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Comprehensive Profiling of Terpenes and Terpenoids in Different Cannabis Strains Using GC × GC-TOFMS

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Abstract: Cannabis contains a wide range of terpenes and terpenoids that are mainly responsible for their distinctive aroma and flavor. These compounds have also demonstrated therapeutic effects either alone and/or as synergistic compounds with other terpenes, terpenoids, and/or cannabinoids. Several studies have attempted to fully characterize terpenes and terpenoids in cannabis; however, most of these studies used one-dimensional gas chromatography, which often results in the co-elution of the compounds. In the present study, we analyzed terpenes and terpenoids in the dried flowers of six cannabis strains using a two-dimensional gas chromatograph time-of-flight mass spectrometer (GC × GC-TOFMS). A total of 146 terpenes and terpenoids were detected across all six cannabis strains with an enhanced separation of 16 terpenes and terpenoids in the second dimension. Additionally, we achieved enhanced separation of four terpenes and terpenoids from a standard mixture in the second dimension. Chemical differences were observed in the number and relative abundance of monoterpenes, monoterpenoids, sesquiterpenes, and sesquiterpenoids in all six strains. We were also able to identify four new terpenoids in cannabis, which are reported here for the first time.

Keywords: cannabis; gas chromatography; terpenes; terpenoids

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1. Introduction

Cannabis has been used for recreational and medicinal purposes for at least 5000 years [1] and is still widely consumed in many regions of the world today [2]. It contains a wide variety of compounds that belong to different chemical classes, including terpenes, flavonoids, alkaloids, hydrocarbons, steroids, sugars, and amino acids, among others [3]. More than 500 compounds have been identified in cannabis [4]. Among these, approximately 150 terpenes and 100 cannabinoids have been identified [4,5]. Tetrahydrocannabinol (THC), the principle psychoactive compound [6], and cannabidiol (CBD) are the most abundant cannabinoids found in cannabis [7]. Terpenes are naturally occurring organic compounds found in plants. They consist of isoprene units (C5 units) and are classified according to the number of isoprene units they contain [8]. Monoterpenes (e.g., camphene, pinene, and myrcene) contain two isoprene units, sesquiterpenes (e.g., caryophyllene, humulene, and bisabolene) contain three isoprene units, and diterpenes (e.g., phytane and taxadiene) contain four isoprene units [9]. Terpenoids are the oxygenated forms of terpenes [10] and contain functional groups such as alcohols, ketones, aldehydes, esters, ethers, and/or carboxylic acids [9]. For example, the monoterpenoids borneol and linalool contain two isoprene units along with an alcohol group [10], while the monoterpenoid eucalyptol contains two isoprene units along with an ether group. Sesquiterpenoids contain three isoprene units along with an ether group in caryophyllene oxide, while an alcohol group has been added to three isoprene units in bisabolol and farnesol [9].

Terpenes and terpenoids are mostly responsible for a plants' characteristic smell. For example, linalool and linalyl acetate are the main constituents responsible for the

sweet, floral smell of lavender oil [11], while β-myrcene gives an earthy, musky smell to cannabis [12]. However, several studies have shown that terpenes and terpenoids also produce pharmacological effects, either alone or in a synergistic interaction (also known as an entourage effect) [13,14] with other terpenes or terpenoids, as well as cannabinoids. The entourage effect is not well understood, but the examples of it are interesting. For example, the anthelmintic activity of a carvone and anethole mixture is higher than that of those individual monoterpenoids [15]. β-Caryophyllene (sesquiterpene) interacts synergistically with aromadendrene oxide 2 (sesquiterpenoid) and phytol (diterpenoid) to cause an increase in apoptosis of skin epidermoid cancer and precancerous cells [16]. LaVigne and colleagues demonstrated that terpenes and terpenoids (α-humulene, geraniol, linalool, and β-pinene) enhance the analgesic activity of a synthetic cannabinoid, WIN55,212-2, which suggests that terpenes could be used to enhance the analgesic activity of cannabinoids [17].

The cataloging and discovery of terpenes and terpenoids in cannabis could lead to new therapeutic molecules, whether directly or through an entourage effect. Most profiling of terpenes and terpenoids in cannabis utilize conventional one-dimensional gas chromatography (GC) [18–21]. While this has been broadly helpful in discovering and quantifying the molecules in cannabis, GC can co-elute compounds in a complex mixture, resulting in poor or no identification or an incorrect calculation of their abundance [22]. Two-dimensional gas chromatography (GC × GC) is a powerful technique that provides better separation of the compounds in a second, chemically orthogonal dimension [23]. GC × GC can detect 2–5 times more compounds from a complex mixture than GC [24,25]. To date, there are only two published studies on cannabis profiling using GC × GC [26,27]. Franchina and colleagues mostly focused on the simultaneous profiling of multi-class components in cannabis [27], while Marchini and colleagues used GC × GC to profile the volatile compounds from hashish and one dried cannabis herb [26]. In the present study, we used GC × GC-TOFMS to profile the terpenes and terpenoids extracted from six strains of dried cannabis.

2. Materials and Methods

2.1. Cannabis Strains and Chemical Standards

The dried flowers of six cannabis strains—Pink Kush, Afghani Drifter, Blue Dream, Ultra Sour, Western Frost, and Reeferman's Rockstar—were purchased from a local cannabis store (Vancouver, BC, Canada). Methanol (≥99.8%) and toluene (≥99.5%) were purchased from Fisher Scientific (Ottawa, ON, Canada). Two terpene standard mixtures, Can-Terp Mix 1H and Can-Terp Mix 2H (Supplementary Table S1), each containing 21 terpenes (1000 µg/mL each in methanol), were purchased from Spex CertiPrep (Metuchen, NJ, USA) and kept at −20 °C. A C_8–C_{20} n-alkane standard solution (40 mg/L each in hexane) was purchased from Sigma-Aldrich (Oakville, ON, Canada) and kept at −20 °C.

2.2. Preparation of Extracts and Standard Solutions

A mixture of methanol and toluene (1:1 *v*/*v*) was prepared and used as a diluent and extraction solvent for the preparation of standard solutions and cannabis extracts, respectively. Each cannabis strain (2.0 g) was ground and passed through a 500 µm sieve. The sieved cannabis was stored at 4 °C in hermetic vials until extracted. For the preparation of the extracts, 0.2 g of the sieved cannabis was transferred to a 20 mL clear glass GC vial and extracted using 10 mL extraction solvent by sonication for 20 min at room temperature using an ultrasonic cleaner (Vevor, Rancho Cucamonga, CA, USA). The extract was then transferred to microcentrifuge tubes (made of polypropylene) and centrifuged (Eppendorf centrifuge 5424) at 10,000 rpm for 3 min at room temperature. The supernatant from the microcentrifuge tubes was transferred into a 20 mL clear glass GC vial and used for analysis. The extracts were prepared in triplicate for each strain. For the preparation of the blank, 10 mL of extraction solvent was directly transferred into a 20 mL clear glass GC vial and processed the same as cannabis strains to nullify the contamination from the vials and microcentrifuge tubes.

The C_8–C_{20} n-alkane standard solution was prepared at a concentration of 0.04 mg/L and used for the calculation of the linear retention index using the formula:

$$I_X = 100n + 100(t_X - t_n)/(t_{n+1} - t_n)$$

where I_X is the retention index of compound X (terpene or terpenoid), t_X is the retention time of compound X, t_n and t_{n+1} are retention times of the n-alkanes eluting immediately before and after the compound X. The terpene standard solution was prepared by mixing Can-Terp Mix 1H and Can-Terp Mix 2H in equal volumes and diluting to the final concentration of 10 µg/mL with diluent in a 10 mL glass vial using a single channel pipette. Each extract and standard solution were freshly prepared before analysis.

2.3. Analytical Instrument

All the standards and extracts were analyzed using GC × GC-TOFMS from Markes International (Sacramento, CA, USA), equipped with an Agilent 7890B GC and BenchTOF-Select mass spectrometer (SepSolve Analytical, Waterloo, ON, Canada). The two-dimensional column set consisted of an Rxi-5ms (crossbond 5% diphenyl/95% dimethyl polysiloxane) of dimensions 30 m × 0.25 mm i.d. × 0.50 µm d_f (Restek Corporation, Bellefonte, PA, USA) as a first dimension (1D) column, and a DB-HeavyWax (polyethylene glycol) of dimensions 5 m × 0.25 mm i.d. × 0.20 µm d_f (Agilent Technologies, Mississauga, ON, Canada) as a second dimension (2D) column. The carrier gas was helium at a constant flow of 0.7 mL/min and 21 mL/min in the 1D and 2D, respectively. The liquid injections were performed in the split mode (5:1) using an injection volume of 1 µL and an inlet temperature of 280 °C. The oven temperature program was set at 50 °C (1 min isotherm) increased to 280 °C (50 min isotherm) at a rate of 3 °C/min. The GC was equipped with an INSIGHT flow modulator (SepSolve analytical), and a modulation period of 2.6 s was used. The transfer line and ion source temperature were set at 300 °C and 280 °C, respectively. A mass range of 45 to 450 m/z was monitored at an acquisition rate of 50 spectra/s, and the ionization energy was set at 70 eV. The blank was run on GC × GC-TOFMS using the same analytical parameters.

2.4. Data Analysis

Chromatographic data acquisition and data processing were performed using ChromSpace software (SepSolve Analytical, ver. 2.1.4, Waterloo, ON Canada). For peak detection, a deconvolution algorithm and a forward NIST 11 mass spectral library search, with a minimum match factor of 600 (of 1000), were applied. The contaminants eluted in the blank (Supplementary Table S2), from the columns and extraction solvents, were removed from the data. A signal-to-noise (S/N) cut-off of 20 was used. The peaks were identified at levels 1, 2, and 3 of metabolite identification confidence according to the Metabolomics Standards Initiative criteria [28]. For level 1, the identity of 23 compounds was confirmed using standards. For level 2, the peaks were putatively identified by library matching with a forward match score of ≥700 (of 1000) and/or by matching the retention indices, calculated from a series of C_8–C_{20} n-alkanes, against the NIST Standard Reference Database (NIST Chemistry WebBook, SRD 69) and Adams database [29]. A ±20 retention index window tolerance was used. For level 3, a chemical class was assigned to any peak with a forward match score of ≥600 (of 1000) and with the top 5 hits for the same chemical class.

For each cannabis extract, the identified peaks were checked in all three technical replicates based on their retention time. For this purpose, maximum first- and second-dimension retention time deviations were set at ±0.070 min and ±0.15 s, respectively. A 100% frequency of observation (FOO) criterion was applied to select the most consistent features (i.e., those present in all of three technical replicates). The same method and criterion were used for all the cannabis extracts.

3. Results and Discussion

3.1. Enhanced Separation of Terpenes and Terpenoids in the Second Dimension Using GC × GC-TOFMS

3.1.1. Enhanced Separation of Terpenes and Terpenoids in the Standard Mixture

A mixture of 44 standards of terpenes and terpenoids (list given in Table S1) were analyzed using GC × GC-TOFMS, which included 12 monoterpenes, 20 monoterpenoids, 6 sesquiterpenes, and 6 sesquiterpenoids. Of these, we were able to separate 35 compounds, as shown in Figure 1a, which included 10 monoterpenes, 13 monoterpenoids, 6 sesquiterpenes, and 6 sesquiterpenoids. Monoterpenes and sesquiterpenes are non-polar and have different chemical formulas; thus, they were eluted in separate regions in the first dimension. However, the oxygenated derivatives, monoterpenoids and sesquiterpenoids, being polar, were eluted in distinct regions, well separated from monoterpenes and sesquiterpenes, respectively, in the second dimension using a polar 2D column. The exception to this general rule is the monoterpenoid eucalyptol, which was eluted in the monoterpene region due to its low boiling point compared to other monoterpenoids. There were nine standards, two monoterpenes and seven monoterpenoids, that could not be separated under the configurations used in this study.

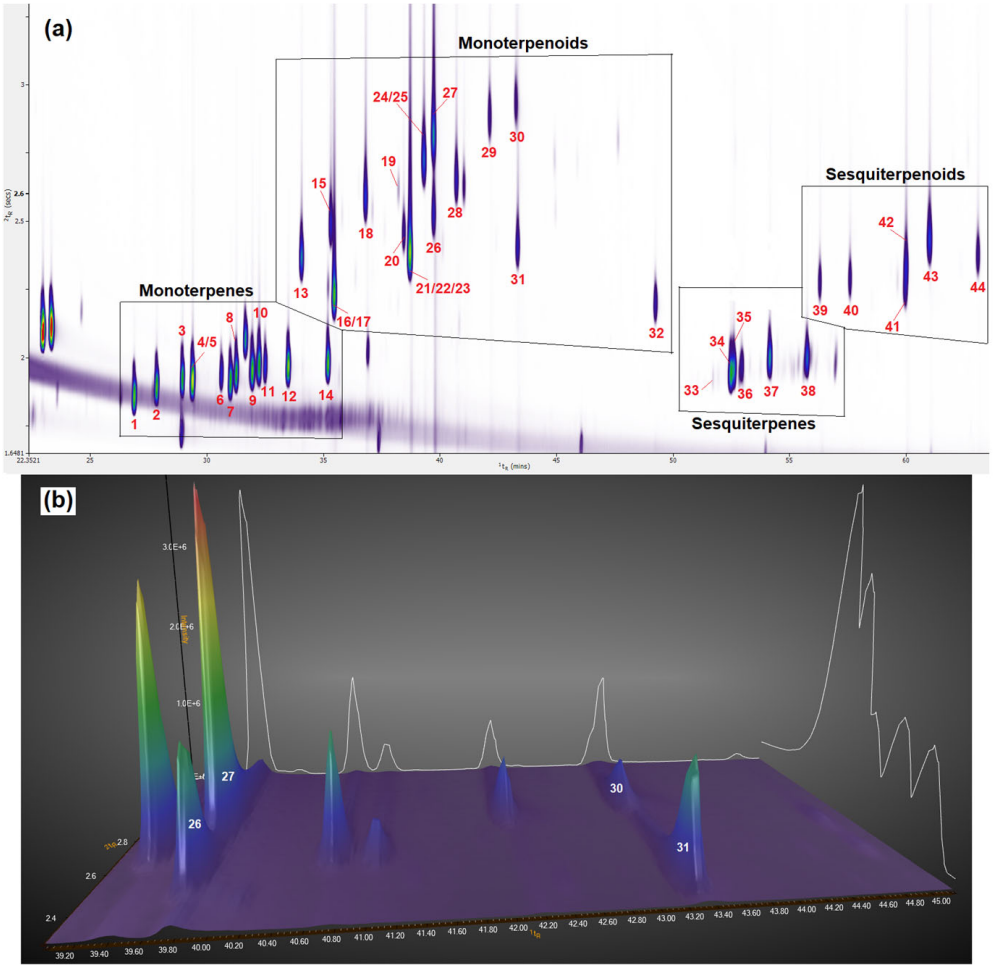

Figure 1. (**a**) GC × GC-TOFMS chromatogram of a standard mixture containing 44 terpenes and terpenoids. (**b**) Expansion of the surface chart showing the enhanced separation of hexahydrothymol (26) and (−)-borneol (27), and geraniol (30) and (+)-pulegone (31) in the second dimension. Compound numbers are given according to Table S1.

Of the 35 resolved standard compounds, GC × GC provided enhanced separation of four standards in the monoterpenoid region, hexahydrothymol, (−)-borneol, geraniol, and pulegone. These standards co-eluted in the first dimension; however, they were well separated in the second dimension, as shown in a surface chart in Figure 1b. For example, hexahydrothymol and (−)-borneol were eluted at a similar retention time (RT) in the first dimension (39.72 min and 39.76 min, respectively), but were well separated in the second dimension with RTs of 2.52 s and 2.81 s, respectively. Similarly, geraniol and (+)-pulegone were well separated in the second dimension with RTs of 2.92 s and 2.40 s, respectively, and were co-eluted in the first dimension due to their similar RTs (43.28 min and 43.31 min, respectively) in our study. Jin and colleagues reported on the separation of geraniol and pulegone in their GC analysis [20] due to their use of a more polar column (5% phenyl-arylene/95% dimethyl polysiloxane).

The nine unresolved standards included two monoterpenes and seven monoterpenoids. The two monoterpenes, β-myrcene and β-pinene, were co-eluted in both the first and second dimensions. Of the seven monoterpenoids, (+)-fenchone and L-(−)-fenchone, isoborneol, (+)-borneol, and camphor, 1S-(−)-camphor, and 1R-(+)-camphor are stereoisomers of each other, and so for this column configuration, they were co-eluted in both the first and second dimensions. As these compounds remained unseparated, we used the name of all the co-eluted compounds without R/S designation for peak identification in the cannabis strain analysis.

All the standards in the sesquiterpene and sesquiterpenoid regions were well separated in the first dimension, except for the sesquiterpenoids caryophyllene oxide and guaiol (standards 41 and 42, respectively, in Figure 1a). Both these standards were co-eluted in the first dimension with RTs of 59.98 min and 59.99 min, respectively. However, they had enhanced separation in the second dimension with RTs of 2.26 s and 2.33 s, respectively, which resulted in their detection and identification in the standard mixture.

3.1.2. Enhanced Separation of Terpenes and Terpenoids in Six Cannabis Strains

We applied the same GC × GC-TOFMS method to analyze the terpenes and terpenoids extracted from six cannabis strains. As expected, the monoterpenes, monoterpenoids, sesquiterpenes, and sesquiterpenoids were eluted in separate regions in the chromatograms of all six cannabis extracts using GC × GC, as explained above. Figure 2 shows a chromatogram of an extract from Afghani Drifter as a representation of the separation in each terpene and terpenoid region.

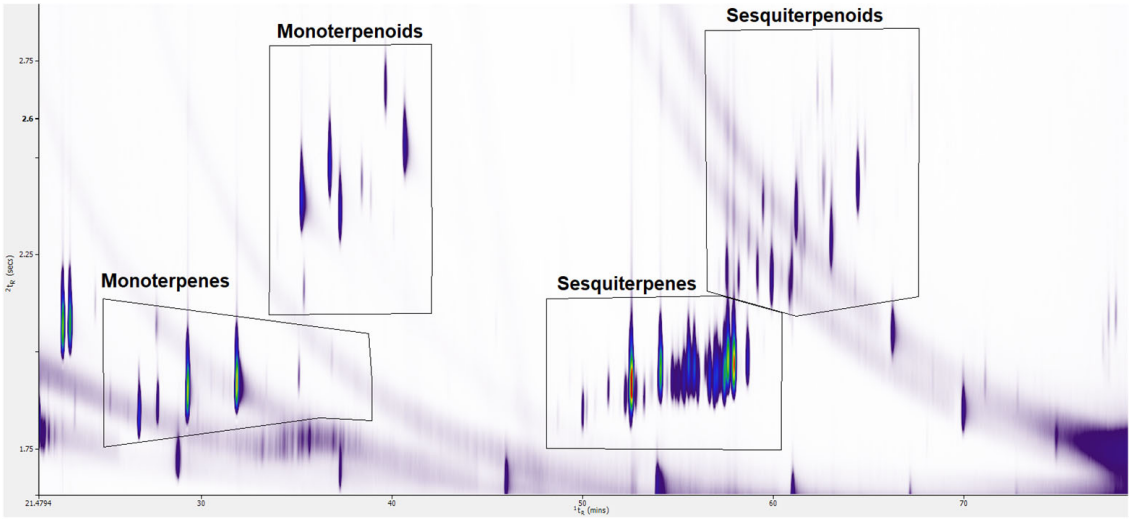

Figure 2. GC × GC-TOFMS chromatogram of Afghani Drifter showing separation of the monoterpene, monoterpenoid, sesquiterpene, and sesquiterpenoid regions in different elution zones.

GC × GC-TOFMS provided enhanced separation of a total of 16 terpenes and terpenoids, across all six cannabis strains, in the second dimension that were co-eluted in the first dimension. Of these, two were monoterpenes and 14 were sesquiterpenes and sesquiterpenoids. All of these 16 terpenes and terpenoids are marked with an asterisk (*) in Table 1. As a representation of the enhanced separation in the monoterpene, sesquiterpene, and sesquiterpenoid regions, the GC × GC-TOFMS chromatogram of Ultra Sour is shown in Figure 3. In the monoterpene region, α-terpinolene and *p*-cymenene were co-eluted in the first dimension but were separated in the second dimension with RTs of 1.97 s and 2.26 s, respectively, as shown in Figure 3a. In the sesquiterpene and sesquiterpenoid regions (Figure 3b), sesquiterpenoid *trans*-nerolidol was eluted at the same RT with an unidentified sesquiterpene (compound **87** in Table 1) but was separated in the second dimension with an RT of 2.25 s compared to 2.01 s for compound **87**. α-Bisabolol was co-eluted with an unknown sesquiterpenoid (compound **125** in Table 1) in the first dimension; however, it was separated in the second dimension with an RT of 2.34 s as compared to 2.74 s for compound **125** (Figure 3c).

The number of compounds with enhanced separation in the second dimension constitutes up to 11% of the total compounds detected across all six cannabis strains. These compounds would have remained undetected using GC due to their co-elution in the first dimension. The percentages of compounds with enhanced separation in the second dimension in each cannabis strain were: 7.6% in Pink Kush, 11.8% in Afghani Drifter, 6.5% in Blue Dream, 10.3% in Ultra Sour, and 6.7% in Reeferman's Rockstar.

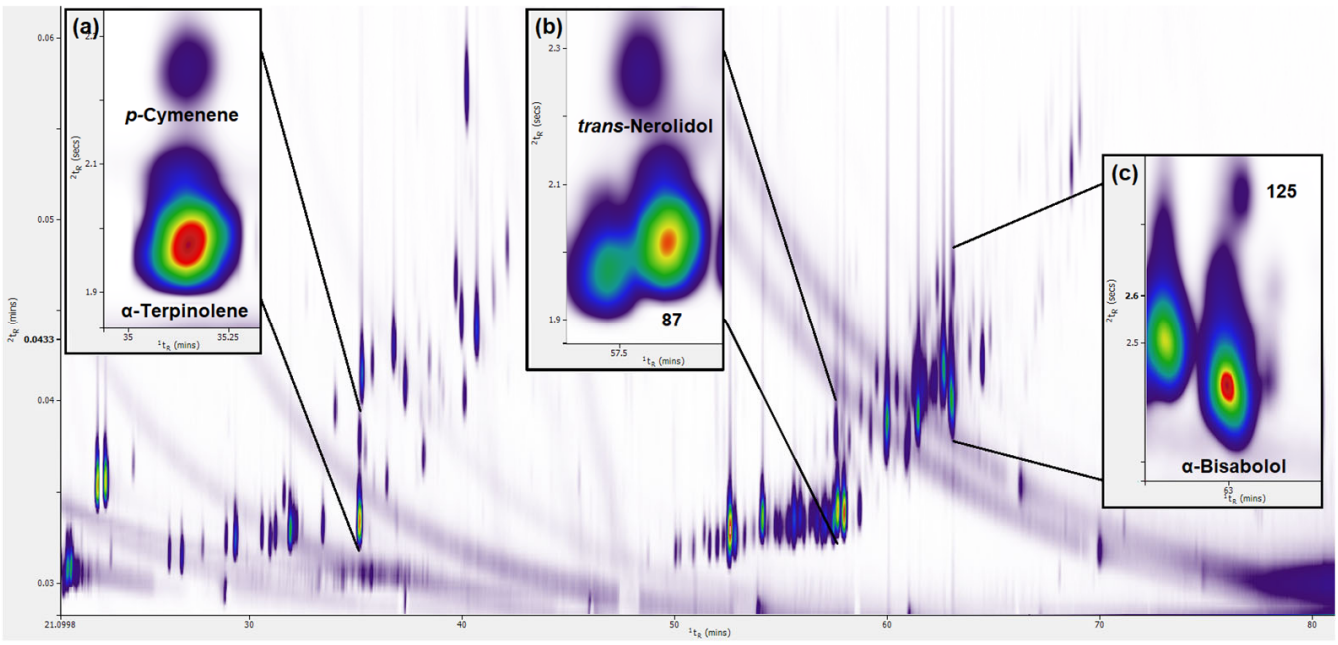

Figure 3. GC × GC-TOFMS chromatogram of Ultra Sour. (**a**) Expansion chromatogram showing enhanced separation of the monoterpenes α-terpinolene and *p*-cymenene. (**b**) Expansion chromatogram showing enhanced separation of an unidentified sesquiterpene (**87**) and a sesquiterpenoid, *trans*-nerolidol. (**c**) Expansion chromatogram showing enhanced separation of sesquiterpenoids α-bisabolol and an unidentified sesquiterpenoid (**125**). Compound numbers are given according to Table 1.

Table 1. List of terpenes and terpenoids detected in six cannabis strains.

S. No.	Compound Name [1]	Chemical Formula	RI (Exp) [4]	RI (Lib)	$^{1}t_R$ (min) [5]	$^{2}t_R$ (s) [6]	Pink Kush (%) [5]	Afghani Drifter (%) [5]	Blue Dream (%) [5]	Western Frost (%) [5]	Reeferman's Rockstar (%) [5]	Ultra Sour (%) [5]	Previously Reported in Cannabis
1	α-Thujene	$C_{10}H_{16}$	934	934	26.19	1.87 ± 0.01	-	-	-	-	-	0.27	[18,26,27]
2	α-Pinene [2]	$C_{10}H_{16}$	945	940	26.75 ± 0.02	1.86 ± 0.02	0.29	1.14	20.38	5.53	0.86	0.64	[19,26,27,30,31]
3	Camphene [2]	$C_{10}H_{16}$	962	951	27.72 ± 0.02	1.89 ± 0.02	0.06	0.31	0.39	0.33	0.22	0.07	[18,20,26]
4	Sabinene [2]	$C_{10}H_{16}$	983	977	28.85 ± 0.02	1.92 ± 0.01	-	-	0.03	0.01	-	0.21	[19,20,27,31]
5	β-Myrcene/β-Pinene *	$C_{10}H_{16}$	991	990	29.29 ± 0.01	1.92 ± 0.02	1.35	7.90	25.50	17.01	2.12	1.58	[19,26,27,30]
6	Monoterpene—unidentified	-	1002	-	29.85 ± 0.01	1.87 ± 0.01	-	-	-	-	-	0.02	-
7	α-Phellandrene [2]	$C_{10}H_{16}$	1015	1005	30.56	1.95 ± 0.01	-	-	-	-	-	0.33	[26,27,30,31]
8	3-Carene [2]	$C_{10}H_{16}$	1022	1010	30.95	1.92 ± 0.01	-	-	-	-	-	0.36	[18,26,30,31]
9	α-Terpinene [2]	$C_{10}H_{16}$	1027	1018	31.19 ± 0.01	1.95 ± 0.01	-	-	-	-	-	0.45	[18,26,30]
10	*o*-/*p*-Cymene	$C_{10}H_{14}$	1034	1026	31.59 ± 0.01	2.07 ± 0.01	-	-	-	-	-	0.18	[26,32,33]
11	Limonene [2]	$C_{10}H_{16}$	1039	1030	31.87 ± 0.01	1.95 ± 0.02	1.84	9.30	2.10	5.75	6.48	2.84	[18,26,30]
12	β-Phellandrene	$C_{10}H_{16}$	1042	1045	32.04	1.96 ± 0.01	-	-	0.34	0.36	-	0.49	[5,18,30]
13	Eucalyptol [2]	$C_{10}H_{18}O$	1044	1046	32.15 ± 0.03	1.97	-	-	0.30	-	-	0.06	[18,26,27]
14	β-Ocimene [2]	$C_{10}H_{16}$	1050	1050	32.44 ± 0.01	1.96 ± 0.02	-	-	0.90	0.14	-	0.04	[18,26,30]
15	γ-Terpinene [2]	$C_{10}H_{16}$	1068	1064	33.43 ± 0.01	1.96 ± 0.01	-	-	0.06	0.04	0.02	0.42	[18,26,27,30]
16	*cis*-Sabinene hydrate [2]	$C_{10}H_{18}O$	1078	1074	34.01	2.32 ± 0.03	-	0.03	0.13	0.08	0.03	0.20	[20,26,31]
17	α-Terpinolene **	$C_{10}H_{16}$	1099	1093	35.13 ± 0.02	1.97 ± 0.02	0.03	0.13	0.02	0.08	0.07	8.76	[18,26,30]
18	*p*-Cymenene (Benzene, 1-methyl-4-(1-methylethenyl)-) **	$C_{10}H_{12}$	1100	1095	35.16 ± 0.01	2.26 ± 0.01	-	-	-	-	-	0.32	[20,26,34]
19	Linalool [2]	$C_{10}H_{18}O$	1102	1104	35.26 ± 0.01	2.44 ± 0.03	2.27	2.45	1.38	1.91	2.98	2.07	[18,20,26,30]
20	Fenchone [2]	$C_{10}H_{16}O$	1104	1096	35.39 ± 0.02	2.19 ± 0.02	0.07	0.14	0.06	-	0.16	0.08	[18,20,26,27]
21	*trans*-Sabinene hydrate	$C_{10}H_{18}O$	1111	1099	35.75 ± 0.01	2.49 ± 0.03	-	-	0.09	-	-	0.23	[5,26,34]
22	Monoterpenoid—unidentified	-	1124	-	36.44 ± 0.02	2.12 ± 0.01	-	-	-	-	-	0.17	-
23	Fenchol [2]	$C_{10}H_{18}O$	1130	1123	36.74 ± 0.02	2.53 ± 0.03	0.94	1.68	0.44	2.15	1.64	0.90	[18–20,26]
24	*trans*-*p*-Menth-2-en-1-ol	$C_{10}H_{18}O$	1135	1137	37.03 ± 0.01	2.48 ± 0.01	-	-	-	-	-	0.04	-
25	*trans*-2-Pinanol	$C_{10}H_{18}O$	1140	1135	37.29 ± 0.01	2.40 ± 0.03	0.66	1.24	0.34	1.58	1.29	0.65	[18]
26	Ipsdienol	$C_{10}H_{16}O$	1151	1150	37.84 ± 0.02	2.69 ± 0.05	0.05	-	-	-	-	-	[5,26,35]
27	Monoterpenoid—unidentified	-	1153	-	37.96 ± 0.04	2.61	-	-	-	-	-	0.02	-
28	Monoterpenoid—unidentified	-	1155	-	38.05 ± 0.01	2.36 ± 0.01	-	-	-	-	-	0.03	-
29	Monoterpenoid—unidentified	-	1157	-	38.16 ± 0.02	2.20 ± 0.01	-	-	-	-	-	0.21	-
30	Monoterpenoid—unidentified	-	1162	-	38.42 ± 0.01	2.48 ± 0.03	0.05	0.19	0.08	0.23	0.17	0.10	-
31	*trans*-β-Terpineol [2]	$C_{10}H_{18}O$	1171	1161	38.90 ± 0.02	2.44 ± 0.03	0.05	0.08	-	0.12	0.10	0.04	[5]

Table 1. *Cont.*

S. No.	Compound Name [1]	Chemical Formula	RI (Exp) [4]	RI (Lib)	$^{1}t_R$ (min) [5]	$^{2}t_R$ (s) [6]	Pink Kush (%) [5]	Afghani Drifter (%) [5]	Blue Dream (%) [5]	Western Frost (%) [5]	Reeferman's Rockstar (%) [5]	Ultra Sour (%) [5]	Previously Reported in Cannabis
32	Monoterpenoid—unidentified	-	1175	-	39.08 ± 0.02	2.41 ± 0.01	-	-	-	-	-	0.04	-
33	(−)-Borneol [2]	$C_{10}H_{18}O$	1187	1171	39.68 ± 0.01	2.73 ± 0.04	0.46	0.45	0.24	0.84	0.56	0.39	[18,20,26,36]
34	*trans*-Carveol	$C_{10}H_{16}O$	1192	1201	39.95 ± 0.02	2.66 ± 0.01	-	-	-	-	-	0.57	[26]
35	Terpinen-4-ol	$C_{10}H_{18}O$	1194	1206	40.08	2.36 ± 0.03	0.03	0.03	-	0.05	0.04	0.29	[20,26,27,31]
36	*p*-Cymen-8-ol	$C_{10}H_{14}O$	1197	1193	40.19	3.42 ± 0.01	-	-	-	-	-	1.00	[26,27,34]
37	α-Terpineol [2]	$C_{10}H_{18}O$	1206	1192	40.67	2.58 ± 0.03	1.11	1.48	0.45	1.53	1.54	1.82	[20,26,34]
38	*trans*-Piperitol	$C_{10}H_{18}O$	1222	1208	41.46 ± 0.02	2.74 ± 0.02	-	-	-	-	-	0.10	[37]
39	Citronellol	$C_{10}H_{20}O$	1231	1232	41.89 ± 0.01	2.67 ± 0.02	-	-	0.02	0.08	0.02	-	[5,34,38]
40	*trans*-Chrysanthenyl acetate	$C_{12}H_{18}O_2$	1235	1233	42.10 ± 0.01	2.90 ± 0.01	-	-	-	-	-	0.19	[39]
41	*trans*-Verbenyl acetate	$C_{12}H_{18}O_2$	1301	1293	45.33 ± 0.01	3.16 ± 0.01	-	-	-	-	-	0.18	-
42	Bornyl acetate	$C_{12}H_{20}O_2$	1303	1287	45.46 ± 0.02	2.10 ± 0.02	0.01	0.02	-	0.03	0.07	0.02	[5,26]
43	Monoterpenoid—unidentified	-	1328	-	46.59 ± 0.01	2.81 ± 0.01	-	-	-	-	-	0.02	-
44	Monoterpenoid—unidentified	-	1334	-	46.91 ± 0.03	3.47 ± 0.01	-	-	-	-	-	0.08	-
45	Monoterpenoid—unidentified	-	1354	-	47.71 ± 0.01	3.26	-	-	-	-	-	0.03	-
46	Monoterpenoid—unidentified	-	1356	-	47.96 ± 0.01	2.28 ± 0.03	-	-	-	-	0.05	-	-
47	Verbenone [3]	$C_{10}H_{14}O$	1367	-	48.44 ± 0.02	2.80 ± 0.05	0.01	-	-	-	0.05	0.01	[26]
48	α-Cubebene	$C_{15}H_{24}$	1372	1374	48.69 ± 0.02	1.86 ± 0.02	0.02	0.02	-	-	0.04	0.01	[5,26,34]
49	α-Ylangene	$C_{15}H_{24}$	1400	1406	50.01 ± 0.02	1.87 ± 0.02	0.29	0.30	0.06	0.11	0.22	0.15	[5,26,27]
50	α-Copaene	$C_{15}H_{24}$	1404	1416	50.22 ± 0.02	1.88 ± 0.02	0.12	0.07	-	0.04	0.10	0.09	[26,27,34]
51	β-Copaene	$C_{15}H_{24}$	1414	1414	50.66 ± 0.02	1.89 ± 0.02	0.03	0.05	-	-	0.04	0.02	[40]
52	*cis*-α-Bergamotene	$C_{15}H_{24}$	1419	1415	50.87 ± 0.02	1.89 ± 0.01	-	-	-	-	-	0.12	[5,18,26]
53	α-Guaiene	$C_{15}H_{24}$	1430	1438	51.36 ± 0.02	1.93 ± 0.02	0.24	0.20	0.05	0.14	0.31	0.12	[5,18,30]
54	*trans*-α-Bergamotene	$C_{15}H_{24}$	1436	1436	51.61 ± 0.02	1.90	0.03	-	-	-	0.03	0.19	[5,18,26,34]
55	Sesquiterpene—unidentified	-	1441	-	51.82 ± 0.02	1.94	0.05	-	-	-	0.01	-	-
56	α-Himachalene	$C_{15}H_{24}$	1444	1444	51.98 ± 0.03	1.90 ± 0.02	-	0.03	-	-	0.01	0.27	[41]
57	β-Gurjunene	$C_{15}H_{24}$	1450	1450	52.25 ± 0.02	1.91 ± 0.02	0.15	0.43	0.17	0.12	0.20	0.17	[21]
58	*trans*-β-Caryophyllene [2]	$C_{15}H_{24}$	1457	1455	52.56 ± 0.02	1.94 ± 0.02	15.80	16.79	14.82	10.40	12.38	12.79	[20,26,30]
59	*cis*-β-Farnesene	$C_{15}H_{24}$	1463	1457	52.81 ± 0.01	1.92 ± 0.02	0.19	0.20	0.10	0.14	0.09	1.64	[18,26,34]
60	Sesquiterpene—unidentified	-	1467	-	52.97 ± 0.01	1.91 ± 0.01	-	-	0.05	0.02	-	0.05	-
61	Sesquiterpene—unidentified	-	1473	-	53.23 ± 0.03	1.92 ± 0.02	0.12	0.19	0.06	0.04	0.10	0.17	-
62	β-Selinene	$C_{15}H_{24}$	1482	1485	53.65 ± 0.03	1.94 ± 0.02	0.14	0.12	-	0.04	0.08	0.10	[18,20,26,34]
63	Humulene [2]	$C_{15}H_{24}$	1492	1495	54.09 ± 0.02	1.98 ± 0.02	6.38	6.63	3.60	2.61	4.56	5.43	[19,26,30,34]

Table 1. *Cont.*

S. No.	Compound Name [1]	Chemical Formula	RI (Exp) [4]	RI (Lib)	$^{1}t_R$ (min) [5]	$^{2}t_R$ (s) [6]	Pink Kush (%) [5]	Afghani Drifter (%) [5]	Blue Dream (%) [5]	Western Frost (%) [5]	Reeferman's Rockstar (%) [5]	Ultra Sour (%) [5]	Previously Reported in Cannabis
64	Sesquiterpene—unidentified	-	1499	-	54.42 ± 0.01	1.94 ± 0.02	0.08	0.12	-	0.02	0.07	0.05	-
65	Cuparene	$C_{15}H_{22}$	1500	1505	54.45 ± 0.03	2.04 ± 0.01	-	-	-	-	-	0.03	[20,31]
66	Valencene [2]	$C_{15}H_{24}$	1506	1491	54.71 ± 0.02	1.96 ± 0.02	0.83	0.63	0.19	0.27	0.68	0.30	[42]
67	Eremophila-1(10),11-diene	$C_{15}H_{24}$	1511	1503	54.89 ± 0.02	1.95 ± 0.02	0.42	0.28	0.09	-	0.32	0.51	[26]
68	α-Farnesene	$C_{15}H_{24}$	1514	1507	55.03 ± 0.01	1.94 ± 0.02	-	0.38	0.24	0.57	-	-	[18,20,34]
69	Sesquiterpene—unidentified	-	1517	-	55.16 ± 0.04	1.96	0.26	-	-	-	0.16	0.07	-
70	β-Cadinene	$C_{15}H_{24}$	1521	1526	55.32 ± 0.02	1.96 ± 0.02	1.02	1.18	0.38	0.43	0.8	0.55	[18]
71	α-Selinene	$C_{15}H_{24}$	1526	1526	55.55 ± 0.01	1.99 ± 0.02	3.35	3.23	0.82	1.33	3.41	0.78	[18,20,26,34]
72	β-Bisabolene	$C_{15}H_{24}$	1528	1517	55.62 ± 0.02	1.98 ± 0.01	-	-	-	-	-	0.94	[5,26,34]
73	Sesquiterpene—unidentified	-	1534	-	55.85 ± 0.02	1.99 ± 0.02	2.86	2.04	0.57	0.93	2.74	1.03	-
74	γ-Selinene	$C_{15}H_{24}$	1538	1532	56.05 ± 0.02	1.95 ± 0.02	0.64	0.72	0.31	0.26	0.35	0.45	[43]
75	Sesquiterpene—unidentified	-	1546	-	56.37 ± 0.02	1.99 ± 0.01	-	-	-	-	-	0.24	-
76	δ-Cadinene	$C_{15}H_{24}$	1547	1530	56.43 ± 0.04	1.99 ± 0.02	0.32	0.31	-	-	0.25	0.25	[5,26,34]
77	Sesquiterpene—unidentified	-	1545	-	56.35 ± 0.06	1.96 ± 0.01	-	-	-	0.06	-	-	-
78	Sesquiterpene—unidentified	-	1549	-	56.49 ± 0.03	1.99 ± 0.02	-	-	0.09	0.21	-	-	-
79	Sesquiterpenoid—unidentified	-	1550	-	56.54	2.20	0.04	-	-	-	0.04	-	-
80	Sesquiterpene—unidentified	-	1553	-	56.66 ± 0.02	1.96 ± 0.02	1.49	1.72	0.41	0.70	1.36	0.70	-
81	α-Bisabolene [2]	$C_{15}H_{24}$	1559	1545	56.92 ± 0.02	1.95 ± 0.02	4.11	1.45	1.25	1.42	1.98	0.76	[18,26]
82	Sesquiterpene—unidentified	-	1562	-	57.05 ± 0.02	1.97 ± 0.02	2.13	2.06	0.42	1.03	2.86	1.26	-
83	*trans*-Sesquisabinene hydrate	$C_{15}H_{26}O$	1564	1559	57.14 ± 0.02	2.21 ± 0.02	0.11	0.03	-	0.04	0.07	0.06	[38,44]
84	Sesquiterpene—unidentified	-	1566	-	57.21 ± 0.01	1.98 ± 0.02	0.90	0.62	0.16	0.32	0.7	0.32	-
85	Sesquiterpene—unidentified	-	1571	-	57.43 ± 0.02	1.97 ± 0.02	3.43	4.33	1.14	1.72	3.38	2.17	-
86	*trans*-Nerolidol [2,**]	$C_{15}H_{26}O$	1574	1567	57.56 ± 0.02	2.25 ± 0.04	-	0.38	0.10	-	-	0.52	[20,26,27]
87	Sesquiterpene—unidentified **	-	1576	-	57.62 ± 0.01	2.01 ± 0.02	15.48	9.75	2.78	8.46	14.92	8.61	-
88	α-Calacorene	$C_{15}H_{20}$	1578	1562	57.74 ± 0.01	2.17 ± 0.02	0.31	0.06	0.02	0.03	0.17	0.06	[26]
89	Selina-3,7(11)-diene [3]	$C_{15}H_{24}$	1583	-	57.94 ± 0.02	1.99 ± 0.02	15.06	12.96	4.05	12.89	18.50	10.76	[18,26,34,43]
90	Sesquiterpenoid—unidentified	-	1589	-	58.18 ± 0.01	2.23 ± 0.02	0.17	0.23	0.06	0.11	0.26	0.14	-
91	Sesquiterpene—unidentified	-	1592	-	58.30 ± 0.07	1.98 ± 0.02	0.05	0.03	-	-	-	0.02	-
92	Sesquiterpenoid—unidentified	-	1597	-	58.51 ± 0.01	2.75 ± 0.02	0.06	-	-	-	0.29	-	-
93	Sesquiterpene—unidentified	-	1600	-	58.66 ± 0.01	2.01 ± 0.02	0.88	1.23	0.24	0.42	0.54	0.35	-
94	Sesquiterpenoid—unidentified	-	1613	-	59.16 ± 0.02	2.24 ± 0.02	0.11	0.28	0.10	0.11	0.21	0.14	-
95	*trans*-Longipinocarveol **	$C_{15}H_{24}O$	1620	1618	59.46 ± 0.02	2.41 ± 0.03	0.24	0.17	-	-	0.12	0.14	[45]

Table 1. *Cont.*

S. No.	Compound Name [1]	Chemical Formula	RI (Exp) [4]	RI (Lib)	$^{1}t_R$ (min) [5]	$^{2}t_R$ (s) [6]	Pink Kush (%) [5]	Afghani Drifter (%) [5]	Blue Dream (%) [5]	Western Frost (%) [5]	Reeferman's Rockstar (%) [5]	Ultra Sour (%) [5]	Previously Reported in Cannabis
96	Sesquiterpene—unidentified **	-	1621	-	59.49	2.05 ± 0.02	0.02	0.01	-	-	0.24	-	-
97	Caryophyllene oxide [2]	$C_{15}H_{24}O$	1632	1617	59.92 ± 0.03	2.24 ± 0.05	0.73	0.77	2.61	4.04	0.52	0.62	[19,20,26,27]
98	Sesquiterpenoid—unidentified	-	1642	-	60.32 ± 0.04	2.21 ± 0.07	-	-	-	-	0.02	0.10	-
99	Sesquiterpenoid—unidentified	-	1647	-	60.56 ± 0.24	2.34 ± 0.13	-	-	-	-	-	0.23	-
100	Sesquiterpenoid—unidentified	-	1654	-	60.82 ± 0.03	2.23 ± 0.06	0.21	-	-	-	-	0.29	-
101	Sesquiterpenoid—unidentified	-	1657	-	60.96 ± 0.02	2.23 ± 0.04	-	0.21	-	-	0.18	0.38	-
102	Aromadendrene oxide	$C_{15}H_{24}O$	1661	1672	61.07	2.68 ± 0.01	-	-	-	-	-	0.04	-
103	Eudesm-7(11)-en-4-ol	$C_{15}H_{26}O$	1663	1675	61.18 ± 0.02	2.40 ± 0.03	0.58	0.65	0.35	0.28	0.50	0.26	[46]
104	Cedren-13-ol, 8-	$C_{15}H_{24}O$	1666	1688	61.31 ± 0.02	2.44 ± 0.03	0.34	0.04	-	-	-	-	[44]
105	Sesquiterpenoid—unidentified	-	1666	-	61.31 ± 0.01	2.48	-	-	-	-	0.25	0.25	-
106	Sesquiterpenoid—unidentified	-	1669	-	61.42	2.32 ± 0.02	-	-	3.41	5.01	-	4.39	-
107	Sesquiterpenoid—unidentified	-	1669	-	61.44 ± 0.01	2.54	0.21	-	-	-	0.11	-	-
108	Sesquiterpenoid—unidentified	-	1670	-	61.48 ± 0.02	2.45 ± 0.05	0.15	-	-	-	-	-	-
109	Bulnesol **	$C_{15}H_{26}O$	1673	1672	61.61	2.38 ± 0.03	0.05	0.09	0.54	0.72	0.14	0.79	[26,30,47]
110	α-Santalol **	$C_{15}H_{24}O$	1675	1675	61.66 ± 0.01	2.55 ± 0.04	0.05	-	-	-	-	-	[48]
111	Sesquiterpenoid—unidentified **	-	1677	-	61.75 ± 0.01	2.42 ± 0.02	-	-	0.24	0.41	-	0.50	-
112	Sesquiterpenoid—unidentified **	-	1679	-	61.77 ± 0.02	2.65 ± 0.01	-	-	-	-	-	0.02	-
113	Sesquiterpenoid—unidentified	-	1679	-	61.84 ± 0.01	2.41	-	-	-	-	-	0.08	-
114	Sesquiterpenoid—unidentified **	-	1682	-	61.93 ± 0.07	2.64 ± 0.04	0.04	-	-	-	0.05	0.04	-
115	Sesquiterpenoid—unidentified **	-	1682	-	61.96	2.42	-	-	0.05	-	-	0.13	-
116	Sesquiterpenoid—unidentified	-	1686	-	62.11	2.42 ± 0.02	-	-	0.34	0.48	-	0.36	-
117	Sesquiterpenoid—unidentified	-	1689	-	62.23	2.43 ± 0.02	-	-	0.37	0.53	-	0.45	-
118	Sesquiterpenoid—unidentified	-	1692	-	62.31 ± 0.02	2.72 ± 0.05	-	0.11	-	0.13	0.03	0.21	-
119	Sesquiterpenoid—unidentified	-	1693	-	62.39 ± 0.02	2.42 ± 0.04	0.18	0.06	-	-	0.08	-	-
120	Sesquiterpenoid—unidentified	-	1693	-	62.41 ± 0.02	2.58 ± 0.01	-	-	-	-	-	0.10	-
121	Sesquiterpenoid—unidentified	-	1698	-	62.55 ± 0.02	2.65 ± 0.05	0.05	-	-	-	-	-	-
122	Sesquiterpenoid—unidentified	-	1699	-	62.62 ± 0.02	2.45 ± 0.03	0.19	0.08	3.20	3.85	0.20	4.27	-
123	Sesquiterpenoid—unidentified	-	1706	-	62.88 ± 0.02	2.57 ± 0.01	0.10	-	-	-	0.07	-	-
124	α-Bisabolol **	$C_{15}H_{26}O$	1709	1700	63.01 ± 0.01	2.34 ± 0.04	6.95	0.76	2.72	-	4.11	5.88	[19,20,26,30]
125	Sesquiterpenoid—unidentified **	-	1711	-	63.06 ± 0.01	2.74 ± 0.04	0.30	0.11	0.09	-	0.15	0.17	-
126	Sesquiterpenoid—unidentified **	-	1714	-	63.22 ± 0.03	2.36 ± 0.03	-	0.05	-	0.08	0.22	0.07	-
127	Sesquiterpenoid—unidentified **	-	1715	-	63.26 ± 0.03	2.52 ± 0.09	0.14	0.03	-	-	0.08	0.06	-

Table 1. *Cont.*

S. No.	Compound Name [1]	Chemical Formula	RI (Exp) [4]	RI (Lib)	$^{1}t_R$ (min) [5]	$^{2}t_R$ (s) [6]	Pink Kush (%) [5]	Afghani Drifter (%) [5]	Blue Dream (%) [5]	Western Frost (%) [5]	Reeferman's Rockstar (%) [5]	Ultra Sour (%) [5]	Previously Reported in Cannabis
128	Sesquiterpenoid—unidentified	-	1722	-	63.50 ± 0.03	2.61 ± 0.02	-	-	-	-	0.03	-	-
129	Sesquiterpenoid—unidentified	-	1726	-	63.66 ± 0.03	2.33 ± 0.02	-	-	-	-	0.02	-	-
130	Sesquiterpenoid—unidentified	-	1729	-	63.76	2.59 ± 0.02	-	-	-	-	0.02	-	-
131	Sesquiterpenoid—unidentified	-	1731	-	63.85 ± 0.01	2.52 ± 0.02	-	-	0.05	0.15	-	0.17	-
132	Sesquiterpenoid—unidentified	-	1731	-	63.87 ± 0.02	2.38 ± 0.01	0.05	-	-	-	0.03	-	-
133	Sesquiterpenoid—unidentified	-	1736	-	64.02 ± 0.03	2.68	-	-	-	-	-	0.04	-
134	Farnesol	$C_{15}H_{26}O$	1734	1740	63.99	2.49 ± 0.01	0.030	-	-	-	0.06	-	[5,18,26]
135	5β, 7β*H*, 10α-Eudesm-11-en-1α-ol	$C_{15}H_{26}O$	1746	1748	64.43 ± 0.02	2.48 ± 0.03	1.89	0.91	0.30	0.66	1.46	0.70	-
136	Sesquiterpenoid—unidentified	-	1756	-	64.80 ± 0.01	2.55 ± 0.03	0.19	0.09	-	0.14	0.20	0.07	-
137	Sesquiterpenoid—unidentified	-	1768	-	65.27 ± 0.05	2.37 ± 0.02	-	-	-	-	0.04	-	-
138	Sesquiterpenoid—unidentified	-	1799	-	66.48	2.42 ± 0.02	-	-	-	-	0.02	-	-
139	Sesquiterpenoid—unidentified	-	1812	-	66.93 ± 0.01	2.61 ± 0.02	-	-	-	-	0.02	-	-
140	Sesquiterpenoid—unidentified	-	1817	-	67.11 ± 0.01	2.73 ± 0.02	-	-	-	-	0.03	-	-
141	Sesquiterpenoid—unidentified	-	1821	-	67.25 ± 0.01	2.67 ± 0.02	0.05	-	-	-	0.09	-	-
142	Sesquiterpenoid—unidentified	-	1845	-	68.13 ± 0.02	3.01 ± 0.03	0.05	-	-	-	0.06	-	-
143	Sesquiterpenoid—unidentified	-	1859	-	68.63	3.11 ± 0.03	-	-	0.13	0.31	-	-	-
144	Sesquiterpenoid—unidentified	-	1869	-	69.03	3.25 ± 0.05	-	-	-	0.13	-	0.12	-
145	Sesquiterpenoid—unidentified	-	1875	-	69.25	2.91 ± 0.03	-	-	-	-	0.02	-	-
146	Phytol [3]	$C_{20}H_{40}O$	1994	-	77.95 ± 0.02	2.13 ± 0.02	0.19	0.12	-	0.21	0.11	0.13	[5,27,34,38]

[1] The compounds are reported according to their elution order on a non-polar column Rxi-5ms (30 m × 0.25 mm i.d. × 0.50 µm d_f). [2] Compounds with confirmed identity using standard mixture. [3] Compounds with no RI match. [4] Retention indices calculated using a series of C_8–C_{20} n-alkanes on a non-polar column Rxi-5ms (30 m × 0.25 mm i.d. × 0.50 µm d_f). [5] Mean retention time ± standard deviation. Standard deviation <0.01 is not shown in Table 1. [6] Percent relative abundance = (peak area of a compound/total peak area of all the compounds detected in a sample) × 100. * Identity not confirmed due to co-elution of β-myrcene and β-pinene in the standard mixture. ** Compounds with enhanced resolution in the second dimension.

3.2. Identification of Terpenes and Terpenoids in Six Cannabis Strains

A total of 146 terpenes and terpenoids were detected across the six cannabis strains, including monoterpenes, monoterpenoids, sesquiterpenes, sesquiterpenoids, and diterpenoids. The total number of terpenes and terpenoids detected in Pink Kush, Afghani Drifter, Blue Dream, Ultra Sour, Reeferman's Rockstar, and Western Frost were 79, 68, 62, 116, 90, and 67, respectively. The compound names, chemical formulas, retention times, retention indices, and percent relative abundances of the detected compounds in all six cannabis strains are summarized in Table 1.

Among the 146 detected compounds, the identity of 23 compounds was confirmed by comparing their retention times and mass spectra with authentic standards, and 51 compounds were assigned putative names based on the mass spectral match and/or RI match with the NIST 2011 library. Of the 23 confirmed compounds, nine were monoterpenes (α-pinene, camphene, sabinene, α-phellandrene, 3-carene, α-terpinene, limonene, β-ocimene, γ-terpinene), eight were monoterpenoids (eucalyptol, *cis*-sabinene hydrate, linalool, fenchone, fenchol, β-terpineol, (−)-borneol, α-terpineol), three were sesquiterpenes (*trans*-caryophyllene, valencene, humulene), and three were sesquiterpenoids (*trans*-nerolidol, α-bisabolol, caryophyllene oxide). The 51 putatively identified compounds included six monoterpenes, 13 monoterpenoids, 22 sesquiterpenes, nine sesquiterpenoids, and one diterpenoid. The unidentified compounds have been classified as monoterpenes, monoterpenoids, sesquiterpenes, or sesquiterpenoids, as shown in Figure 2, based on the elution regions on the chromatograms and using the Metabolomics Standards Initiative criteria for level 3 of metabolite identification confidence.

Of the 51 putatively identified compounds, four terpenoids were found for the first time in cannabis in this study. These included *trans*-*p*-menth-2-en-1-ol (monoterpenoid), *trans*-verbenyl acetate (monoterpenoid), aromadendrene oxide (sesquiterpenoid), and 5β, 7β*H*, 10α-eudesm-11-en-1α-ol (sesquiterpenoid). However, the stereoisomers of *trans*-*p*-menth-2-en-1-ol and aromadendrene oxide have previously been reported in cannabis and hemp. For example, *cis*-*p*-menth-2-en-1-ol, a stereoisomer of *trans*-*p*-menth-2-en-1-ol, has been reported to be present in the essential oil from dried inflorescences and floral bracts of industrial hemp [44]. The stereoisomers of aromadendrene oxide (allo-aromadendrene, aromadendrene epoxide, and isoaromadendrene epoxide) have been found in the essential oils of various cannabis chemotypes [49] and industrial hemp [44]. All these newly identified compounds have previously been found in the essential oils of flowers and aerial parts of other medicinal plants [50–55]. It should be noted that these compounds are only putative identifications and were not confirmed using standards. All the other identified compounds have previously been reported in cannabis herbs and/or hemp (references given in Table 1).

Even though each study design was different, we were able to detect around four times more terpenes and terpenoids using GC × GC as compared to the reported GC studies [4,18–20,30,35,38,43,44,46,47,49,56–59]. The number of terpenes and terpenoids detected in the reported GC studies ranged from 23 to 109. The use of GC × GC in detecting a higher number of compounds was also demonstrated by Marchini and colleagues. They were able to detect three times more compounds in the extracts of cannabis herb and hashish as compared to the conventional GC-MS [26].

We also detected 32 more compounds in our samples compared to a GC × GC study on cannabis and hashish by Marchini and colleagues [26]. However, the number of volatile compounds in cannabis and cannabis derivatives depends on various factors, such as extraction method, chromatographic method, and type of cannabis species and strain used, as well as the nature of the cannabis flowers used, i.e., fresh or dried. Marchini and colleagues found 16 more monoterpenoids than in our study as they used hashish, which contains a higher number of oxygenated terpenes than cannabis herb [26].

In addition to terpenes and terpenoids, other organic compounds such as halogenated compounds, ketones, hydrocarbons, alcohols, esters, carboxylic acids, sulfoxides, ethers, aldehydes, amines, and fatty acids were also detected. We did not consider these com-

pounds for identification as the focus of our study was to identify the terpenes and terpenoids in our samples. A solvent blank was run after every three replicates of a cannabis strain to confirm that there is no carryover from other strains. Clear blank chromatograms were obtained, which indicated that there was no cross-contamination between the samples of different cannabis strains.

The number of compounds detected in each terpene and terpenoid region varied in all six cannabis strains. In Pink Kush, Afghani Drifter, Blue Dream, Ultra Sour, and Western Frost, the number of sesquiterpenes (33, 32, 25, 37, and 28, respectively) was highest followed by sesquiterpenoids (28, 19, 17, 34, and 18, respectively), monoterpenoids (12, 11, 11, 28, and 11, respectively), and monoterpenes (5, 5, 10, 16, and 9, respectively). In Reeferman's Rockstar, the number of sesquiterpenoids was 36, which was the highest followed by 33 sesquiterpenes, 14 monoterpenoids, and 6 monoterpenes. The number of monoterpenes detected in all six strains was the least compared to other categories. The most likely reason for this is that monoterpenes are the most volatile compounds and are present in lower abundances in dried cannabis samples as compared to fresh samples. Ultra Sour had the most complex terpene and terpenoid profile with the maximum number of compounds present in all the terpene and terpenoid regions as compared to other cannabis strains. Phytol was the only diterpenoid present in each cannabis strain, except Blue Dream, which did not contain any diterpenoid.

The relative abundance (%) of the terpenes and terpenoids detected in all six cannabis strains was calculated and are given in Table 1. We did not have the standards for all the identified compounds to quantify them; therefore, we used their relative abundance (%) in each cannabis strain for these comparisons. The abundances of monoterpenes, monoterpenoids, sesquiterpenes, and sesquiterpenoids varied across the different cannabis strains as depicted in Figure 4. All the cannabis strains, except Blue Dream, contained sesquiterpenes in the highest abundance compared to other chemical classes. The relative abundances of sesquiterpenes in Pink Kush, Afghani Drifter, Ultra Sour, Reeferman's Rockstar, and Western Frost were found to be 77.64%, 68.72%, 51.84%, 72.43%, and 44.96%, respectively. Though the number of sesquiterpenes was highest in Blue Dream, it was dominated by monoterpenes with a relative abundance of 49.72%. The most abundant sesquiterpene in Pink Kush, Afghani Drifter, and Ultra Sour was found to be *trans*-β-caryophyllene with relative abundances of 15.80%, 16.79%, and 12.79%, respectively, while selina-3,7(11)-diene was found to be the most abundant sesquiterpene in Reeferman's Rockstar with the relative abundance of 18.50%. The most abundant monoterpene in Blue Dream was found to be β-myrcene/β-pinene (25.50%). Though Western Frost was dominated by sesquiterpenes, it also contained monoterpene β-myrcene/β-pinene (17.01%) as the most abundant compound. None of these dominant compounds constituted more than one-fourth of the total content of terpenes and terpenoids in any cannabis strain.

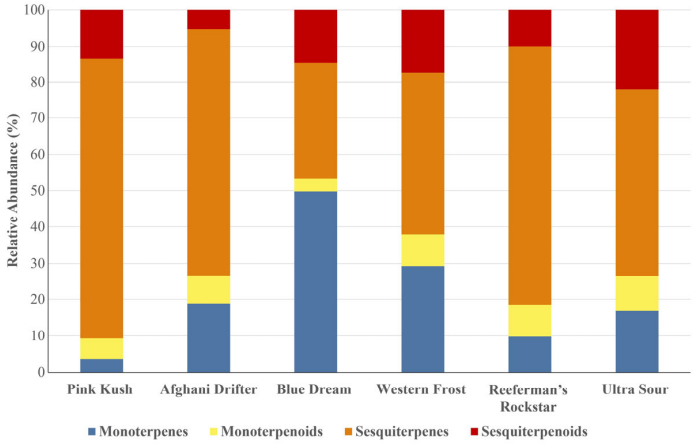

Figure 4. Relative abundance (%) of monoterpenes, monoterpenoids, sesquiterpenes, and sesquiterpenoids regions in six cannabis strains.

Another chemical difference among all six cannabis strains included the relative abundances (%) of α-pinene, β-myrcene/β-pinene, limonene, and selina-3,7(11)-diene. For α-pinene, the highest abundance was recorded in Blue Dream (20.38%) compared to lower values in Western Frost (5.53%), Afghani Drifter (1.14%), Reeferman's Rockstar (0.86%), Ultra Sour (0.64%), and Pink Kush (0.29%). β-Myrcene/β-pinene was most abundant in Blue Dream (25.50%), followed by Western Frost (17.01%), but was significantly lower in other strains ranging from 1.35% to 7.90%. In the case of limonene, the highest abundance was recorded in Afghani Drifter (9.30%) contrary to the lowest abundance in Pink Kush (1.84%). The abundance of selina-3,7(11)-diene ranged from the lowest value of 4.05% in Blue Dream to the highest value of 18.50% in Reeferman's Rockstar.

Furthermore, there were some compounds that were present in only one strain but were absent in other strains. Of the identified compounds, α-thujene (0.27%), α-phellandrene (0.33%), 3-carene (0.36%), α-terpinene (0.45%), *o*-/*p*-cymene (0.18%), *p*-cymenene (0.32%), *trans*-*p*-menth-2-en-1-ol (0.04%), *trans*-carveol (0.57%), *p*-cymen-8-ol (1.00%), *trans*-piperitol (0.10%), *trans*-chrysanthenyl acetate (0.19%), *trans*-verbenyl acetate (0.18%), *cis*-α-bergamotene (0.12%), cuparene (0.03%), β-bisabolene (0.94%), and aromadendrene oxide (0.04%) were detected only in Ultra Sour, whereas ipsdienol (0.05%) was only present in Pink Kush.

Most of the compounds identified in this study have previously been reported to possess therapeutic effects [13,14,16,32,40–42,51–55,59]. For instance, linalool is neuroprotective [60], antiinflammatory [61], and antidepressant [62]; pinene possesses an anxiolytic effect [63], ameliorates memory impairment [64], and acts as an analgesic [65]; bulnesol has anticancer [66] and antianxiety properties [67]; humulene acts as an anti-bacterial and antitumor [68,69] agent and inhibits gastric lesions in gastritis [70]; myrcene possesses sedative, analgesic, and antipsychotic properties [13]; and limonene has antibacterial, anxiolytic, immunostimulant, and antidepressant effects [14]. The presence, amounts, and/or ratios of the terpenes, terpenoids, and/or phytocannabinoids alter the therapeutic effects of cannabis.

4. Conclusions

The present study demonstrated that GC × GC-TOFMS provides enhanced separation and thus identification of compounds that may otherwise have been co-eluted in GC analysis. A total of 146 terpenes and terpenoids were detected across all six cannabis strains. Of these, the identity of 23 compounds was confirmed using a standard mixture and 51 were assigned putative names. GC × GC provided enhanced separation in the second dimension for 16 terpenes and terpenoids among cannabis strains and four terpenes and terpenoids from a standard mixture. We were also able to putatively identify four new terpenoids, which are reported for the first time in cannabis in the present study. The number and relative abundance (%) of compounds in each terpene and terpenoid region varied across all six cannabis strains. This study can be further extended to other cannabis strains to identify more terpenes and terpenoids in cannabis. In addition, the possible therapeutic effects of the compounds identified in this study can be evaluated.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/separations10090500/s1. Table S1: List of terpenes in the standard mixtures; Table S2: List of contaminants removed during data analysis.

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