RT (min) F-L-S	F (%)	L (%)	S (%)	K ^{exp} F-L-S	K ^{lit} F-L-S
Eucalyptol	7.10	nd	0.24	nd	1036	1035
Tetradecane	14.49	nd	0.26	nd	1400	1400
1-Hexadecene	17.86	nd	0.19	nd	1593	1588
Hexadecane	17.98- 17.97	0.25	0.45	nd	1600- 1600	1600
Heptadecane	19.59- 27.80	0.15	1.98	nd	1700	1700
1-Octadecene	21.02	nd	0.21	nd	1793	1789
Octadecane	21.12- 21.13	0.31	nd	0.19	1800- 1800	1800
Hexadecane, 2,6,10,14- tetramethyl-	21.27	nd	nd	0.24	1810	1811
Neophytadiene	21.71- 21.71- 21.73	0.52	0.46	1.05	1841- 1841- 1842	1842
Nonadecane	22.59	nd	nd	0.14	1900	1900
Methyl palmitate	22.96- 22.96	2.09	0.90	nd	1927	1927
<i>n</i> -Hexadecanoic acid	23.69- 23.56- 23.74	10.46	1.77	12.14	1979- 1970- 1983	1959
Ethyl palmitate	23.90- 23.93	nd	0.55	0.97	1994- 1996	1993
Eicosane	23.97	0.24	nd	nd	1999	2000
2-Octadecanone	24.05	nd	1.72	nd	2005	1992
Linoleic acid, methyl ester	25.27	0.28	nd	nd	2096	2100
Methyl 9,12,15- octadecatrienoate	25.35	nd	0.75	nd	2102	2105
Ethyl linolenate	25.36	0.32	nd	nd	2103	2100
Methyl stearate	25.67	0.30	nd	nd	2128	2127
9,12-Octadecadienoic acid (Z,Z)-	25.96	6.31	nd	nd	2151	2130
Linoleic acid	26.04	nd	nd	22.00	2157	2156
Linolenic acid, ethyl ester	26.22	nd	3.00	nd	2171	2166
Octadecanoic acid	26.25- 26.32	1.45	nd	1.50	2173- 2179	2174
Ethyl linoleate	26.14	0.31	nd	nd	2165	2166
Docosane	26.58- 26.61	nd	0.68	0.97	2199- 2201	2200

Table 2. Continued. Chemical Composition of Chloroform Extracts from the Flower, Leaf, and Thin Stem of *P. caerulea* L.

Compound	RT (min) F-L-S	F (%)	L (%)	S (%)	KI ^{exp} F-L-S	KI ^{lit} F-L-S
Tricosane	27.82- 27.84	3.20	nd	3.10	2301- 2302	2300
Tetracosane	29.00- 28.98- 29.03	3.60	2.60	4.26	2400- 2399- 2403	2400
Linoleic acid, butyl ester	29.85	nd	nd	0.28	2476	2478
Hexacosane	31.23	4.33	nd	nd	2601	2600
Heptacosane	32.30	7.51	nd	nd	2703	2700
Octacosane	33.29- 33.27	3.04	1.55	nd	2801- 2799	2800
Squalene	33.60- 33.59- 33.62	0.56	1.74	0.59	2832- 2831- 2833	2835
(Z)14-Tricosenyl formate	33.66	0.40	nd	nd	2838	2878
Nonacosane	34.34- 34.35	5.16	nd	2.42	2903- 2903	2900
triacontane	35.52- 35.50	1.38	0.34	nd	3000- 2998	3000
β -Tocopherol	36.20- 36.21	1.13	nd	0.20	3048- 3049	3043
γ -Tocopherol	36.42	nd	nd	0.17	3064	3074
Hentriacontane	36.96- 36.98	1.55	nd	0.93	3101- 3102	3100
Vitamin E	37.69- 37.72- 37.71	2.11	9.94	1.41	3144- 3146- 3146	3149
Ergosterol	39.08	0.82	nd	nd	-	3080
Campesterol	39.45- 39.48	0.94	nd	0.38	-	3131
1,2-Epoxy-nonadecane	39.67- 39.65	nd	13.43	1.69	-	-
Cholesta-6,22,24-trien, 4,4-dimethyl-	40.14	4.78	nd	nd	-	-
Stigmasterol	40.17	nd	nd	3.76	-	3170
γ -Sitosterol	41.44- 41.36- 41.45	6.58	0.95	3.80	-	3351
Handianol	42.22	0.25	nd	nd	-	3465
Total Identified	-	70.33	43.71	62.19	-	-

Notes. KI^{exp}, experimental Kovats retention index; KI^{lit}, literature Kovats retention index; RT, retention time; nd, not detected

The leaf chloroform extract showed a distinct chemical profile characterized by oxygenated long-chain compounds and tocopherol constituents. 1,2-Epoxy-nonadecane was the most abundant identified constituent (13.43%), followed by vitamin E (9.94%). Linolenic acid ethyl ester (3.00%) was also detected, together with smaller amounts of other fatty acid derivatives, tocopherols, hydrocarbons, and squalene. Similar lipid-associated constituents have been reported in GC-MS analyses of *P. incarnata*, including

vitamin E, fatty acids, phytol, and sterol-related compounds (Aman *et al.* 2016). These findings indicate some degree of chemical similarity between the chloroform extract of *P. caerulea* and previously investigated *Passiflora* species.

The stem chloroform extract was particularly rich in fatty acid compounds. Linoleic acid was the dominant constituent (22.00%), followed by *n*-hexadecanoic acid (12.14%). Other notable constituents included tetracosane (4.26%), γ -sitosterol (3.80%), stigmasterol (3.76%), tricosane (3.10%), and nonacosane (2.42%). The predominance of linoleic and palmitic acids in the stem fraction is consistent with the recurrent occurrence of these fatty acids in *Passiflora* tissues. Recent metabolic profiling of *Passiflora* leaves and pulp likewise identified palmitic and linoleic acids as important fatty acids, together with β -sitosterol, stigmasterol, and campesterol among the sterol constituents (Song *et al.* 2018).

The chemical profiles differed according to both plant organ and extraction fraction. The hexane extracts were generally characterized by hydrocarbons and highly lipophilic constituents, whereas the chloroform fractions showed appreciable contributions from fatty acids, oxygenated lipids, tocopherols, and sterol-related compounds. Such differences reflect the influence of solvent polarity and extraction selectivity on the recovery of plant metabolites (Lefebvre *et al.* 2021). Because chloroform was applied sequentially to the residue remaining after hexane extraction, these differences should not be interpreted as independent solvent effects; rather, they reflect the combined effects of solvent selectivity and sequential extraction.

Fatty acids were among the major constituents detected in several fractions. Linoleic acid reached 22.00% in the stem chloroform extract and 17.60% in the stem hexane extract, while *n*-hexadecanoic acid accounted for 12.14%, 10.46%, and 4.11% in the stem chloroform, flower chloroform, and stem hexane extracts, respectively. Ethyl linoleolate was also relatively abundant in the flower hexane extract (14.92%). The occurrence of fatty acids and other lipid-associated compounds is in agreement with the presence of similar constituents reported in previous phytochemical investigations of *P. incarnata* and other *Passiflora* species (Aman *et al.* 2016; Song *et al.* 2018; della Cuna *et al.* 2018, 2021).

Tocopherols and sterol-related compounds were also detected, particularly in the chloroform fractions. Vitamin E reached 9.94% in the leaf chloroform extract, while β -tocopherol accounted for 2.35% in the leaf hexane fraction. In the chloroform extracts, γ -sitosterol represented 6.58% of the flower fraction and 3.80% of the stem fraction, while stigmasterol accounted for 3.76% in the stem fraction. These findings further indicate organ-dependent differences in the distribution of lipophilic metabolites in *P. caerulea*, with similar classes of compounds having previously been reported in *P. incarnata* and other *Passiflora* species (Aman *et al.* 2016; Song *et al.* 2018).

Finally, compound identification was based on mass spectral matching together with experimental and literature retention indices. The use of retention indices provides an additional criterion for evaluating library-based assignments; nevertheless, compounds identified without authentic standards should be considered tentatively identified rather than definitively confirmed (Dickschat 2014).

Figures 3a and 3b provide further insight into differences in the distribution of chemical groups among the six extracts of *P. caerulea*. Linoleic acid was particularly abundant in the stem extracts, accounting for 17.60% and 22.00% in the hexane and chloroform fractions, respectively, whereas it was not detected in the leaf extracts. *n*-Hexadecanoic acid reached 10.46% and 12.14% in the flower and stem chloroform extracts, respectively. In the hexane fractions, ethyl linoleolate was particularly abundant

in the flower extract (14.92%), while 1,2-epoxynonadecane occurred at 4.86% and 3.83% in the flower and leaf extracts, respectively.

Tocopherol- and sterol-related compounds also contributed to the differentiation among the extracts. β -Tocopherol (2.35%) and γ -tocopherol (0.59%) were detected in the leaf hexane extract, whereas vitamin E reached 9.94% in the leaf chloroform extract. γ -Sitosterol was detected at 6.58%, 0.95%, and 3.80% in the flower, leaf, and stem chloroform extracts, respectively, while stigmasterol accounted for 3.76% in the stem chloroform extract. To facilitate comparison, the identified constituents listed in Tables 1 and 2 were classified into major chemical groups based on their structural characteristics, and the relative percentages of individual compounds belonging to the same group were summed. The resulting chemical-group distributions are presented in Figs. 3a and 3b.

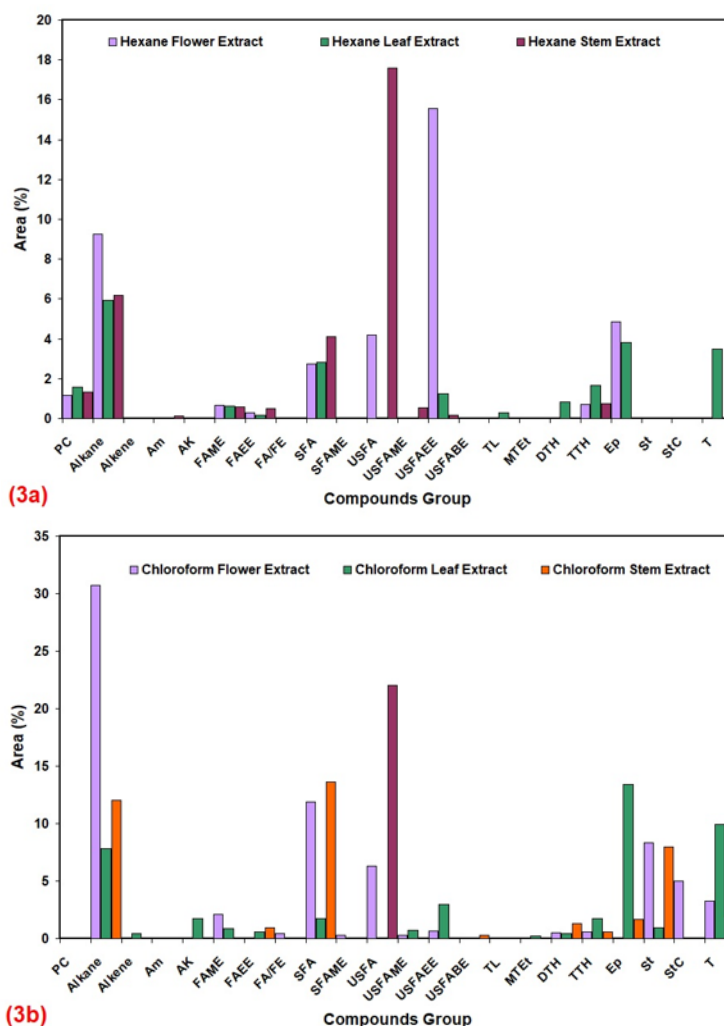


Fig. 3. Distribution of GC-MS identified compounds among chemical groups in the sequential hexane (a) and chloroform (b) extracts of *P. caerulea* flower, leaf, and stem. PC: phenolic compound; Am: amide; AK: aliphatic ketone; FAME: fatty acid methyl ester; FAEE: fatty acid ethyl ester; FA/FE: fatty acid/formate ester; SFA: saturated fatty acid; SFAME: saturated fatty acid methyl ester; USFAME: unsaturated fatty acid methyl ester; USFAEE: unsaturated fatty acid ethyl ester; USFABE: unsaturated fatty acid butyl ester; TL: terpenoid lactone; MTET: monoterpene ether; DTH: diterpene hydrocarbon; TTH: triterpene hydrocarbon; Ep: epoxy/epoxide lipid; St: sterol; StC: sterol-related compound; T: tocopherol.

This classification provides an additional perspective on the chemical profiles of the six extracts, allowing the flower, leaf, and stem extracts to be compared at the chemical-class level rather than solely on the basis of individual compounds. Overall, Figs. 3a and 3b demonstrate differences among the extracts according to both plant organ and extraction solvent, with lipid-derived compounds, tocopherols, sterol-related compounds, and other non-polar constituents contributing to the observed chemical variation.

As shown in Fig. 3a, the relative contributions of the major chemical groups differed substantially among plant organs and extraction fractions. In the hexane extracts, hydrocarbons and lipid-associated compounds constituted important components of the detected profiles. The flower extract was characterized particularly by unsaturated fatty acid ethyl esters, hydrocarbons, unsaturated fatty acids, and epoxy/epoxide lipids, whereas the leaf extract contained relatively greater contributions from hydrocarbons, epoxy/epoxide lipids, tocopherols, saturated fatty acids, and triterpene hydrocarbons. The stem extract showed a distinct profile, with unsaturated fatty acids and hydrocarbons representing major groups. These differences indicate that the distribution of lipophilic constituents varied substantially among plant organs.

The chloroform extracts also exhibited clear organ-specific differences (Fig. 3b). The flower fraction was characterized mainly by hydrocarbons, saturated fatty acids, unsaturated fatty acids, sterols, and sterol-related compounds. In the leaf chloroform fraction, epoxy/epoxide lipids and tocopherols represented important chemical groups, together with hydrocarbons and triterpene hydrocarbons. The stem chloroform fraction was characterized predominantly by unsaturated and saturated fatty acids, hydrocarbons, and sterols. Thus, although the three organs contained several common classes of lipophilic constituents, their relative contributions differed considerably among the organs.

The observed differences between the hexane and chloroform fractions are consistent with the selectivity of sequential solvent extraction. Hexane, because of its very low polarity, preferentially solubilizes highly lipophilic constituents such as hydrocarbons and other non-polar lipid-associated compounds. Chloroform can recover a somewhat broader range of lipophilic and moderately non-polar constituents, including fatty acids, oxygenated lipids, sterols, and related compounds. In the present study, however, chloroform was applied sequentially to the residue remaining after hexane extraction. Therefore, the differences between the two fractions should not be interpreted solely as independent solvent effects; rather, they reflect the combined effects of solvent selectivity, sequential extraction, and the distribution of metabolites within the individual plant organs (Lefebvre *et al.* 2021).

The group-level distribution also supports the organ-dependent variation observed for individual constituents in Figs. 3a and 3b. Linoleic acid was particularly prominent in the stem extracts, accounting for 17.6% and 22.0% of the identified peak area in the hexane and chloroform stem fractions, respectively. Similarly, *n*-hexadecanoic acid contributed substantially to the flower and stem chloroform fractions, whereas ethyl linoleolate was particularly abundant in the flower hexane extract. Tocopherols were especially prominent in the leaf chloroform fraction, while sterols made an appreciable contribution to the chloroform flower and stem profiles. Similar classes of fatty acids, hydrocarbons, tocopherols, sterols, and other lipid-associated constituents have been reported in previous investigations of *Passiflora* species (Aman *et al.* 2016; Song *et al.* 2018; della Cuna *et al.* 2018, 2021).

Overall, Figs. 3a and 3b demonstrate that the chemical composition of *P. caerulea* extracts cannot be described solely in terms of the extraction solvent. Both plant organ and

the sequential extraction procedure contributed to the observed chemical differentiation. The predominance of hydrocarbons, fatty acids and their derivatives, oxygenated lipids, tocopherols, and sterol-related compounds confirms the predominantly lipophilic character of the constituents detected under the applied GC-MS conditions. The group-level approach therefore provides a useful complementary interpretation of the individual compound data presented in Tables 1 and 2.

The percentages in Figs. 3a and 3b represent relative contributions calculated from the chromatographic peak areas of the identified constituents and should therefore not be interpreted as absolute concentrations of the corresponding compound groups. In addition, GC-MS assignments based on mass-spectral matching and retention indices should be regarded as tentative when authentic reference standards are not available. Retention indices provide an important complementary criterion for evaluating library-based identifications, but definitive structural confirmation generally requires comparison with authentic standards and/or additional analytical techniques (Koo *et al.* 2014; Bizzo *et al.* 2023).

CONCLUSIONS

1. Gas chromatography-mass spectrometry (GC-MS) analysis demonstrated clear differences in the chemical profiles of *Passiflora caerulea* flower, leaf, and stem extracts obtained by sequential hexane and chloroform extraction. The hexane fractions were generally characterized by long-chain hydrocarbons and other highly lipophilic constituents, whereas the chloroform fractions showed appreciable contributions from fatty acids, oxygenated lipids, tocopherols, and sterol-related compounds.
2. Among the major constituents, linoleic acid was particularly abundant in the stem hexane and chloroform extracts, whereas *n*-hexadecanoic acid was prominent in the stem chloroform extract. Ethyl linoleolate was particularly abundant in the flower hexane extract, while 1,2-epoxynonadecane and vitamin E were prominent constituents of the leaf chloroform extract.
3. Overall, the results demonstrate substantial organ- and extraction-dependent variation in the lipophilic chemical composition of *P. caerulea*. These findings indicate that both plant organ and the sequential extraction procedure substantially influence the chemical profiles of *P. caerulea* extracts.

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Conflict of Interest

The authors declare no conflicts of interest.

Use of Generative AI

The authors would like to declare that generative AI (ChatGPT-4.0) was used solely to assist in checking and refining the English language of the manuscript.

REFERENCES CITED

- Adams, R. P. (2001). *Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy*, 3rd Edition, Allured Publishing Corp., Carol Stream, IL, USA.
- Aman, U., Subhan, F., Shahid, M., Akbar, S., Ahmad, N., Ali, G., Fawad, K., and Sewell, R. D. E. (2016). “*Passiflora incarnata* attenuation of neuropathic allodynia and vulvodynia apropos GABA-ergic and opioidergic antinociceptive and behavioural mechanisms,” *BMC Complement. Altern. Med.* 16, article 77. <https://doi.org/10.1186/s12906-016-1048-6>
- Bizzo, H. R., Brilhante, N. S., Nolvachai, Y., and Marriott, P. J. (2023). “Use and abuse of retention indices in gas chromatography,” *J. Chromatogr. A* 1708, article 464376. <https://doi.org/10.1016/j.chroma.2023.464376>
- Costa, G. M., Gazola, A. C., Zucolotto, S. M., Castellanos, L., Ramos, F. A., Reginatto, F. H., and Schenkel, E. P. (2016). “Chemical profiles of traditional preparations of four South American *Passiflora* species by chromatographic and capillary electrophoretic techniques,” *Rev. Bras. Farmacogn.* 26(4), 451-458. <https://doi.org/10.1016/j.bjp.2016.02.005>
- da Silva Francischini, D., Lopes, A. P., Segatto, M. L., Stahl, A. M., and Zuin, V. G. (2020). “Development and application of green and sustainable analytical methods for flavonoid extraction from *Passiflora* waste,” *BMC Chem.* 14, 56. <https://doi.org/10.1186/s13065-020-00710-5>
- della Cuna, F. S., Giovannini, A., Braglia, L., Sottani, C., Grignani, E., and Preda, S. (2021). “Chemical composition of the essential oils from leaves and flowers of *Passiflora sexocellata* and *Passiflora trifasciata*,” *Nat. Prod. Commun.* 16(4), 1-7. <https://doi.org/10.1177/1934578X211007691>
- della Cuna, F. S., Calevo, J., Giovannini, A., Boselli, C., and Tava, A. (2018). “Characterization of the essential oil of the bat-pollinated *Passiflora mucronata*,” *Nat. Prod. Commun.* 13(12), 1-4. <https://doi.org/10.1177/1934578X1801301236>
- Dickschat, J. S. (2014). “Capturing volatile natural products by mass spectrometry,” *Nat. Prod. Rep.* 31, 838-861. <https://doi.org/10.1039/C3NP70080A>
- Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S., and Ju, Y.-H. (2014). “Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*,” *J. Food Drug Anal.* 22(3), 296-302. <https://doi.org/10.1016/j.jfda.2013.11.001>
- El-Askary, H. I., Haggag, M. Y., Abou-Hussein, D. R., Hussein, S. M., and Sleem, A. A. (2017). “Bioactivity-guided study of *Passiflora caerulea* L. leaf extracts,” *Iran. J. Pharm. Res.* 16(Suppl.), 46-57.
- Garmidolova, A., Halkoglu-Hristova, P., and Georgiev, V. (2025). “Exploring the multifunctionality of *Passiflora caerulea* L.: From traditional remedies to modern applications,” *Appl. Sci.* 15(6), article 3251. <https://doi.org/10.3390/app15063251>
- Gerasimova, A., Nikolova, K., Petkova, N., Ivanov, I., Dincheva, I., Tumbarski, Y., Yanakieva, V., Todorova, M., Gentscheva, G., Gavrilova, A., Yotkovska, I., Nikolova, S., Slavov, P., and Harbaliev, N. (2024). “Metabolic profile of leaves and pulp of *Passiflora caerulea* L. (Bulgaria) and their biological activities,” *Plants* 13(13), article 1731. <https://doi.org/10.3390/plants13131731>

- Ghahremaninejad, F., Naqinezhad, A., and Amirgholipour, K. V. (2012). "Plant diversity of five important wetlands of Babol Mazandaran province, Iran," *Taxon. Biosystem.* 13-24.
- Guimarães, C. S., Garcia, L. B., Oliveira, Y. T., Lima, P. M., Damasceno, G. A. B., Gomes, A. F., and Amaral, J. G. (2026). "Exploring the diversity of *Passiflora* species: A comprehensive review of its phytochemistry and biological activities," *Phytochem. Rev.* <https://doi.org/10.1007/s11101-026-10319-6>
- HosseiniHashemi, S. K., Baseri, H., Mehjabin, J. J., Alam, M. K., HossinAshrafi, S. K., Lotfi, M. (2025). "Phytochemical profile and antioxidant activity of *Juglans regia* L. green husk extracts from Tuyserkan region, Iran," *BioResources* 20(3), 7265-7277. <https://doi.org/10.15376/biores.20.3.7265-7277>
- Hosseinihashemi, S. K., Salem, M. Z. M., Hossein Ashrafi, S. K., and Latibari, A. J. (2016). "Chemical composition and antioxidant activity of extract from the wood of *Fagus orientalis*: Water resistance and decay resistance against *Trametes versicolor*," *BioResources* 11(2), 3890-3903. <https://doi.org/10.15376/biores.11.2.3890-3903>
- Hosseinihashemi, S. K., Safdari, V., and Kanani, S. (2013). "Comparative chemical composition of *n*-hexane and ethanol extractives from the heartwood of black locust," *Asian J. Chem.* 25(2), 929-933. <https://doi.org/10.14233/ajchem.2013.13159>
- Julian, D., and Konig, W. A. (1988). *The Atlas of Spectral Data of Sesquiterpene Hydrocarbons*, E. B. Verlag, Hamburg, Germany.
- Koo, I., Shi, X., Kim, S., and Zhang, X. (2014). "iMatch2: Compound identification using retention index for analysis of gas chromatography-mass spectrometry data," *J. Chromatogr. A* 1337, 202-209. <https://doi.org/10.1016/j.chroma.2014.02.049>
- Kováts, E. (1958). "Characterization of organic compounds by gas chromatography. Part 1. Retention indices of aliphatic halides, alcohols, aldehydes and ketones," *Helv. Chim. Acta.* 41(7), 1915-1932. <https://doi.org/10.1002/hlca.19580410703>
- Lefebvre, T., Destandau, E., and Lesellier, E. (2021). "Selective extraction of bioactive compounds from plants using recent extraction techniques: A review," *J. Chromatogr. A* 1635, article 461770. <https://doi.org/10.1016/j.chroma.2020.461770>
- Mozaffarian, V. (2004). *Trees and Shrubs of Iran*, Farhang Moaser Publisher, Tehran, Iran.
- Oca, M. L., Rubio, L., Sarabia, L. A., and Ortiz, M. C. (2016). "Dealing with the ubiquity of phthalates in the laboratory when determining plasticizers by gas chromatography/mass spectrometry and PARAFAC," *J. Chromatogr. A* 1464, 124-140. <https://doi.org/10.1016/j.chroma.2016.07.074>
- Ospina-Calvo, B., De Gerónimo, E., Villarruel, F. D., Aparicio, V. C., Ashworth, L., Erra-Balsells, R., and Cabrerizo, F. M. (2024). "Distribution of photoactive β -carboline alkaloids across *Passiflora caerulea* floral organs," *Photochem. Photobiol.* 100(1), 87-100. <https://doi.org/10.1111/php.13837>
- Ożarowski, M., and Karpiński, T. M. (2021). "Extracts and flavonoids of *Passiflora* species as promising anti-inflammatory and antioxidant substances," *Curr. Pharm. Des.* 27(22), 2582-2604. <https://doi.org/10.2174/1381612826666200526150113>
- Sabzikar, A., Hosseinihashemi, S. K., Shirmohammadli, Y., and Jalaligoldeh, A. (2020). "Chemical composition and antimicrobial activity of extracts from thyme and rosemary against *Staphylococcus aureus* and *Candida albicans*," *BioResources* 15(4), 9656-9671. <https://doi.org/10.15376/biores.15.4.9656-9671>

- Song, Y., Li, Z., Wang, Y., Ma, H., Zhang, X., and Liu, X. (2018). "Nutritional composition and antioxidant properties of the fruits of a Chinese wild *Passiflora foetida*," *Molecules* 23(2), article 459. <https://doi.org/10.3390/molecules23020459>
- Wosch, L., dos Santos, K. C., Imig, D. C., and Santos, C. A. M. (2017). "Comparative study of *Passiflora* taxa leaves: II. A chromatographic profile," *Rev. Bras. Farmacogn.* 27(1), 40-49. <https://doi.org/10.1016/j.bjp.2016.06.007>

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APPENDIX

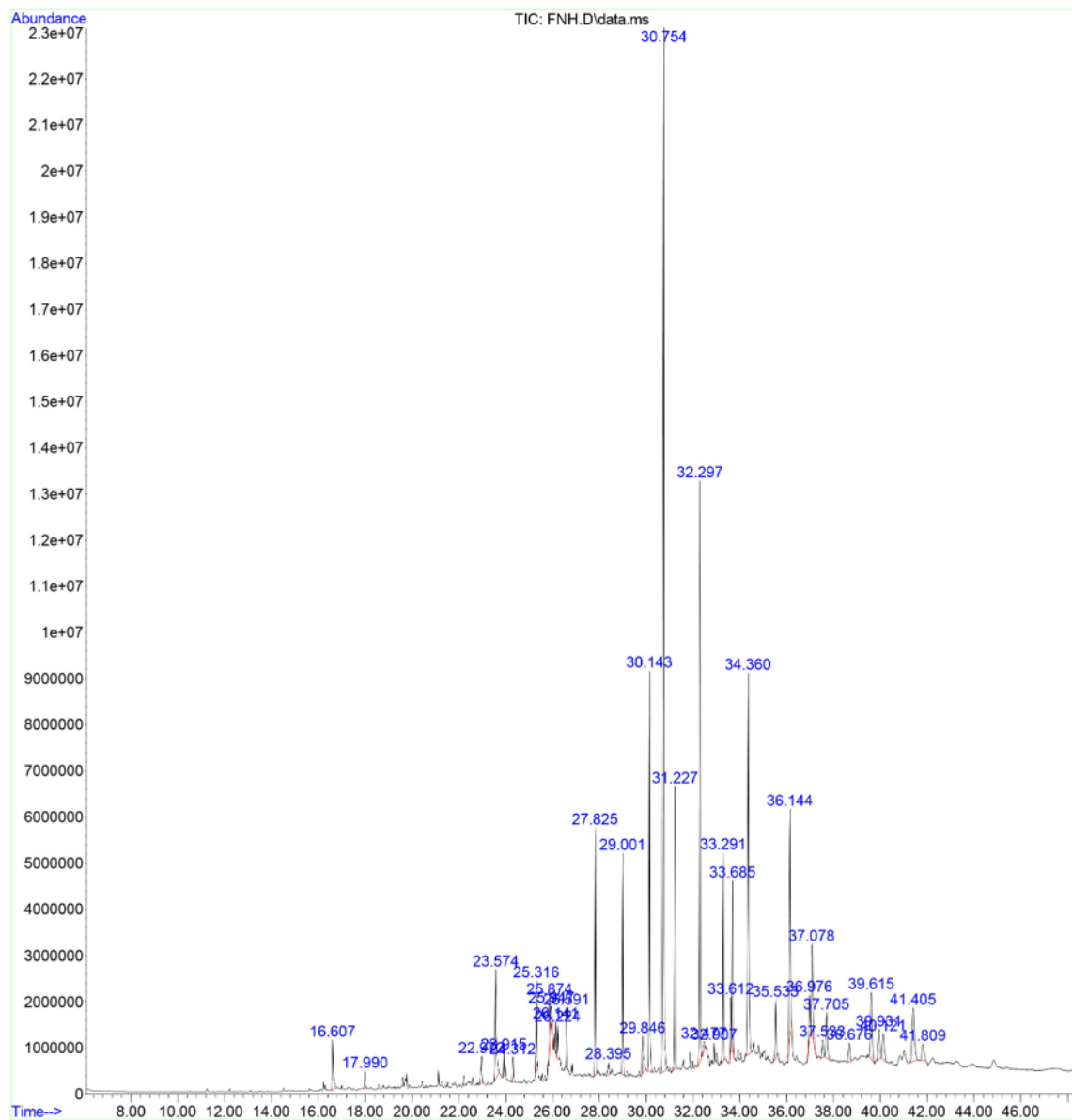
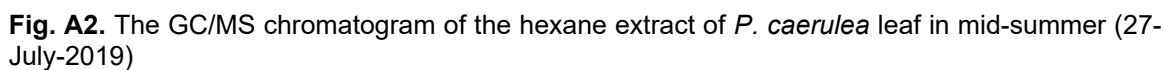


Fig. A1. The GC/MS chromatogram of the hexane extract of *P. caerulea* flower in mid-summer (27-July-2019)



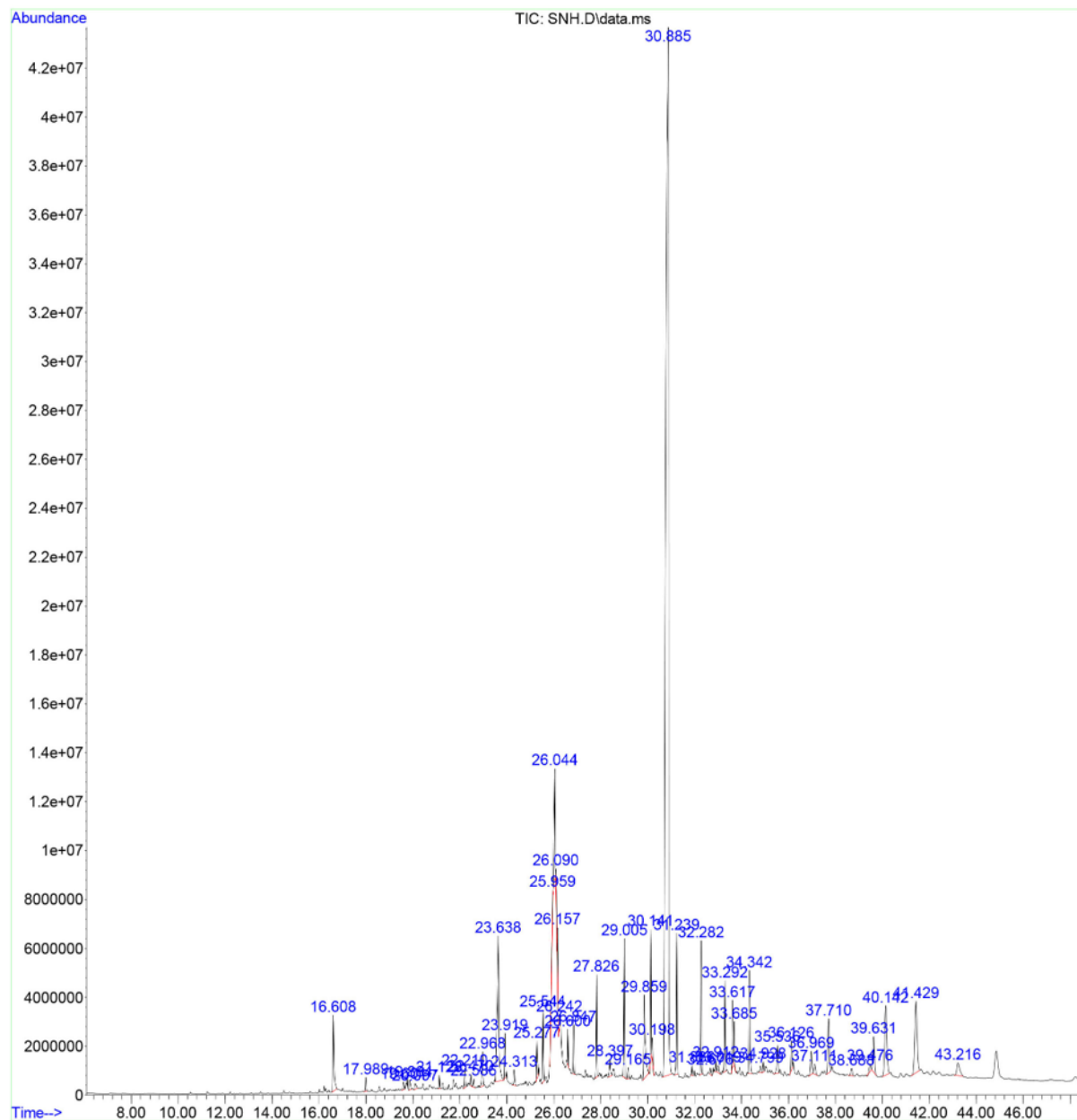


Fig. A3. The GC/MS chromatogram of the hexane extract of *P. caerulea* stem in mid-summer (27-July-2019)

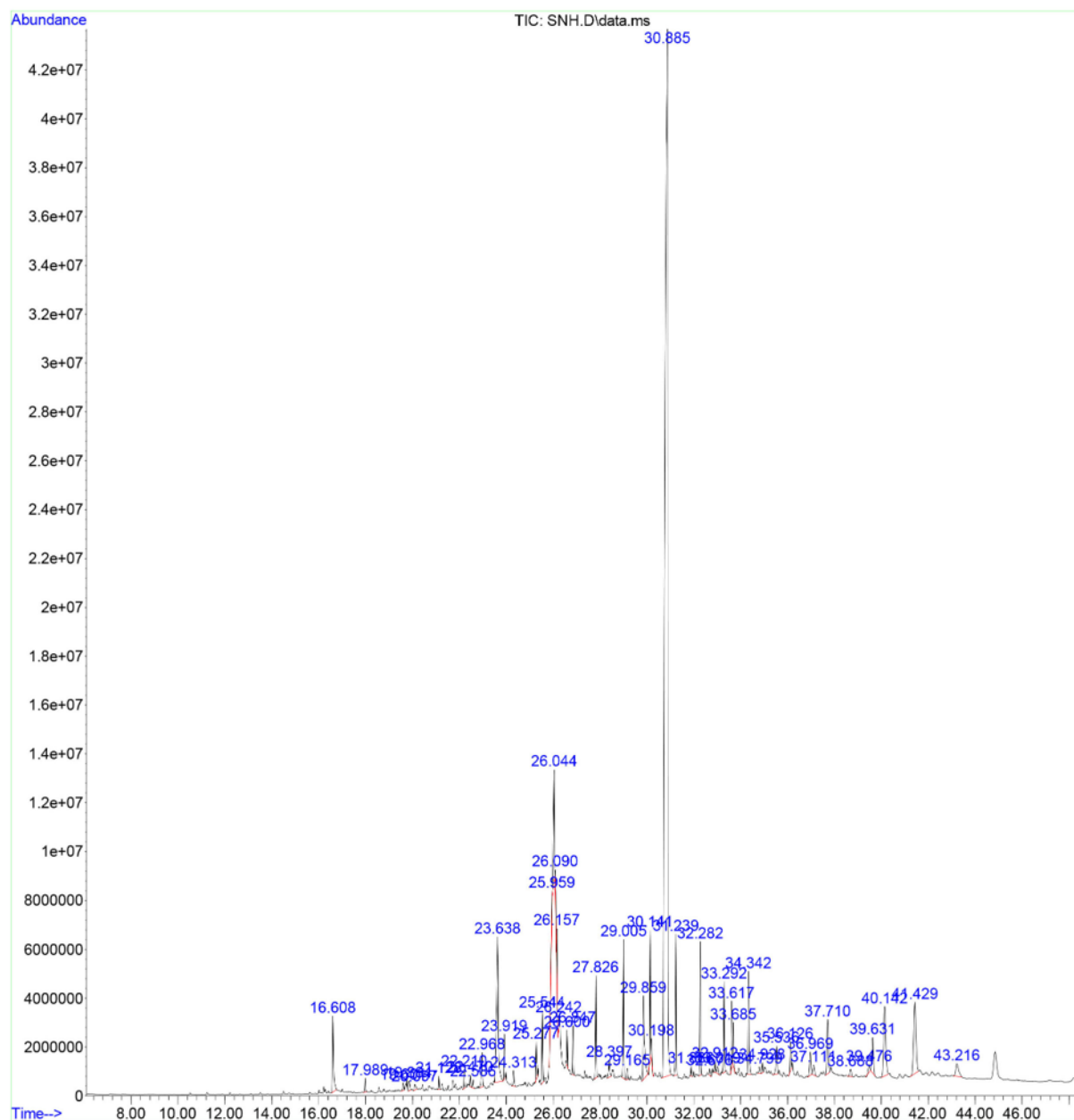


Fig. A4. The GC/MS chromatogram of the chloroform extract of *P. caerulea* flower in mid-summer (27-July-2019)

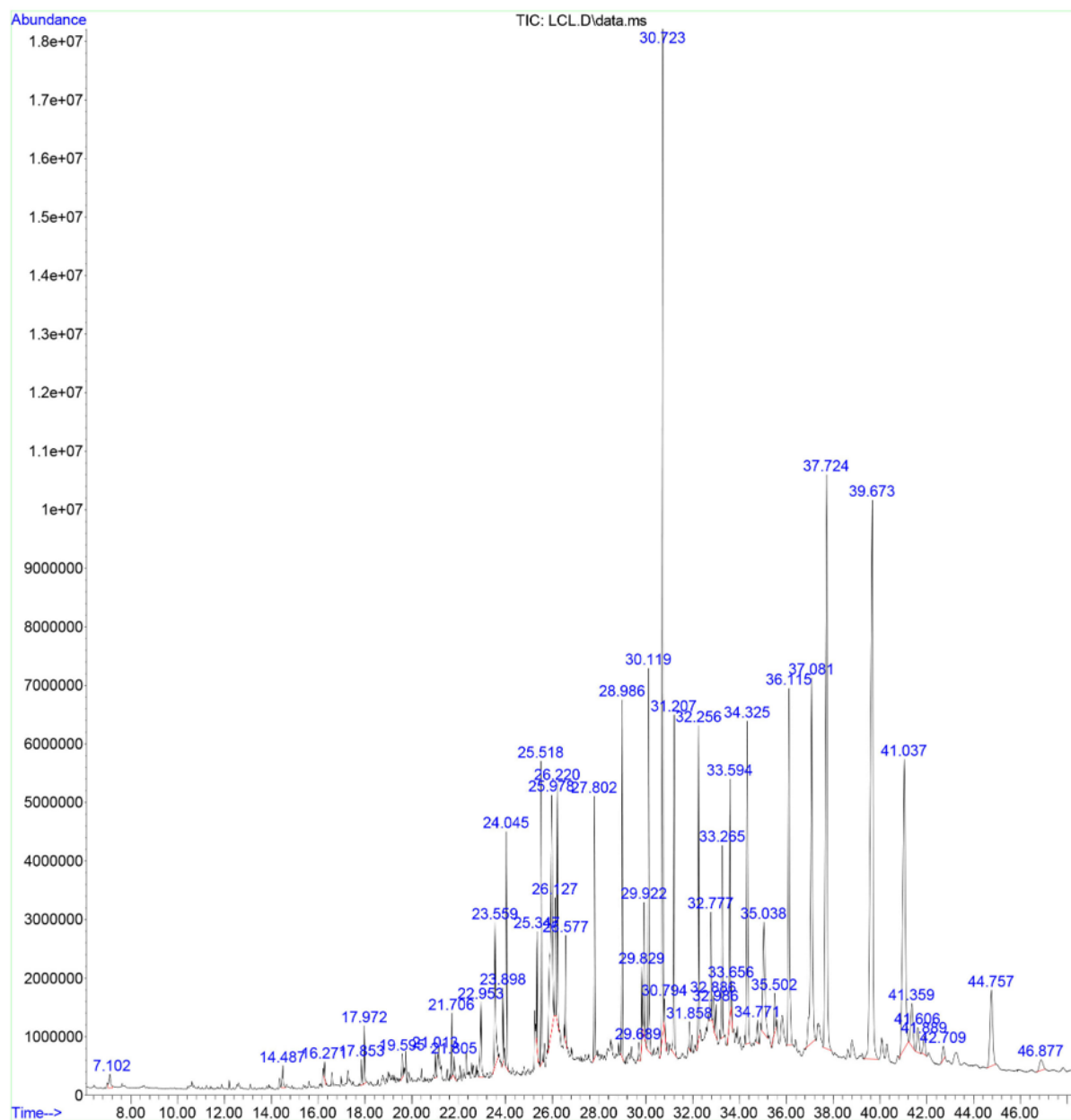


Fig. A5. The GC/MS chromatogram of the chloroform extract of *P. caerulea* leaf in mid-summer (27-July-2019)

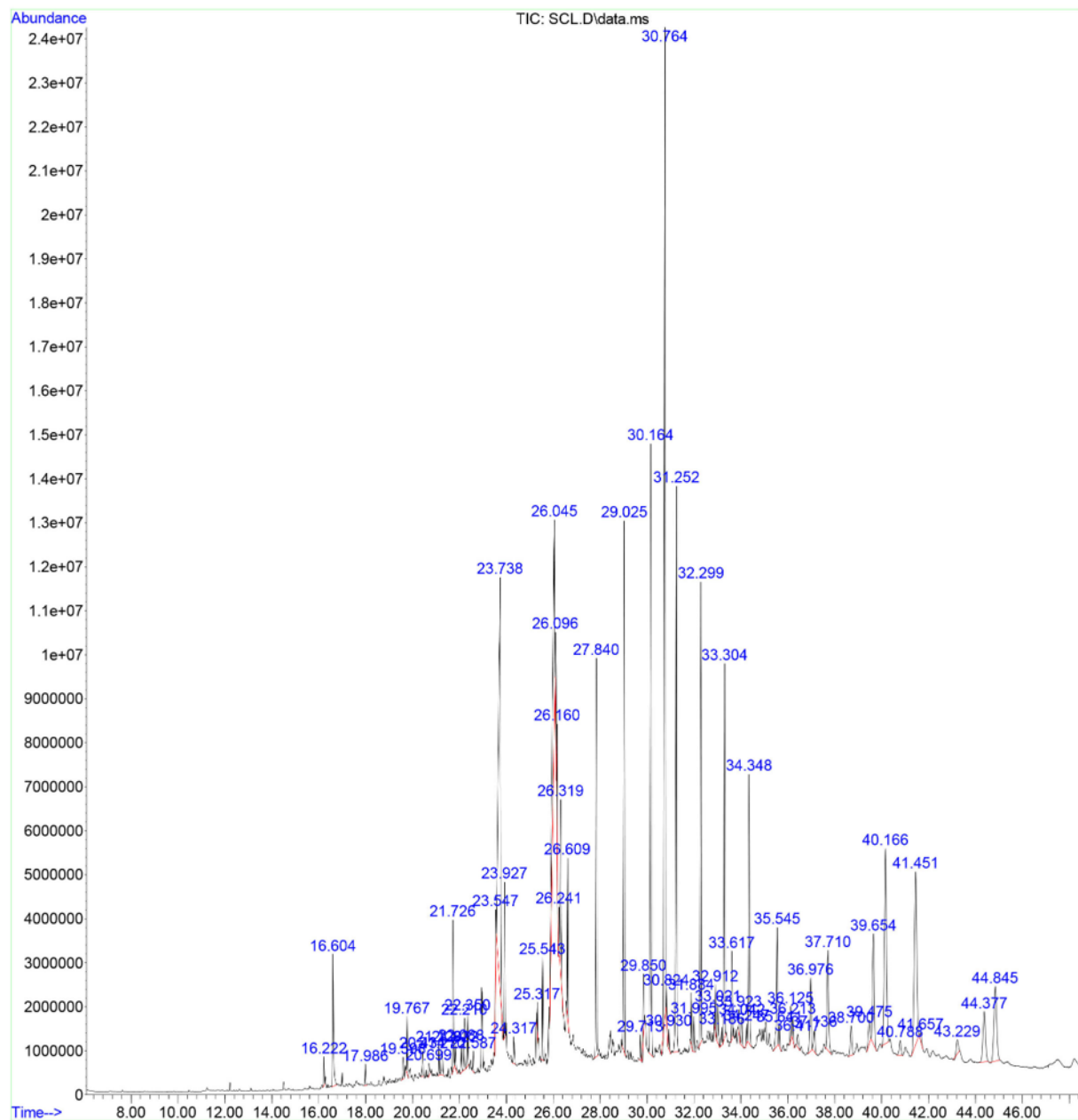


Fig. A6. The GC/MS chromatogram of the chloroform extract of *P. caerulea* stem in mid-summer (27-July-2019)