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Comprehensive chemical and enantiomeric characterization of commercial turpentine oils by GC–MS

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ABSTRACT

Recent reports from families of individuals with Angelman syndrome (AS), a rare neurodevelopmental disorder characterised by severe neurological impairment and epilepsy, have described perceived neurological improvements following the topical application of a commercial turpentine oil (TO). These observations prompted the present analytical study, which aimed to characterise and quantify the terpenoid composition of 2 commercial TOs (Diamond and Creekwood), together with 6 additional commercial TOs, using gas chromatography-mass spectrometry (GC–MS). Analysis of 6 TOs reported to originate from *Pinus pinaster* revealed similar combined percentages of α- and β-pinene (≈75%), except for one oil that exhibited a more complex profile, a lower α-pinene content (≈30%), and the presence of eucalyptol, which is uncommon in TOs from this species. The Creekwood oil contained a higher proportion of α-pinene (≈76%) and a markedly lower β-pinene content (≈3%) than the Diamond oil (≈62% and ≈26%, respectively). Chiral GC–MS analysis further revealed contrasting enantiomeric distributions, with (–)-α-pinene predominating in the Diamond oil and (+)-α-pinene in the Creekwood oil, while (–)-β-pinene predominated in both samples. For quantitative analysis, the analytical method was successfully validated, demonstrating suitable detection (1.5–5.0 mg/L) and quantification (5.0–16.7 mg/L) limits, good linearity (r^2>0.991), and acceptable precision and accuracy (<15%). These findings demonstrate substantial chemical variability among commercial TOs and provide a framework for future studies on the biological effects of pine resin terpenoids in neuronal models relevant to AS.

1. Introduction

Angelman syndrome (AS) is a rare neurodevelopmental disorder caused by the loss of function of the maternally inherited *UBE3A* gene in neurons due to genomic imprinting. The disorder affects approximately 1 in 12,000–20,000 individuals and is characterised by severe intellectual disability, absence of speech, motor impairment, ataxia, and epilepsy. Seizures affect a large proportion of individuals with AS and often remain difficult to control despite available treatments [1,2]. Consequently, the identification of compounds capable of modulating neuronal excitability remains an area of considerable interest.

Natural terpenoid compounds have attracted attention for their diverse biological activities, including anti-inflammatory, antioxidant, and neuroprotective effects [3–7]. Among these, the monoterpenes α-pinene and β-pinene, which are major constituents of pine resins, have been reported to exhibit neuromodulatory properties in experimental systems [8–14]. Various studies have reported that several terpenoids, such as α-pinene, β-pinene, and linalool, display antidepressant and anxiolytic properties. Due to their ability to cross the blood-brain barrier, they help modulate key neurotransmitter systems (such as the GABAergic, dopaminergic, serotonergic, and noradrenergic pathways) and upregulate synaptic plasticity-related protein expression, which contributes to the reduction of depression and anxiety symptoms [15–18]. Historical medical literature from the nineteenth century also

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describes the use of pine resin–derived preparations in the treatment of neurological conditions, including epilepsy, suggesting that resin terpenoids may have biologically relevant effects on neuronal activity [19].

Turpentine oil (TO), sometimes referred to as gum turpentine, is obtained by steam distillation of pine oleoresin. Although not strictly classified as a traditional essential oil, it represents a terpene-rich volatile fraction derived from resins of several species of the genus *Pinus*. These oils consist predominantly of monoterpenes, particularly α-pinene and β-pinene (Fig. 1), together with smaller amounts of other volatile terpenoids. However, the composition of TOs may vary considerably depending on the botanical origin of the resin, environmental conditions, and distillation processes. Such variability may influence not only the relative abundance of the major constituents but also the stereochemical distribution of terpenoids, making detailed chemical characterisation essential before any comparison of their biological properties [8,20,21].

Recently, caregivers of individuals with AS have reported perceived beneficial changes in neurological symptoms following topical application of one commercially available turpentine oil (Diamond G Forest 100% Pure Gum Spirits of Turpentine). When this product became temporarily unavailable, another commercially available TO (Creekwood Naturals 100% Pure Gum Spirits of Turpentine) marketed under a similar description was used, but comparable effects were not reported. Although these observations cannot be interpreted as clinical evidence of efficacy, they raised the possibility that TOs marketed under similar descriptions may differ substantially in their chemical composition.

These observations prompted the hypothesis that commercially available TOs may represent chemically distinct mixtures of terpenoids, potentially differing not only in the relative abundance of their major constituents but also in the enantiomeric distribution of key terpenoids.

The aim of the present study was therefore to perform a comparative chemical characterisation of 8 commercially available TOs using gas chromatography-mass spectrometry (GC–MS). Particular attention was given to the identification and quantification of the major terpenoid constituents as well as to the chiral composition of the principal monoterpenes. This work provides the first comparative chemical characterisation of these products and establishes a chemical foundation for future investigations into the potential neurobiological effects of pine resin terpenoids in AS models.

2. Materials and methods

2.1. TO samples

The TOs used by the AS families, named “Diamond G Forest 100% Pure Gum Spirits of Turpentine” (Diamond, *Pinus elliottii*, USA) and “Creekwood Naturals 100% Pure Gum Spirits of Turpentine” (Creekwood, species unspecified; may come from *Pinus elliottii* or *Pinus palustris*, USA), were obtained online from the Amazon shopping platform. The remaining 6 TOs were acquired from European online retailers following a search on the Google search engine (TOs 1 to 6, *Pinus pinaster*, Europe). All 8 TOs were obtained by steam distillation extraction method. All oils were purchased between October 2024 and May 2025 and stored in sealed amber vials at −20 °C until analysis. Table S1 in the Supporting Information lists, for each of the 8 TO samples, the commercial name, declared botanical source, declared country of origin, lot/batch number, and expiry date.

2.2. Chemical reagents, analytical standards and materials

HPLC-grade *n*-hexane (99%) was obtained from Carlo Erba Reagents (France). The analytical standards for the terpenoids (+)-α-pinene (98%), (−)-α-pinene (98%), (+)-β-pinene (≥98.5%), (−)-β-pinene (99%), myrcene (technical grade), 3-carene (90%), R-(+)-limonene (97%), *p*-cymene (99%), eucalyptol (99%), estragole (98%), α-terpineol (90%), terpinolene (≥95%), β-caryophyllene (≥98%), and (−)-caryophyllene oxide (analytical standard), the internal standard (IS) bromopentafluorobenzene (99%) and the standard mixture of *n*-alkanes C7-C30 (certified reference material, 1 mg/mL of each component in *n*-hexane) were obtained from Sigma-Aldrich (USA). The analytical standards for the terpenoids DL-camphene (98%), β-phellandrene (>95%) and α-humulene were obtained from LGC Labor GmbH (Germany), Toronto Research Chemicals (Canada) and PhytoLab GmbH & Co. KG (Germany), respectively. The IUPAC names and CAS numbers for all reference standards have been added to Table S2 in the Supporting Information.

In addition to standard laboratory equipment, the following equipment was used: an analytical balance (Sartorius M-power model, Germany), a vortex mixer (Velp, Italy), micropipettes of 0.5–10, 2–20, 20–200, and 100–1000 µL (Eppendorf, Germany), and 2 mL amber glass vials (12 × 32 mm) with their respective 1 mm thick polytetrafluoroethylene/silicone septum caps (ALWSCI Technologies, China).

2.3. Experimental procedure

2.3.1. Preparation of standard solutions

Stock solutions of all terpenoids were prepared at a concentration of 10 g/L in *n*-hexane, from which two mixed solutions of these terpenoids were prepared at a concentration of 1 g/L in *n*-hexane (one solution with (+)-α-pinene, (−)-β-pinene, 3-carene, limonene, eucalyptol, α-terpineol, β-caryophyllene, and caryophyllene oxide, and another solution with camphene, β-myrcene, *p*-cymene, β-phellandrene, α-terpinolene, estragole, and α-humulene). For the IS, a stock solution of 10 g/L in *n*-hexane was prepared, from which the working solutions were made. Calibration standards and additional working solutions were obtained by serial dilution of the mixture solutions and the IS stock solution. Throughout the experimental procedure, all stock solutions were stored in amber glass vials at −20 °C and replaced weekly.

2.3.2. Instrumental set-up

The GC–MS analyses were performed using a 6890 Network gas chromatography system coupled to a 5973 Network quadrupole

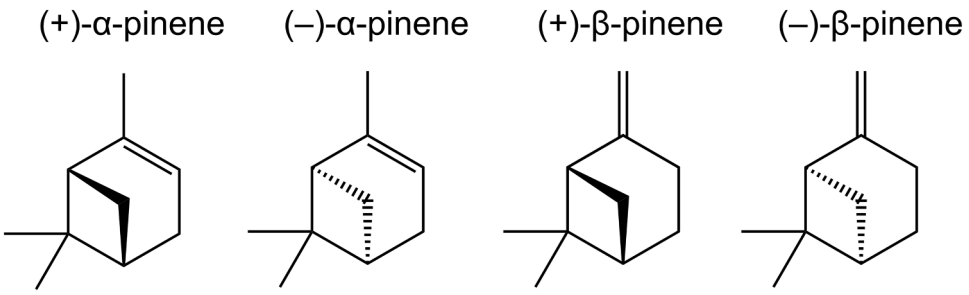

Fig. 1. Chemical structures of α-pinene and β-pinene enantiomers (KingDraw (v3.0), 2022, kingdraw.cn/en/).

selective mass detector, with a 7683 Series automatic injector (Agilent Technologies, USA). Helium (99.9992%; Gasin, Portugal) was used as the carrier gas at a flow rate of 1.5 mL/min, and the capillary column employed was a Mega-5MS (30 m x 0.25 mm x 0.25 μm; 5% phenyl, 95% methylpolysiloxane; Mega, Legnano (MI), Italy). Injection was performed in splitless mode with a volume of 1 μL, and the injector temperature was set at 250 °C.

A 26.5 min chromatographic run was carried out, starting with the oven at 50 °C for 1 min, followed by a temperature ramp of 5 °C/min up to 90 °C, held for 1 min, then increased at 20 °C/min to 200 °C, held for 2 min, and finally raised at 25 °C/min to 300 °C, held for 5 min. The analysis was performed with a solvent delay of 3.5 min. The transfer line, ion source, and quadrupole temperatures were maintained at 300 °C, 250 °C, and 200 °C, respectively. GC–MS analysis was conducted in continuous scan mode from 45 to 550 *m/z* using electron ionization at 70 eV.

For the chiral analysis by GC–MS, a Cyclosil-B capillary column was used (30 m x 0.25 mm x 0.25 μm; 30% heptakis (2,3-di-O-methyl-6-O-t-butyl dimethylsilyl)-β-cyclodextrin in DB-1701; Agilent Technologies, USA). The operating conditions were: carrier gas flow of 1.3 mL/min; injector temperature at 200 °C; total run time of 26 min (starting with the oven at 55 °C for 1 min, followed by a temperature ramp of 5 °C/min up to 180 °C); and transfer line temperature maintained at 200 °C. The remaining conditions were identical to the previous method.

The data obtained were processed using the MSD ChemStation software (G1701EA, version E.02.00.493, Agilent Technologies, USA). For compound identification, the mass spectra obtained were compared with those in the mass spectral libraries Wiley7n.l and NIST2.0, the t_R values were compared with those of analytical standards (only for the compounds whose standards were previously mentioned), and the RIs were calculated for each compound relative to a standard mixture of *n*-alkanes C7–C30, using Eq. (1):

$$RI = 100n + 100\left(\frac{t_x - t_n}{t_{n+1} - t_n}\right) \quad (1)$$

where t_x is the t_R of the target compound (x), t_n and t_{n+1} are the t_R values of the adjacent *n*-alkanes that elute immediately before and after the target compound, respectively, with *n* being the number of carbon atoms of the *n*-alkane that elutes before it. Only identifications with a forward match score greater than 90% were accepted, together with retention index comparison. Peaks were detected and integrated using the standard ChemStation procedures and manually inspected when necessary. Background subtraction was applied only when required to improve spectral quality or peak identification. Peak deconvolution was not performed because the chromatographic separation was considered sufficient for the identified terpenoids.

2.4. Method validation assays

To validate the analytical method, several parameters were evaluated, namely sensitivity (limit of detection (LOD) and limit of quantification (LOQ)), linearity (determination coefficient (r^2), lack-of-fit test (LoF), and goodness-of-fit test (GoF)), precision (relative standard deviation (RSD)) and accuracy (Bias), in accordance with the International Council for Harmonization (ICH) guidelines [22,23].

Sensitivity was evaluated by determining the LOD and the LOQ. The LOD values were determined from the analysis of successive dilutions of the 1 g/L terpenoid mixture solutions until a signal-to-noise ratio of 3:1 was achieved. The LOQ values were established based on a signal-to-noise ratio of 10:1.

Linearity was assessed through the construction of calibration curves. Thus, from the two terpene mixture solutions (1 g/L) and the IS stock solution (10 g/L), calibration standards of 20, 50, 100, 150, 200, 250, 300, 350, and 400 mg/L were prepared, in triplicate, each containing IS at 20 mg/L as final concentration. Using these solutions, calibration curves were then constructed for each terpene, and the linearity of each one was evaluated using the r^2 as well as the LoF and GoF tests, performed according to Eqs. (2) and (3), respectively.

$$F_{LoF} = p\left(\frac{\sum(\bar{y}_i - \hat{y}_i)^2 \Big/ n-2}{\sum(y_i - \bar{y}_i)^2 \Big/ n(p-1)}\right) \quad (2)$$

$$F_{GoF} = \frac{\sum(\bar{y}_i - \hat{y}_i)^2 \Big/ p-1}{\sum(y_i - \hat{y}_i)^2 \Big/ n-p} \quad (3)$$

where y_i represents the value of the chromatographic peak area at concentration (i), $\bar{y}_i$ is the mean area of the replicates at concentration (i), $\hat{y}_i$ is the value of the area at concentration (i) calculated from the calibration curve, *n* indicates the number of calibrators in the calibration curve, and *p* corresponds to the number of replicates for each calibrator. The lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ) were defined as the lowest and highest calibration standard, respectively.

Intra-day and inter-day precision and accuracy were evaluated. For this purpose, 3 concentration levels of the analyte were prepared: the low concentration corresponding to 3 times the LLOQ (60 mg/L with IS at 20 mg/L), the medium concentration corresponding to approximately 30–50% of the working range (140 mg/L with IS at 20 mg/L), and the high concentration corresponding to at least 75% of the ULOQ (320 mg/L with IS at 20 mg/L). Each concentration was prepared in triplicate to assess intra-day precision and accuracy, and over 3 days to assess inter-day precision and accuracy. Precision was calculated using Eq. (4) and accuracy using Eq. (5). For these parameters, acceptance criteria were defined as follows: RSD for precision and bias for accuracy should not exceed 15% at any concentration.

$$RSD(\%) = \left(\frac{\text{standard deviation}}{\text{mean}}\right) \quad (4)$$

$$Bias(\%) = \left(\frac{c_E - c_T}{c_T}\right) \times 100 \quad (5)$$

where C_E is the measured concentration and C_T is the nominal (spiked) concentration.

2.5. Quantification of terpenoids in the TO samples

The concentrations of terpenoids in TO samples were determined using calibration curves. Accordingly, 20 μL of each oil were weighed into vials, followed by the addition of 2 μL of IS (10 g/L), and the volume was adjusted to 1 mL with *n*-hexane. Dilutions of the oils (1:10 and 1:100) were also prepared, as well as solutions 10 times more concentrated (200 μL of oil with IS), in order to ensure that the concentrations of the terpenoids fell within the working range.

Table 1
Total terpenoids identified by GC–MS in the studied TO samples through matching with the mass spectra library and the calculated RI values. Compounds shown in bold were confirmed by analysis of authentic reference standards.

t_R (min)	Compound	RI_{exp}	RI_{lit} [24,25]	Classification
5.835	tricyclene	924	921 - 923	monoterpene
5.903	thujene	926	924 - 928	monoterpene
6.142	**α-pinene**	936	932 - 936	monoterpene
6.535	**camphene**	951	946 - 950	monoterpene
7.124	sabinene	974	969 - 973	monoterpene
7.293	**β-pinene**	981	974 - 978	monoterpene
7.534	**β-myrcene**	990	988 - 989	monoterpene
8.034	α-phellandrene	1009	1002 - 1004	monoterpene
8.078	**δ-3-carene**	1010	1008 - 1011	monoterpene
8.275	1,4-cineol	1017	1012 - 1017	oxygenated monoterpene
8.324	α-terpinene	1019	1014 - 1017	monoterpene
8.565	***p*-cymene**	1027	1020 - 1024	monoterpene
8.672	**limonene**	1031	1024 - 1030	monoterpene
8.735	**β-phellandrene**	1033	1025 - 1030	monoterpene
8.782	**eucalyptol**	1035	1026 - 1032	oxygenated monoterpene
9.134	trans-β-ocimene	1047	1044 - 1048	monoterpene
9.510	γ-terpinene	1060	1054 - 1060	monoterpene
10.352	**α-terpinolene**	1089	1086 - 1087	monoterpene
10.528	*p*-cymenene	1095	1088 - 1089	monoterpene
10.747	linalool	1104	1095 - 1099	oxygenated monoterpene
10.771	α-pinene oxide	1105	1097 - 1099	oxygenated monoterpene
11.222	fenchol	1131	1114 - 1115	oxygenated monoterpene
11.628	trans-pinocarveol	1154	1135 - 1140	oxygenated monoterpene
12.147	borneol	1184	1165 - 1166	oxygenated monoterpene
12.252	4-terpineol	1190	1174 - 1177	oxygenated monoterpene
12.353	*p*-cymen-8-ol	1196	1179 - 1184	oxygenated monoterpene
12.463	**α-terpineol**	1203	1186 - 1188	oxygenated monoterpene
12.497	**estragole**	1206	1195 - 1196	phenylpropanoid
12.650	verbenone	1219	1204 - 1206	oxygenated monoterpene
13.050	linalyl acetate	1255	1254 - 1255	oxygenated monoterpene
13.482	bornyl acetate	1293	1284	oxygenated monoterpene
14.057	α-cubebene	1357	1345 - 1351	sesquiterpene
14.125	α-longipinene	1364	1350 - 1352	sesquiterpene
14.274	ylangene	1382	1370 - 1373	sesquiterpene
14.330	α-copaene	1388	1374 - 1376	sesquiterpene
14.492	sativene	1408	1390	sesquiterpene
14.667	longifolene	1431	1407	sesquiterpene
14.716	**β-caryophyllene**	1438	1417 - 1420	sesquiterpene
14.996	**α-humulene**	1476	1452 - 1453	sesquiterpene
15.106	γ-muurolene	1490	1478 - 1481	sesquiterpene
15.178	germacrene D	1500	1481 - 1484	sesquiterpene
15.265	α-muurolene	1513	1498 - 1500	sesquiterpene
15.399	δ-cadinene	1533	1522 - 1523	sesquiterpene
15.446	calamenene	1540	1523 - 1528	sesquiterpene
15.515	cadina-1,4-diene	1550	1533	sesquiterpene
15.592	α-calacorene	1562	1540 - 1544	sesquiterpene
15.926	**caryophyllene oxide**	1610	1581 - 1582	oxygenated sesquiterpene
16.138	α-humulene epoxide II	1638	1605 - 1608	oxygenated sesquiterpene

3. Results and discussion

3.1. Identification of terpenoids in the TO samples

The identification of terpenoids was carried out through the comparison of the mass spectra obtained for each chromatographic peak with the mass spectra from mass spectral libraries, and by comparing the retention indices (RIs) of the terpenoids obtained (RI_{exp}) with the values reported in the literature (RI_{lit}) [24,25] for the corresponding compounds. Additionally, the identity of 15 compounds was confirmed by comparison of their retention times (t_R) with those of the corresponding analytical standards, as listed in Table 1.

A large proportion of the terpenoids present in TOs exist in the form of constitutional isomers, which are molecules with the same chemical formula but different structures. Due to this similarity, these compounds can produce similar mass spectra, thus leading to false identifications. To overcome this problem, the RIs can be used to help identify the isomers as well as other volatile compounds when the sample is very complex. Therefore, for compounds such as α-pinene, camphene, and α-cubebene, the difference between RI_{exp} and RI_{lit} is <10, indicating a strong likelihood that the mass spectra obtained for these chromatographic peaks correspond to the terpenoids identified by the libraries [26]. For the remaining compounds (such as fenchol, longifolene, and α-humulene epoxide II), the difference between the RI_{exp} and RI_{lit} exceeds 10, which may reduce the reliability of the identification. Such discrepancies may arise from differences in chromatographic conditions (*e.g.*, temperature programme), column ageing, or the assignment of structural isomers that exhibit very similar mass spectra. Consequently, these compounds (*e.g.*, fenchol, longifolene, and α-humulene epoxide II) are reported as tentative identifications despite good spectral library matches. However, despite these differences, the RIs proved useful in the identification of the terpenoids, particularly of those isomers whose mass spectra are identical, as they allow verification of the elution order of these compounds through comparison with the literature. The total number and percentage of terpenoids identified by GC–MS in each TO sample are indicated in Table 2.

The results obtained allowed the identification of 89.3 to 99.1% of the compounds present in the 8 TOs. Of the total identified compounds, monoterpenes dominate the composition of all the analysed TOs (54.2 to 96.9%), with the percentage of sesquiterpenes in the TO 2 sample (31.5%) also standing out compared to the other oils (0.1 to 9.6%). The TO 2 sample is also the one with the lowest percentage of monoterpenes (54.2%).

Table 2
Total terpenoids identified by GC-MS in each TO sample.

	Diamond	Creekwood	TO 1	TO 2	TO 3	TO 4	TO 5	TO 6
% Total Identified	98.9	99.1	95.6	89.3	96.4	94.8	95.3	95.3
monoterpenes	96.9	95.0	82.9	54.2	83.6	81.8	82.5	81.9
oxygenated monoterpenes	0.2	1.3	2.9	2.8	2.9	2.9	2.7	3.0
sesquiterpenes	0.1	2.1	9.3	31.5	9.6	8.6	9.0	8.7
oxygenated sesquiterpenes	<0.1	-	0.6	0.7	0.5	1.5	1.1	1.7
phenylpropanoids	1.7	0.7	-	-	-	-	-	-
% Not Identified	1.1	0.9	4.4	10.7	3.6	5.2	4.7	4.7
Total No of Terpenoids Identified	17	27	28	35	27	29	30	31

3.2. Characterisation by GC–MS of the TO samples of Pinus pinaster

Regarding the terpenoids identified in each of the 8 TOs, these are listed in Table 3 with the respective percentages obtained by GC–MS, along with the percentage limits obtained by GC–MS referenced in the European Pharmacopoeia (Ph. Eur.) for the TO from *Pinus pinaster* [27].

The chromatograms obtained by GC–MS of the 8 TOs can be found in Fig. 2. The chromatograms shown in Figure S1 of the Supporting Information are displayed using a logarithmic intensity scale to facilitate the visualization of lower-intensity peaks.

According to the supplier, TOs 1 to 6 originate from the pine species *Pinus pinaster*. Among these oils, TO samples 1, 3, 4, 5, and 6 have

Table 3
Percentages obtained from relative GC-MS peak areas for the terpenoids identified in each TO sample, and comparison with the data provided by the Ph. Eur. for TO from *Pinus pinaster*.

Compounds	GC–MS %								
	Diamond	Creekwood	TO 1	TO 2	TO 3	TO 4	TO 5	TO 6	Ph. Eur. [19]
tricyclene	0.2	0.7	0.1	-	-	0.1	0.1	0.1	-
thujene	0.2	0.1	-	-	-	-	-	-	-
α-pinene	62.1	76.3	60.6	29.3	61.5	60.8	61.5	60.8	70.0 – 85.0
camphene	1.2	1.8	1.1	0.6	1.2	1.1	1.1	1.1	0.5 – 2.0
sabinene	0.1	-	-	-	-	-	-	-	-
β-pinene	26.3	2.6	14.3	13.4	16.9	16.4	16.1	16.4	5.0 – 20.0
β-myrcene	0.7	0.7	0.6	3.6	0.6	0.5	0.6	0.6	0.4 – 1.5
α-phellandrene	0.2	-	-	-	-	-	-	-	-
δ-3-carene	-	5.6	-	2.1	-	-	-	-	≤1.0
1,4-cineol	-	0.3	0.1	-	-	-	-	-	-
α-terpinene	-	0.2	-	0.1	-	-	-	-	-
p-cymene	0.2	0.4	0.2	0.2	0.1	0.2	0.2	0.2	-
limonene	1.5	3.1	4.8	3.1	2.1	1.9	1.9	1.9	1.0 – 7.0
β-phellandrene	4.2	2.1	0.6	0.7	0.6	0.5	0.6	0.5	-
eucalyptol	-	-	-	1.1	-	-	-	-	-
trans-β-ocimene	-	-	-	0.4	-	-	-	-	-
γ-terpinene	-	0.2	-	0.1	-	-	-	-	-
α-terpinolene	0.1	1.2	0.6	0.6	0.7	0.3	0.5	0.3	-
p-cymenene	-	0.1	-	-	-	-	-	-	-
linalool	-	0.4	-	0.1	-	-	-	-	-
α-pinene oxide	0.1	-	0.1	-	0.1	0.3	0.2	0.3	-
fenchol	-	0.1	0.2	0.1	0.2	0.2	0.1	0.1	-
trans-pinocarveol	-	-	-	0.2	-	-	-	-	-
borneol	-	0.1	0.2	0.1	0.2	0.2	0.2	0.2	-
4-terpineol	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	-
p-cymen-8-ol	-	0.1	0.1	-	0.1	0.2	0.1	0.2	-
α-terpineol	-	0.3	1.9	0.9	1.9	1.8	1.7	1.8	-
estragole	1.7	0.7	-	-	-	-	-	-	-
verbenone	-	-	-	-	-	-	0.1	0.1	-
linalyl acetate	-	-	-	0.1	-	-	-	-	-
bornyl acetate	-	0.1	0.2	0.1	0.3	0.3	0.3	0.3	-
α-cubebene	-	-	0.1	0.6	0.2	0.3	0.3	0.3	-
α-longipinene	-	0.2	0.4	0.2	0.4	0.4	0.3	0.4	-
ylangene	-	-	-	0.3	-	-	-	-	-
α-copaene	-	-	0.4	1.1	0.5	0.5	0.5	0.5	-
sativene	-	0.1	0.1	-	0.1	0.1	0.1	0.1	-
longifolene	-	1.8	3.3	1.9	3.6	3.2	3.1	3.2	0.2 – 4.0
β-caryophyllene	0.1	0.1	3.7	9.6	3.4	2.7	3.3	2.8	0.1 – 3.0
α-humulene	<0.1	-	0.5	1.8	0.5	0.4	0.5	0.4	-
γ-muurolene	-	-	-	2.9	-	0.2	-	0.2	-
germacrene D	-	-	-	6.1	-	-	-	-	-
α-muurolene	-	-	0.1	1.8	0.2	0.2	0.2	0.2	-
δ-cadinene	-	-	0.5	4.6	0.5	0.4	0.5	0.4	-
calamenene	-	-	0.2	0.5	0.2	0.3	0.3	0.3	-
cadina-1,4-diene	-	-	-	-	0.1	0.1	0.1	0.1	-
α-calacorene	-	-	-	0.2	-	-	-	-	-
caryophyllene oxide	<0.1	-	0.6	0.7	0.5	1.5	0.9	1.5	≤1.0
α-humulene epoxide II	-	-	-	-	-	-	0.2	0.2	-

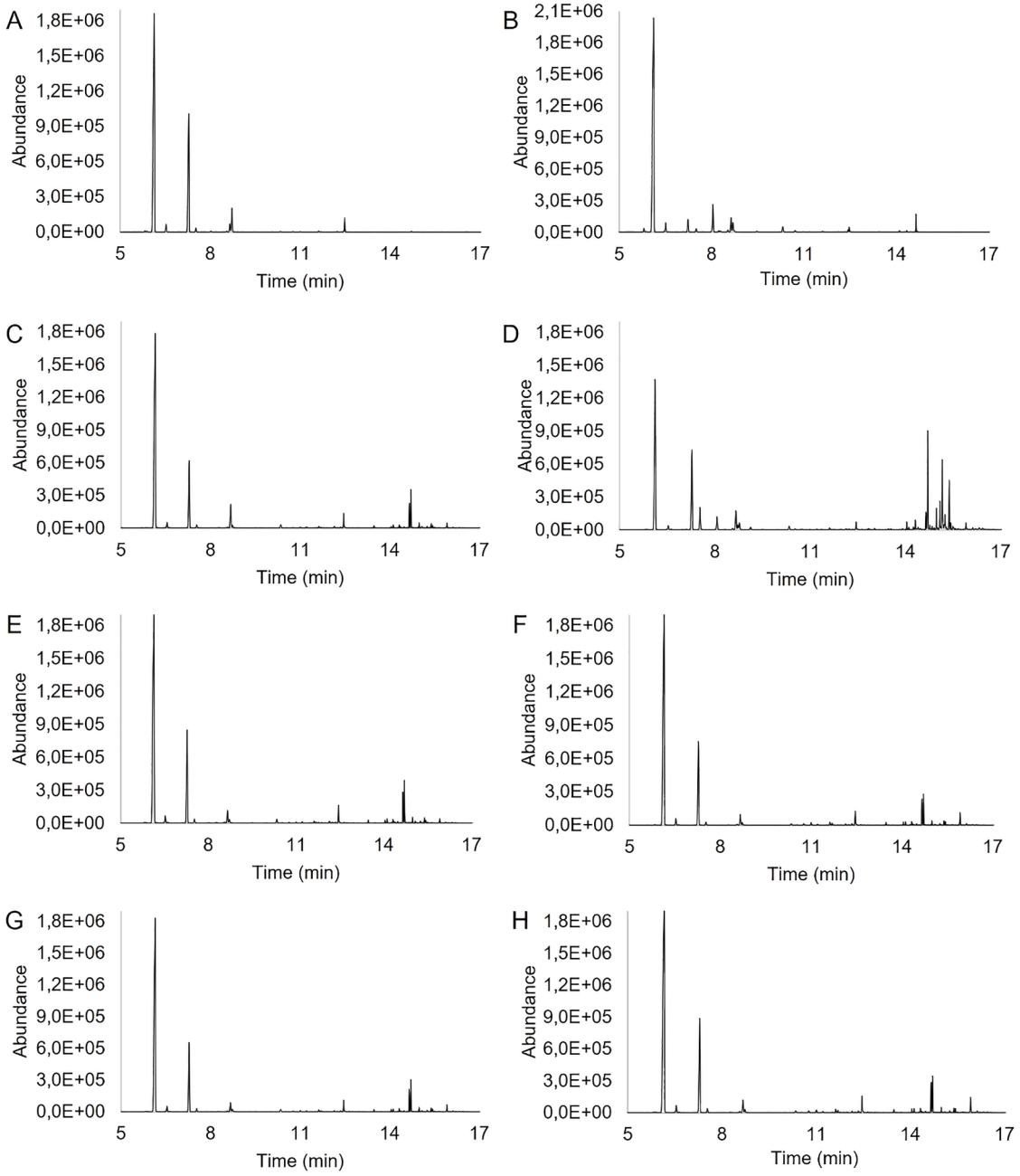

Fig. 2. Chromatograms obtained by GC–MS of the TO samples Diamond (A), Creekwood (B), 1 (C), 2 (D), 3 (E), 4 (F), 5 (G) and 6 (H).

α-pinene (60.6 to 61.5%) and β-pinene (14.3 to 16.9%) as their main compounds, followed by limonene (1.9 to 4.8%), β-caryophyllene (2.7 to 3.7%), longifolene (3.1 to 3.6%), α-terpineol (1.7 to 1.9%), camphene (1.1 to 1.2%), and caryophyllene oxide (0.5 to 1.5%). The TO 2 sample, although originates from the same pine species as the others, has a different composition and is also the one with the highest number of identified terpenoids (35) among the 8 oils analysed. Its main compounds are α-pinene (29.3%) and β-pinene (13.4%), followed by β-caryophyllene (9.6%), germacrene D (6.1%), δ-cadinene (4.6%), β-myrcene (3.6%), limonene (3.1%), γ-muurolene (2.9%), δ-3-carene (2.1%), longifolene (1.9%), α-humulene (1.8%), α-muurolene (1.8%), eucalyptol (1.1%), and α-copaene (1.1%).

As shown in Table 3, with the exception of TO 2, samples TOs 1 and 3-6 exhibited a similar terpenoid composition, despite all 6 oils having been produced in Europe and obtained through steam distillation. Distinctively, the TO 2 sample has a significantly lower percentage of α-pinene (29.3%) compared to the other TOs of this species and compared to the Diamond and Creekwood samples (60.6 to 76.3%). It also contains a considerable percentage of germacrene D (6.1%), δ-3-carene (2.1%), and eucalyptol (1.1%), which are not present in the other 5 oils. The atypical composition of the TO 2 sample may reflect several non-exclusive factors, including natural variability in the botanical source (harvest season, tree age, geographic origin, growing conditions), differences in distillation parameters, storage prior to purchase, or cross-contamination during processing. The present data do not allow these possibilities to be distinguished [28–30].

3.2.1. Authentication of the TOs based on the data provided by the Ph. Eur.

The Pharmacopoeias are books of legal value, composed of monographs that set physical and chemical requirements for medicinal products to ensure their quality and safety. A search was conducted to ascertain whether there was any monograph relating to TO, and two

pharmacopoeias were found, the British and European Pharmacopoeias, whose description and requirements were the same for this oil (Table 3). Thus, the authenticity of the TOs in this study was assessed based on the Ph. Eur. [27].

In this study, the values obtained for these terpenoids in the TOs 1 and 3-6 of *Pinus pinaster* were 60.6 to 61.5% for α-pinene, 1.1 to 1.2% for camphene, 14.3 to 16.9% for β-pinene, 0.5 to 0.6% for β-myrcene, 1.9 to 4.8% for limonene, 3.1 to 3.6% for longifolene, 2.7 to 3.7% for β-caryophyllene, 0.5 to 1.5% for caryophyllene oxide and, in the case of δ-3-carene, it was below the LOD. Comparing these values, it can be observed that, even though some values obtained in this study are slightly above or below the established ranges (which may be the result of factors such as the origin of the sample and climate variation), in general, the results obtained are in agreement with the values of the Ph. Eur.

However, the same is not observed for the TO 2 sample. For this oil, the values obtained for the same terpenoids were 29.3% for α-pinene, 0.6% for camphene, 13.4% for β-pinene, 3.6% for β-myrcene, 2.1% for δ-3-carene, 3.1% for limonene, 1.9% for longifolene, 9.6% for β-caryophyllene, and 0.7% for caryophyllene oxide. Although this oil is also from the *Pinus pinaster* species, it can be seen that it has values that are quite different from those established by the Ph. Eur., namely α-pinene, which is well below the established range (70.0 to 85.0%), and also β-myrcene, δ-3-carene, and β-caryophyllene, which are well above the ranges mentioned (0.4 to 1.5%, ≤1.0%, 0.1 to 3.0%, respectively).

These differences between the chromatographic profile presented by the Ph. Eur. and the profile obtained in this study for the TO 2, suggest that cross-contamination may have occurred. However, the data provided by the Ph. Eur. require updating, since the monograph for the TO obtained from the species *Pinus pinaster* only contains information on the major compounds present in this type of oil, with no data on the minor compounds that are part of the composition of this TO. Therefore, an evaluation based only on the chromatographic profile present in the Ph. Eur. [27] is not sufficient to evaluate the authenticity of the TOs.

3.3. Characterisation by GC–MS of the TO samples

The results of the terpenoids identified in the TOs Diamond and Creekwood used by the AS families are also listed in Table 3. The Diamond sample, from the species *Pinus elliottii* (originating from the USA), stands out for having the lowest number of terpenoids in its composition (17) (Table 2), with the main ones being α-pinene (62.1%) and β-pinene (26.3%), followed by β-phellandrene (4.2%), estragole (1.7%), limonene (1.5%), and camphene (1.2%). The Creekwood sample, although its pine species of origin is unspecified, is known to come from the USA, and its main compounds are α-pinene (76.3%), δ-3-carene (5.6%), limonene (3.1%), β-pinene (2.6%), β-phellandrene (2.1%), camphene (1.8%), longifolene (1.8%), and α-terpinolene (1.2%).

When comparing the results obtained for Diamond and Creekwood, it can be noted that they have different terpenoid compositions, with the Creekwood sample having a higher percentage of α-pinene (76.3%) but a lower percentage of β-pinene (2.6%) than the Diamond sample, and also than the remaining 6 *Pinus pinaster* oils (29.3 to 62.1% for α-pinene; 13.4 to 26.3% for β-pinene). Additionally, compounds were identified that are only present in either the Diamond sample or the Creekwood sample, namely δ-3-carene in Creekwood at a significant percentage (5.6%), and sabinene, α-phellandrene, α-pinene oxide, α-humulene, and caryophyllene oxide in Diamond, although they are found in trace amounts (<0.1 to 0.2%). Knowing that the Diamond oil comes from the *Pinus elliottii* species and that the origin species of the Creekwood oil is not specified (may come from *Pinus elliottii* or *Pinus palustris*), the results obtained indicate the possibility that these TOs are from different species, considering that both come from the USA and were obtained using the same extraction method (steam distillation).

These preliminary results may contribute to a possible explanation for the differing effects described in patients with AS. Beyond the fact that the Diamond sample had a lower number of terpenoids in its composition (17) compared to Creekwood (27), this remarkable therapeutic distinction may be particularly related to the differences observed between the percentages of α-pinene (62.1% in Diamond and 76.3% in Creekwood) and β-pinene (26.3% in Diamond and 2.6% in Creekwood), since these two terpenoids have shown neuroprotective potential in neurodevelopmental disorder [8–10].

3.3.1. Chiral analysis of the TO samples

Knowing that both α-pinene and β-pinene have 2 enantiomers, and that different enantiomers may exhibit different biological properties, potentially affecting patients with AS differently, a chiral analysis of the TOs was performed by GC–MS using a Cyclosil-B column (chromatograms are shown in Figure S2). Table 4 shows the enantiomers that could be separated in all the oils, with the respective percentages obtained by GC–MS. The results demonstrate that, for all TO samples, it was possible to separate the enantiomers of α-pinene, β-pinene and camphene. The identification of the enantiomers of both pinenes was carried out by comparison with the analytical standards of (–)-α-pinene, (+)-α-pinene, (–)-β-pinene, and (+)-β-pinene.

Regarding these compounds, it is observed that, for the Diamond and the 1 to 6 TO samples, the (–) enantiomer predominates for both α-pinene (73.3 to 91.4%) and β-pinene (73.8 to 96.9%). For the Creekwood sample, (–)-β-pinene is also the predominant enantiomer (73.8%), but the opposite occurs for α-pinene, since the predominant form is (+)-α-pinene (92.3%). For camphene, it was not possible to identify which chromatographic peak corresponds to the (–) and (+) enantiomers of this terpenoid due to the absence of standards for comparison. However, it can be observed that, similarly to what happens with α-pinene, the Diamond and the 1 to 6 TO samples have the same chiral profile for camphene, that is, they contain a higher amount of the enantiomer that elutes first (74.4 to 86.5%), whereas Creekwood is the only one with a different profile, having a higher amount of the camphene enantiomer that elutes second (73.5%).

Additionally, the results of the chiral analysis of the oils highlight the hypothesis that the difference between the effects of Diamond and Creekwood oils in patients with AS may also be related to the enantiomeric ratio of α-pinene and camphene, as these oils have opposite chiral

Table 4
Percentages obtained through chiral analysis from relative GC–MS peak areas for the enantiomers identified in TO samples.

t_R (min)	Compound	GC-MS %							
		Diamond	Creekwood	TO 1	TO 2	TO 3	TO 4	TO 5	TO 6
8.781	(–)-α-pinene	77.5	7.7	91.1	73.3	91.4	91.4	91.4	91.3
8.994	(+)-α-pinene	22.5	92.3	8.9	26.7	8.6	8.6	8.6	8.7
9.706	camphene[a]	79.3	26.5	86.2	74.4	85.5	85.7	86.1	86.5
9.858	camphene[b]	20.7	73.5	13.8	25.6	14.5	14.3	13.9	13.5
10.189	(+)-β-pinene	3.9	26.2	5.3	3.1	5.0	5.0	5.1	5.0
10.337	(–)-β-pinene	96.1	73.8	94.7	96.9	95.0	95.0	94.9	95.0

[a] 1st eluting enantiomeric peak
[b] 2nd eluting enantiomeric peak

profiles for these two terpenoids: (–)-α-pinene and the camphene enantiomer that elutes first are predominant in the Diamond oil, while (+)-α-pinene and the camphene enantiomer that elutes second are predominant in the Creekwood oil. It is noteworthy that the Diamond oil also has a higher proportion of (–)-β-pinene (96.1%) compared to the Creekwood oil (73.8%), which may also be an important factor to consider.

These results reinforce the need to systematically investigate the impact of the main terpenoid compounds on neuronal cellular models, namely α-pinene and β-pinene, as well as other detected terpenoids, such as δ-3-carene, sabinene, α-phellandrene, α-pinene oxide, α-humulene, and caryophyllene oxide, which, not being common to both oils, may contribute to distinct cellular responses. It is also important to study the enantiomers of both pinenes, whose uneven distribution between the oils could translate into different biological properties. Together, these findings provide a solid foundation for future studies aimed at clarifying the underlying molecular mechanisms and identifying terpenoid combinations with therapeutic potential in neurogenetic diseases, and particularly in AS. For clarity, a summary comparing the Diamond and Creekwood turpentine oil products, including their declared botanical sources, compositional differences, and claimed therapeutic benefits, is provided in Table S3 of the Supporting Information.

3.4. Method validation

The validation of the analytical method is extremely important to determine whether it is suitable for quantifying the compounds under analysis. For this, a set of parameters is studied, which must comply with certain standards for a method to be considered validated. In this case, the parameters evaluated were sensitivity, linearity, precision and accuracy, which were verified according to the recommendations given by the ICH guidelines [22,23].

3.4.1. Sensitivity

To assess the sensitivity of the methodology used in the analysis of TOs in study, the analytical thresholds, the LOD and the LOQ, were determined using a signal-to-noise ratio of 3:1 and 10:1, respectively. LOD values ranged from 1.5 to 5.0 mg/L, while LOQ values varied from 5.0 to 16.7 mg/L. The lowest limits (LOD of 1.5 mg/L and LOQ of 5.0 mg/L) were found for (–)-β-pinene, α-terpineol, β-caryophyllene, and α-humulene. A large group of five compounds, including (+)-α-pinene, δ-3-carene, eucalyptol, caryophyllene oxide, and *p*-cymene, exhibited LODs of 2.0 mg/L and LOQs of 6.7 mg/L. The compounds β-myrcene, α-terpinolene, and estragole showed LODs of 2.5 mg/L and LOQs of 8.3 mg/L. Lastly, the highest limits (LOD of 5.0 mg/L and LOQ of 16.7 mg/L) were obtained for limonene, camphene, and β-phellandrene. The results obtained are presented in Table S4 of the supplementary materials.

The values obtained for both analytical thresholds in this work are above the values reported in other studies (LODs of 0.03 to 0.39 mg/L and LOQs of 0.24 to 2.50 mg/L) which also validated the methodology for the quantification of some terpenoids, also studied in this work, in citrus oils [31] and in a hydrodistilled TO of *Cannabis sativa* [32]. However, it should be noted that both studies calculated the analytical limits using the standard deviation of replicates, which may overestimate the LODs compared to those estimated by the signal-to-noise ratio.

Comparing the literature studies with this work, some similarities and differences can be observed between the methodologies, such as the type of chromatograph employed (6890 Network Agilent), the same mass detector (quadrupole 5973 Network Agilent) in the study where *Cannabis sativa* TO was analysed [32], the brand and size of the column (Mega-5MS of 30 m in this work, BD-5MS [31] and HP-5MS [32] of 60 m), and different analysis modes (continuous scan mode and selected ion monitoring). The mass detector used by Güzel et al. [31] (inert triple-axis 5975C Agilent) shows higher sensitivity due to its ability to increase the signal and reduce noise, allowing for lower LODs and LOQs. Furthermore, the analyses carried out by Güzel et al. [31] and Joy et al. [32] were performed with different temperature ramps. The *Cannabis sativa* oil analysed by Joy et al. [32] was also extracted by hydrodistillation prior to chromatographic analysis, which was conducted using selected ion monitoring. Thus, based on the results obtained in this work regarding LODs and LOQs, and taking into account the differences between the method developed and those reported in the literature, it is observed that the method under study has acceptable sensitivity for the analysis of these terpenoids in TOs by GC–MS.

3.4.2. Linearity

After the analytical thresholds were established, calibration curves were constructed for the terpenoids, with their parameters shown in Table S5. Two working ranges were established for two groups of terpenoids (corresponding to the two mixture solutions), from 20 to 350 mg/L and from 20 to 400 mg/L, each with 6 concentration levels. For the linearity study, the following parameters were evaluated: the coefficient of determination (r^2) and two statistical tests (the goodness-of-fit test (GoF) and the lack-of-fit test (LoF)) calculated using Excel.

Table S5 shows that the values of r^2 obtained were greater than 0.991 for all terpenoids. However, although an r^2 value close to 1 may indicate a strong linear correlation, this is not sufficient to guarantee that linearity exists. Therefore, other statistical tests were also performed, namely the LoF and GoF tests, which aim to determine whether the model is fitted to the data, thus helping to ascertain whether the model is linear. This is carried out by comparing the calculated experimental F value (F_{exp}) with the tabulated F value (F_{tab}), the latter being consulted from an F distribution table for a 95% confidence level. From the results obtained, it is noted that, for both the LoF and GoF tests, F_{tab} is greater than F_{exp} for the linear regressions of all terpenoids, which means that the models used (linear regressions) are shown to be fitted to the data, thus indicating that they are linear.

3.4.3. Precision and accuracy

To assess intra-day (n = 3) and inter-day (n = 9, 3 replicates over 3 days) precision and accuracy, 3 concentration levels of the analytes were prepared: 60 mg/L (low), 140 mg/L (medium), and 320 mg/L (high).

For intra-day precision, the relative standard deviation (RSD) values ranged from 0.63 to 7.00% at 60 mg/L, from 1.77 to 10.82% at 140 mg/L and from 1.37 to 6.52% at 320 mg/L. For inter-day precision, the RSD values varied from 4.48 to 8.41% at 60 mg/L, from 5.27 to 12.26% at 140 mg/L and from 1.49 to 10.13% at 320 mg/L. For intra-day accuracy, the bias ranged from −12.44 to 2.21%, from −8.53 to 2.85% and from −13.93% to 5.74%, at 60, 140 and 320 mg/L, respectively. For inter-day accuracy, the bias varied from −13.19 to 1.44%, from −11.50 to 4.64% and from −14.46 to 9.51% at 60, 140 and 320 mg/L. All the results obtained can be found in Table S6.

Thus, it is verified that, for all concentration levels of the various terpenoids, both the RSD values and the bias values obtained are below 15% [23], which means that the analytical method under study is suitable for the quantification of these terpenoids.

3.4.4. Quantification of terpenoids in the TO samples

With the method properly validated, it was then possible to quantify the 15 terpenoids in the TOs, namely α-pinene, camphene, β-pinene, β-myrcene, δ-3-carene, *p*-cymene, limonene, β-phellandrene, eucalyptol, α-terpinolene, α-terpineol, estragole, β-caryophyllene, α-humulene, and caryophyllene oxide. The results are shown in Table 5, where the concentrations are presented in mg/g, using the weight of the measured TO volume for each solution.

Based on the results obtained, it is possible to verify that α-pinene is the compound found in the highest amount in all 8 samples (186.0 to 523.9 mg/g), with the TO 2 and Creekwood samples showing the lowest and highest concentrations, respectively. Next, β-pinene is the second most abundant terpenoid in all TOs (99.3 to 173.2 mg/g), except in Creekwood, where β-pinene is the fourth most concentrated compound

Table 5
Concentrations (mg/g) of terpenoids in TO samples calculated using calibration curves and GC–MS analysis.

Compound	Concentration (mg/g)							
	Diamond	Creekwood	TO 1	TO 2	TO 3	TO 4	TO 5	TO 6
α-pinene	357.6	523.9	416.4	186.0	394.9	361.9	400.3	353.8
camphene	8.1	11.4	7.3	4.2	7.1	7.2	6.6	6.6
β-pinene	173.2	16.3	100.7	107.2	106.0	99.3	122.3	104.1
β-myrcene	4.2	5.2	4.2	26.6	3.9	3.9	3.9	3.4
δ-3-carene	-	27.8	-	13.7	-	-	-	-
p-cymene	0.2	1.6	0.1	0.7	0.1	0.1	0.1	0.1
limonene	7.8	18.2	26.1	16.9	12.2	12.3	11.6	10.9
β-phellandrene	15.3	10.3	2.0	4.9	2.6	2.2	2.2	2.0
eucalyptol	-	-	-	6.7	-	-	-	-
α-terpinolene	0.7	6.8	3.8	3.6	3.5	1.5	2.5	1.5
α-terpineol	-	0.1	7.7	3.6	6.9	7.5	6.5	6.5
estragole	8.5	2.8	-	-	-	-	-	-
β-caryophyllene	<0.1	0.1	23.1	78.0	20.3	17.7	19.6	15.7
α-humulene	<0.1	-	2.8	9.9	2.4	2.3	2.4	2.1
caryophyllene oxide	<0.1	-	5.0	5.5	3.5	12.3	6.8	11.0

(16.3 mg/g), with δ-3-carene (27.8 mg/g) and limonene (18.2 mg/g) being the second and third, respectively. The concentrations of this last terpenoid in the remaining oils are also significant (7.8 to 26.1 mg/g), with Diamond and TO 1 having the lowest and highest amounts of this compound, respectively. In the TOs 1 and 3-6 from the *Pinus pinaster* species, the β-caryophyllene concentrations obtained are quite notable (15.7 to 78.0 mg/g), with the highest concentration belonging to the TO 2 sample. This sample is also distinguished from the others by the amounts of β-myrcene (26.6 mg/g), δ-3-carene (13.7 mg/g), eucalyptol (6.7 mg/g), and α-humulene (9.9 mg/g), with δ-3-carene and eucalyptol not being present in any of the other TOs from the same species. Additionally, the Diamond and Creekwood oils stand out for their higher concentration of β-phellandrene (15.3 and 10.3 mg/g, respectively) compared to the oils from *Pinus pinaster* (2.0 to 4.9 mg/g), and also for the amount of estragole (8.5 and 2.8 mg/g, respectively), which is absent in the remaining TOs.

4. Conclusion

A GC–MS method was successfully validated for the qualitative and quantitative characterisation of the major terpenoid constituents of commercial turpentine oils. The method demonstrated acceptable sensitivity, linearity, precision, and accuracy in accordance with ICH guidelines, confirming its suitability for the analysis of these complex terpene mixtures.

The TOs 1 and 3-6 from the *Pinus pinaster* species showed a similar terpenoid composition and, in general, the percentages obtained by GC-MS were in accordance with the reference percentage limits provided by the Ph. Eur. TO 2, despite originating from the same pine species as the previous ones, exhibited a more distinctive composition, a much lower percentage of α-pinene and the presence of eucalyptol.

A comparison of the two oils reported by families of individuals with AS revealed substantial differences in both terpenoid composition and enantiomeric distribution. The Creekwood oil contained a higher number of identified terpenoids (27) and a higher proportion of α-pinene (76.3%), but a markedly lower proportion of β-pinene (2.6%) than the Diamond oil (17, 62.1%, and 26.3%, respectively). Chiral GC–MS analysis further showed contrasting enantiomeric profiles, particularly for α-pinene, with (–)-α-pinene predominating in most oils, whereas (+)-α-pinene was dominant in the Creekwood sample.

The present study was designed as an analytical characterisation of these products and does not evaluate their biological activity, efficacy, or safety. Consequently, the observations reported by families should not be interpreted as evidence of therapeutic benefit, but rather as the rationale for investigating whether the products involved differed chemically. By establishing a detailed chemical and stereochemical framework for these commercially available turpentine oils, this work provides a robust analytical foundation for future mechanistic studies investigating the effects of defined pine resin terpenoids and their stereoisomers in neuronal models of seizure susceptibility, including models relevant to AS. The results must not be interpreted as supporting or recommending the clinical, therapeutic, or self-administered use of turpentine oils in AS or in any other condition. Turpentine oil is a known irritant, and its unsupervised human use may pose health risks.

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Débora S. D. Pombo: Writing – original draft, Methodology, Formal analysis, Investigation, Data curation, Visualization. **Bárbara F. C. Barra:** Methodology, Formal analysis, Visualization. **Madalena Bettencourt:** Writing – review & editing, Validation. **Alexandre Quintas:** Writing – review & editing, Conceptualization, Validation, Resources, Supervision. **Evguenia P. Bekman:** Writing – review & editing, Resources, Supervision, Project administration. **Nuno R. Neng:** Writing – review & editing, Conceptualization, Methodology, Validation, Resources, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2026.467289.

Data Availability

Data will be made available on request.

References

[1] [1] C. Maranga, T.G. Fernandes, E. Bekman, S.T. da Rocha, Angelman syndrome: a journey through the brain, FEBS J. 287 (2020) 2154–2175, https://doi.org/10.1111/febs.15258.

[2] J. Camões dos Santos, C. Appleton, F. Cazaux Mateus, R. Covas, E.P. Bekman, S. T. da Rocha, Stem cell models of Angelman syndrome, Front. Cell Dev. Biol. 11 (2023), https://doi.org/10.3389/fcell.2023.1274040.

[3] W. Hassan, S. Rehman, H. Noreen, S. Gul, N. Neelofar, N. Ali, Gas chromatography mass spectrometric (GCMS) analysis of essential oils of medicinal plants, Adv. Anim. Vet. Sci. 4 (2016) 420–437, https://doi.org/10.14737/journal.aavs/2016/4.8.420.437.

[4] M.R. Loizzo, F. Menichini, R. Tundis, M. Bonesi, F. Conforti, F. Nadjafi, G.A. Statti, N.G. Frega, F. Menichini, *In vitro* biological activity of *salvia leriifolia* benth essential oil relevant to the treatment of Alzheimer's disease, J. Oleo Sci. 58 (2009) 443–446, https://doi.org/10.5650/jos.58.443.

[5] A.W. Septama, I. Jantan, P. Panichayupakaranant, Flavonoids of *Artocarpus heterophyllus* Lam. Heartwood inhibit the innate immune responses of human phagocytes, J. Pharm. Pharmacol. 70 (2018) 1242–1252, https://doi.org/10.1111/jphp.12952.

[6] M.D. Allenspach, C. Valder, C. Steuer, Absolute quantification of terpenes in conifer-derived essential oils and their antibacterial activity, J. Anal. Sci. Technol. 11 (2020) 12, https://doi.org/10.1186/s40543-020-00212-y.

[7] E.P. Romanenko, D.V Domrachev, A.V Tkachev, Variations in essential oils from South Siberian conifers of the Pinaceae Family: new data towards identification and quality control, Chem. Biodivers. 19 (2022), https://doi.org/10.1002/cbdv.202100755.

[8] B. Mercier, J. Prost, M. Prost, The essential oil of turpentine and its major volatile fraction (α- and β-pinenes): a review, Int. J. Occup. Med. Environ. Health 22 (2009) 331–342, https://doi.org/10.2478/v10001-009-0032-5.

[9] K. Weston-Green, H. Clunas, C.J. Naranjo, A review of the potential use of Pinene and Linalool as terpene-based medicines for brain health: discovering novel therapeutics in the flavours and fragrances of cannabis, Front. Psychiatry 12 (2021), https://doi.org/10.3389/fpsyt.2021.583211.

[10] B. Salehi, S. Upadhyay, I. Erdogan Orhan, A. Kumar Jugran, S.L.D. Jayaweera, D. A. Dias, F. Sharopov, Y. Taheri, N. Martins, N. Baghalpour, W.C. Cho, J. Sharifi-Rad, Therapeutic potential of α- and β-pinene: a miracle gift of nature, Biomolecules 9 (2019) 738, https://doi.org/10.3390/biom9110738.

[11] J.S. Laws, S.D. Smid, Characterizing cannabis-prevalent terpenes for neuroprotection reveal a role for α and β-pinenes in mitigating amyloid β-evoked neurotoxicity and aggregation *in vitro*, Neurotoxicology 100 (2024) 16–24, https://doi.org/10.1016/j.neuro.2023.12.004.

[12] B. Tezcan Yavuz, E. Kabartan Cokeli, G. Hacioglu, S. Cirrik, C. Sirin Tomruk, C. Tomruk, Endoplasmic reticulum stress modulation by alpha-Pinene in a rotenone-induced rat model of Parkinson's disease, Eur. J. Pharmacol. 1005 (2025) 178125, https://doi.org/10.1016/j.ejphar.2025.178125.

[13] P. Hashemi, M.R. Moloudi, H. Rahmani, K. Hassanzadeh, Z. Vahabzadeh, E. Izadpanah, Alpha-pinene ameliorates memory deficits in 3-nitropropionic acid-induced rat model of Huntington's Disease, Neurochem. Res. 50 (2025) 144, https://doi.org/10.1007/s11064-025-04393-z.

[14] P. Hashemi, S. Ahmadi, Alpha-pinene moderates memory impairment induced by kainic acid via improving the BDNF/TrkB/CREB signaling pathway in rat hippocampus, Front. Mol. Neurosci. 16 (2023), https://doi.org/10.3389/fnmol.2023.1202232.

[15] N. Zhang, L. Yao, Anxiolytic effect of essential oils and their constituents: a review, J. Agric. Food Chem. 67 (2019) 13790–13808, https://doi.org/10.1021/acs.jafc.9b00433.

[16] D. Donelli, F. Meneguzzo, M. Antonelli, D. Ardissino, G. Niccoli, G. Gronchi, R. Baraldi, L. Neri, F. Zabini, Effects of plant-emitted monoterpenes on anxiety symptoms: a propensity-matched observational cohort study, Int. J. Environ. Res. Public Heal. 20 (2023) 2773, https://doi.org/10.3390/ijerph20042773.

[17] T.J. Mony, F. Elahi, J.W. Choi, S.J. Park, Neuropharmacological effects of terpenoids on preclinical animal models of psychiatric disorders: a review, Antioxidants 11 (2022) 1834, https://doi.org/10.3390/antiox11091834.

[18] A.A.M. Noor, Exploring the therapeutic potential of terpenoids for depression and anxiety, Chem. Biodivers. 21 (2024), https://doi.org/10.1002/cbdv.202400788.

[19] J.W. Begbie, The therapeutic actions and uses of turpentine, Edinb. Med. J. 17 (1871) 39–56.

[20] L.M.L. Nollet, in: H.S. Rathore (Ed.), Green Pesticides Handbook, 1st ed, CRC Press, 2017, https://doi.org/10.1201/9781315153131.

[21] W.C. Evans, D. Evans, Volatile oils and resins. Trease and Evans' Pharmacognosy, Elsevier, 2009, pp. 263–303, https://doi.org/10.1016/B978-0-7020-2933-2.00022-8.

[22] International council for harmonisation of technical requirements for pharmaceuticals for human use, Validation of Analytical Procedures Q2(R2), ICH Harmonised Guideline, 2023.

[23] International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, Bioanalytical method validation and study sample analysis M10, ICH Harmonised Guideline (2022).

[24] V.I. Babushok, P.J. Linstrom, I.G. Zenkevich, Retention indices for frequently reported compounds of plant essential oils, J. Phys. Chem. Ref. Data 40 (2011), https://doi.org/10.1063/1.3653552.

[25] R.P. Adams, Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry, 4th ed., Allured Publishing Corporation, Carol Stream, Illinois, 2007.

[26] P. Christiaens, D. Jenke, J. Baeten, P. Verlinde, J.-M. Beusen, Use of the retention index to secure correct identities in GC/MS, PDA J. Pharm. Sci. Technol. 79 (2025), https://doi.org/10.5731/pdajpst.2025-000008.1.

[27] European Directorate for the Quality of Medicines & HealthCare (EDQM), Turpentine oil (Terebinthinae aetheroleum), in: European Pharmacopoeia. Monograph 07/2014:1627. Corrected 9.4, 11th ed., 2022: pp. 1762–1763.

[28] V. Bampidis, G. Azimonti, M. de L Bastos, H. Christensen, M. Durjava, M. Kouba, M. López-Alonso, S. López Puente, F. Marcon, B. Mayo, A. Pechová, M. Petkova, F. Ramos, Y. Sanz, R.E. Villa, R. Woutersen, P. Brantom, A. Chesson, J. Westendorf, P. Manini, F. Pizzo, B. Dusemund, Safety and efficacy of a feed additive consisting of an essential oil obtained from the oleoresin of *Pinus pinaster* Aiton (pine white oil) for use in all animal species (FEFANA asbl), EFSA J. 21 (2023), https://doi.org/10.2903/j.efsa.2023.7952.

[29] B. Leseche, J. Levisalles, H. Rudler, Constituents of French Turpentine of oil from *Pinus pinaster* (Bordeaux), Planta Med. 46 (1982) 252–253, https://doi.org/10.1055/s-2007-971228.

[30] J.I. Alonso-Esteban, M. Carocho, D. Barros, M.V. Velho, S. Heleno, L. Barros, Chemical composition and industrial applications of Maritime pine (*Pinus pinaster* Ait.) bark and other non-wood parts, Rev. Environ. Sci. Biotechnol. 21 (2022) 583–633, https://doi.org/10.1007/s11157-022-09624-1.

[31] B. Güzel, O. Canlı, S.M. Hocaoğlu, Method development and validation for accurate and sensitive determination of terpenes in bio-based (citrus) oils by single quadrupole gas chromatography-mass spectrometry (GC/MS), Microchem. J. 191 (2023) 108903, https://doi.org/10.1016/j.microc.2023.108903.

[32] N. Joy, D. Jackson, T. Coolong, A validated GC-MS method for major terpenes quantification in hydrodistilled *cannabis sativa* essential oil, Phytochem. Anal. 36 (2025) 2362–2373, https://doi.org/10.1002/pca.3526.