Industrial Crops & Products 213 (2024) 118469

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

Profile and accumulation of essential oils from guayule (*Parthenium argentatum* A. Gray) accessions and hybrids

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ARTICLE INFO

Keywords:
Essential oil
Guayule
GC-QTOF
Monoterpenes
Sesquiterpenes
Industrial extraction

ABSTRACT

For guayule (*Parthenium argentatum* A. Gray) to become a viable market option, it will be necessary to exploit not only the rubber and resin components, but also other high-value plant products such as essential oils (EOs). A detailed EO profiling study was conducted on 16 guayule accessions for first time, which revealed significant differences in EO yield and profile among them. Accession AZ-2 had the highest yield up to 0.9% (dry weight), followed by CFS18–2005, CAL-7, and CFS17–2005. These yields were comparable with those of aromatic plants used in the industry, providing valuable economic insights. The volatile EO composition of the accessions was unique and discriminating. Volatile sesquiterpenes were the most abundant compounds at the beginning of the flowering stage (S1-June sampling), while monoterpenes became more abundant as flowering progressed (S2-July and S3-August sampling). At S1, it was possible to distinguish between hybrid (mariola and non-mariola) and other accessions by the EO profile. By the end of the summer season (S3, August 2023), the EO volatile profile could more easily distinguish the accessions according to their genetic origin, enabling the separation of pure guayule accessions. The extraction of guayule EOs on an industrial scale (1.2 Mg fresh biomass) demonstrated high yield and consistency in terms of volatile profile, underscoring its potential for commercial exploitation.

1. Introduction

Essential oils (EOs) are complex mixtures of organic secondary metabolites localized in the cytoplasm of plant cells. They are characterized by their low molecular weight, typically less than 300 Da, oily fragrance/scent, and high solubility in organic solvents (Bhavaniramya et al., 2019; Sell, 2006). Terpenes make up the majority of the EO components, and can exist as different chemical families such as esters, ethers or oxides, amines, amides, alcohols, aldehydes, ketones, and phenols (Dhifi et al., 2016). EOs display a high degree of variability in their composition due to both intrinsic factors related to the environment including the plant's maturity and the time of harvest (Marotti et al., 1994), as well as extrinsic factors such as the methods used for their extraction (Gonçalves et al., 2021).

Of the ~3000 known EOs only about 300 are used commercially, and there is a growing demand from major end-use industries such as food and beverage, cosmetics, and aromatherapy. EOs are known for their antifungal, antibacterial, anti-inflammatory, and antiviral properties (Brochot et al., 2017; Kakouri et al., 2022). In the agriculture sector, they can be used as fungicides, insecticides, bioherbicides, and for plant growth and germination (Gonçalves et al., 2021; Gupta et al., 2023). According to Murti et al. (2023), the global EO market was valued at USD 23.74 billion in 2023 and is expected to grow at a compound annual growth rate of 7.9% through 2030. Currently, the leading EOs in the market are citrus, peppermint, lavender, lime, and rosemary EOs, although research and development is expanding rapidly, indicating a promising global market outlook in the coming years (Specialty & Chemicals, 2023).

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https://doi.org/10.1016/j.indcrop.2024.118469
Received 28 January 2024; Received in revised form 20 March 2024; Accepted 25 March 2024
Available online 1 April 2024
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In this scenario, the valorization of EOs from guayule (*Parthenium argentatum* A. Gray), a plant known mainly for its rubber (Coffelt and Ray, 2010; Cornish and Backhaus, 1990; Schloman, 1988) and resin (Gallego et al., 2023; Jara et al., 2019; Rozalén et al., 2021a, b) content, could contribute to the economic feasibility of this plant (Dehghanizadeh et al., 2021; Sfeir et al., 2014). So far, the guayule essential oil studies are limited to three references.

In the first study, Haagen-Smit and Siu (1994) conducted an analysis on guayule accession 593, and found that the average EO yield for the whole plant was 0.4%, with the highest content (based on fresh weight) found in the leaves (1.0%), followed by the flowers (0.7%), the bark (0.2%), and the wood (0.1%). Scora and Kumamoto (1979), reported an EO yield of 2.8% from the leaves of an unknown accession and finally, Nik et al. (2008) found that the chemical composition of guayule flower EOs in accession CAL-7 was dependent on water treatments, with yields of 0.4–1.0% (dry weight).

The differences in the chemical composition of guayule EOs in the aforementioned studies are noteworthy. α-Pinene was the most abundant compound in all EOs, accounting for 60% of the total EO composition in the 593 accession (Haagen-Smit and Siu, 1944), which contrasts sharply with the abundance of 16.7% and 6.6% reported by Scora and Kumamoto (1979) y and Nik et al. (2008), respectively. The other isomer, β-pinene varied among 2–3.4% (Haagen-Smit and Siu, 1944; Nik et al., 2008) at regular irrigation conditions and up to 13.6% within Scora and Kumamoto (1979) study. Nik et al. (2008) also identified other minor compounds including γ-eudesmol and β-eudesmol, elemol, germacrene D, and bicyclogermacrene.

The purpose of the present study was to comparatively profile EOs of various guayule accessions. Sixteen accessions, including nine pure accessions and seven hybrids, were cultivated under identical edaphoclimatic conditions, and the accumulation of EOs was analyzed during three samplings within the same cultivation cycle (June to August 2023) using a laboratory-scale Clevenger-type steam distillation apparatus. Furthermore, an industrial-scale steam-distillation extraction process was conducted on a single hybrid accession to determine the EO profile using a real-world production process.

2. Materials and methods

2.1. Germplasms

Sixteen guayule accessions were used for EO profiling (Table 1), including 9 pure guayule accessions and 7 hybrids, which included the AZ-3 hybrid accession used only for industrial-scale extraction. All accessions were obtained from the USDA-ARS National Plant Germplasm System and were germinated and then transplanted at three months of age, except for accessions AZ-6 and 11591 (CL-1), which were obtained from the CIRAD (France) as four-month-old plants.

2.2. Field location and sampling

For the laboratory-scale study, the field sampled was located in Santa Cruz de la Zarza (Toledo, Spain) on a 0.5 ha experimental plot located at coordinates 39º53′47.26" N; 3º07′51.87" W with an average elevation of 763 m above sea level. The plot has been previously described by Jara et al. (2023) and consisted of 9 blocks, each 3 m wide and 8 m long, spaced 1.5 m apart. The 15 accessions were six years old and were planted at a density of 33,333 plants/ha. Plants were sampled monthly from early June 2023 to August 2023 (S1=12 June 2023; S2=12 July 2023, and S3=21 August 2023) (see Table 1). Three homogeneous representative adjacent plants of each accession were randomly selected in each sampling and harvested at 10 cm above the ground. The plants were quickly transported to the laboratory for immediate processing and distillation.

For the industrial-scale study, the field sampled (0.8 ha) was located in Villarrubia de Santiago (Toledo, Spain) at coordinates 40º2′56.61" N; 3º20′40.32" W with an average elevation of 538 m above sea level. A subplot of 1125 m^2 (7.5 m × 150 m) containing five lines of the AZ-3 accession, spaced 1.5 m apart, at a density of 39,216 plants/ha, were mechanically harvested (12 June 2023) to obtain 1.5 Mg of fresh biomass, which was transported by truck to the distillation plant.

Both fields (44 km distance apart) share the same agro-climatic features characterized by a developing arid climate, relatively cold winters and warm summers. The mean annual temperature is 15.4ºC and the lowest mean temperature is ~9.5ºC during the coldest month. The average annual precipitation is 240 mm, concentrated in the spring and autumn, causing winters to be somewhat dry and summers very dry. The temperature, rainfall, and irrigation in the experimental plot containing the 15 accessions during the experimental period is described in more detail in Fig. 1.

2.3. Plant processing and essential oil distillation

For the laboratory experiments, 200 g of each of the three plants (including stems, flowers, and leaves) were mixed together so that the sample was representative. The procedure was carried out in duplicate. The samples for each batch were crushed in a Thermomix for 15 s at speed 6 (Thermomix TM 31, Vorwerk, Madrid, Spain). The extraction of EOs was performed by steam distillation of 200 g of crushed plant material with 700 mL of distilled water (fixed by previous laboratory data) in a Clevenger-type steam distillation apparatus for 2 h. The oil was collected in an Eppendorf tube and centrifuged at 15,000 rpm for 2 min

Table 1
Guayule accessions studied in Santa Cruz de la Zarza and Villarrubia de Santiago (Toledo, Spain).

Guayule Accession	Species	Line	Ilut Group*	Seed code	Origin	Year	Ploidy
N-565	*P. argentatum*	From 4265-I synthesis	G1	PI 478655 15i SO	ERP, 4265-I	1950	3x,4x,5.7x
11591 (CL-1)	*P. argentatum*	Parent USDA 11591	G1	CL1	CIRAD	2017	3x
A48118	*P. argentatum*	-	G2	PI 478655 15i SO	Durango, México	1948	3x,4x,4.5x
CAL-7	*P. argentatum*	3x germplasm selected before 1981	G2	W6 715715i SO	USDA-CA	1982	4x
11600	*P. argentatum*	Hybrid 4265-I x unknown 2x plant	G2	PI 478641 15i SO	ERP, 4265-I	1950	3x,4x
AZ-6	*P. argentatum*	Improved	G2	CL6	CIRAD	2017	4x
CFS18–2005	*P. argentatum*	U Unreleased wild cultivar collection from Firestone	G2	PARL 805 15i SO	Texas, USA	UL	4x
593	*P. argentatum*	Released variety	G3	PI 478639 13i SO	IRC	1926	4x
CFS17–2005	*P. argentatum*	Big Bend National Park	G4	PARL 804 15i SO	Texas, USA	2005	4x
R-1100	*P. hybrid*	Hybrid guayule x mariola	Mariola	W6 2252 13i SO	Coahuila, México	1979	4x
R-1101	*P. hybrid*	Hybrid guayule x mariola	Mariola	W6 2253 15i SO	Coahuila, México	1977	4x
R-1103	*P. hybrid*	Hybrid guayule x mariola	Mariola	W6 2255 13i SO	Durango, México	1981	4x
AZ-2	*P. hybrid*	Hybrid guayule x unknown	Non-mariola	PI 599675 13i SO	USDA-AZ	1997	4x
CAL-1	*P. hybrid*	*P. argentatum Gray x P. tomentosum var. tomentosum*	Non-mariola	PI 478666 13i SO	USDA-CA	1982	3x,4.5x,7x
CAL-2	*P. hybrid*	*P. argentatum Gray x P. fruticosum Less.*	Non-mariola	PI 478666 13i S	USDA-CA	1982	3x,4x
For industrial-scale extraction							
AZ-3	*P. hybrid*	Hybrid guayule x mariola	Mariola	PI 599676 12i SO	USDA-AZ	1997	4x,6x

*(Ilut et al., 2017)

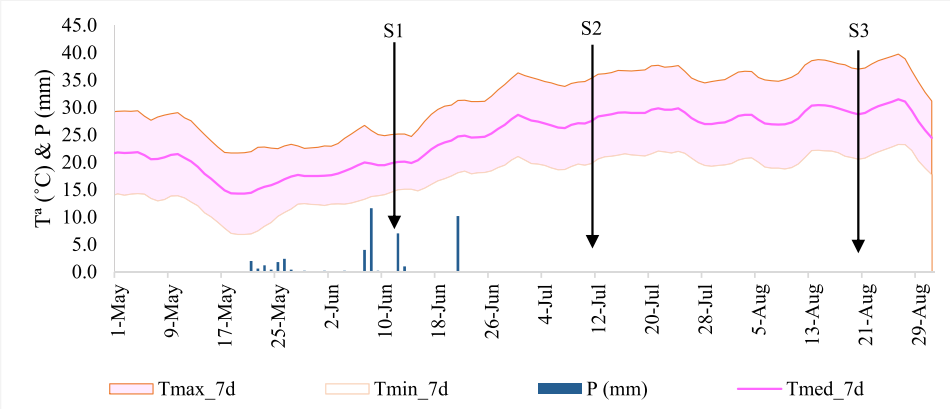


Fig. 1. Climatological characteristics of the test area during the sampling periods. Relationship of temperature and precipitation on the X and Y axes. Maximum temperature (Tmax), average temperature (Tmed), minimum temperature (Tmin) and precipitation (P). Lines represent temperature and bars represent precipitation.

to improve phase separation, and the resulting volume was measured with a precision pipette. All samples were distilled in duplicate.

For industrial-scale extraction, two different experiments were carried out with the 1.5 Mg of fresh biomass of accession AZ-3, the only accession available in such quantities, using different particle sizes (60–80 mm and 20 mm), and crushed as described by Bados et al. (2023) with a 90 kW pre-shredder to reduce the plant material. This apparatus presses the material against a monorotor via a hydraulic feeder with a pusher stroke of 1100 mm. The rotor (φ 450 mm, length 1400 mm) contained 102 embedded blades (40 × 40 mm) that shredded the biomass by passing it through different mesh screens depending on the size required. The plant material was placed in a perforated basket for distillation and filled to 95% of its volume (480 kg for the larger size and 693 kg for the smaller size). The basket was placed into the distillation unit by crane and the distillation process was carried out for 2.5 h with an average steam flow of 115 kg/h. The distillation system is comprised by a 1.8 m^3 stainless steel still, a biomass boiler to produce the steam (0.5 bar), a cooling system with a double cooling coil and a stainless steel Florentain flask to separate the essential oil and the hydrolate.

2.4. Volatile compound analysis by GC-QTOF-MS

Samples were diluted 1:20 (EO:methanol) and injected into a GC 7280 (Agilent, Santa Clara, CA, USA) gas chromatograph equipped with an HP5MS column (30 m × 0.25 mm, 0.25 μm; Agilent 19091S-433UI) connected to an accurate-mass QTOF-MS (Agilent 7200) mass spectrometer for identification. Samples (1 μL) were injected into the GC using an autosampler in split mode (1:100). The injector's temperature was established at 250ºC and the helium carrier gas flow was constant at 1 mL/min. The oven temperature was set to 70ºC (10 min), 3ºC/min at 95ºC, 4ºC/min at 170ºC, 20ºC/min to 300ºC and kept at 300ºC (2 min). The transfer line temperature was 300°C. The N_2 collision gas was fixed at 1 mL/min, the ionization energy at 70 eV, and electron ionization source temperature at 260°C. Spectra were acquired with a scan range of 35–350 *m/z*. Compound identification was performed using the National Institute of Standards and Technology database (version 14) and Agilent MassHunter qualitative analysis software.

2.5. Data analysis

IBM SPSS Statistics v25 (Templeton, 2011) was used to analyze the profile and accumulation of EOs across the three different samplings (S1: June 2023; S2: July 2023; S3: August 2023). Both chemical family composition and temporal evolution were compared using analysis of variance (ANOVA) and Tukey's test at 95% confidence level. Similarly, ANOVA was used to determine the differences in yield between accessions within the same sampling period, as well as the differences between the three samplings for each accession. The compounds identified in the EOs (40) were reduced by principal component analysis (PCA) to select those that could explain the main differences in the profiles between both accessions and samplings. The selected compounds were then used to classify the guayule accessions based on genotype (Ilut et al., 2017) and their temporal evolution (S1-S3).

3. Results and discussion

For the laboratory-scale characterization of EOs, an analysis of 15 guayule accessions was undertaken without the influence of extrinsic factors that could limit their comparison. Accordingly, all plants were grown on the same plot with homogeneous edaphoclimatic conditions and were harvested at the same maturity stage and extracted under the same conditions. It would therefore be expected that the only independent variable affecting the EO yield and chemical composition would be the accession itself. The three samplings (from early June 2023 to August 2023) were selected to overlap with the maximum flowering stage of the plants (end of June to the first week of July).

3.1. Essential oil extraction yields

As shown in Fig. 2, the EO yield (% dry weight) differed between pure guayule accessions (Fig. 2**a**) and hybrids (Fig. 2**b**) of the same sampling and was more pronounced in S3. The yield ranged from 0.1% in S1 (accession N565) to 0.9% in S3 (accession AZ-2). There was a significant increase (up to 48%) in EO yield from S1 (June 2023) to S2 (July 2023) for all accessions except for the R1100 and AZ-2 hybrids, which showed no significant differences in EO yield. The trend of increase was not observed at S3 (August 2023), as accessions AZ-6 and CFS18–2005 (both pure accessions) showed a decrease in EO yield of 54.8% and 47.7%, respectively, and 5 accessions showed an increase in yield (A48118<CAL-7<R1101<CFS17–2005<AZ-2) ranging from 29.2% for accession A48118 to 86.3% for AZ-2. No significant changes were observed for the remaining guayule accessions analyzed from S2 to S3 (Fig. 2).

The highest EO yields were found for AZ-2 (0.9%), CFS18–2005 (0.9%), CAL-7 (0.8%), and CFS17–2005 (0.7%), all at S3, except for CFS18–2005, which reached its maximum yield at S2. While there are limited studies on guayule EOs, Haagen-Smit and Siu (1944) reported a yield of 0.4% for accession 593 when the whole plant was distilled, which is similar to the results obtained in the present study at S2 (yield of 0.4%). The EO yields obtained from most of the guayule accessions tested are similar to other aromatic plants used in industry, for example, *Cymbopogon citratus* (0.4–0.6%), *Ocimum basilicum* (0.4–0.7%), *Origanum majorana* (0.8%) and *Achillea millefolium* (0.7%) (Kakouri et al.,

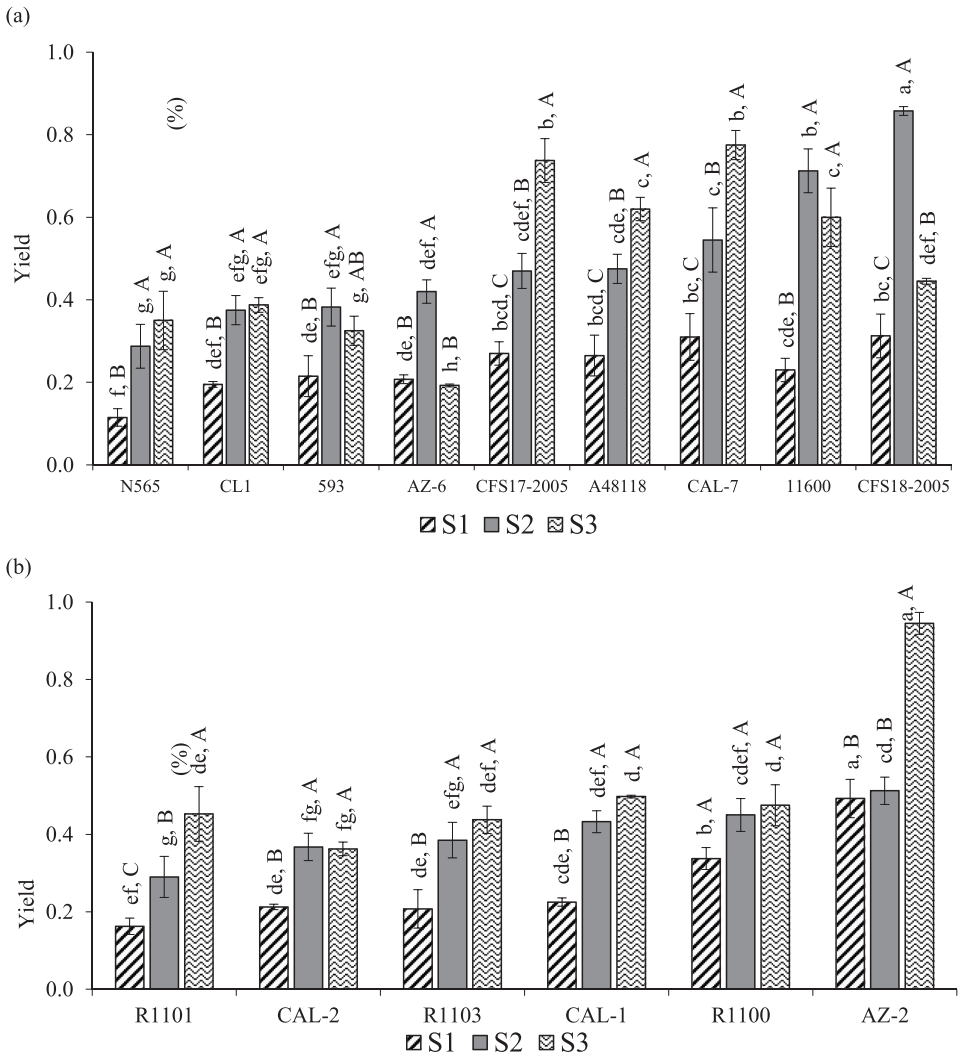


Fig. 2. Yield (%) of essential oils in (a) pure accessions and (b) hybrid accessions over three months. The percentage refers to 100 g of wet weight. Different letters indicate significant differences at 95% confidence level. Uppercase letters refer to an ANOVA comparing differences between the three samplings for each accession; lowercase letters refer to an ANOVA comparing differences between accessions within the same sampling time, irrespective of whether it is a pure or hybrid accession. S1, June 2023; S2, July 2023; S3, August 2023.

2022; Ranjan et al., 2023). The accessions showing the highest yields (AZ-2 and CFS18–2005) were comparable with the yields from *Rosmarinus officinalis* and *Salvia officinalis* yields, with EO yields of 1.0% and 1.1%, respectively (Ranjan et al., 2023). The relatively high yields of EOs in guayule are encouraging and should open up possibilities for industrial exploitation beyond rubber production.

3.2. Essential oil characterization

Forty volatile EO compounds were identified and were grouped as terpenes, oxygenated terpenes, sesquiterpenes, and oxygenated sesquiterpenes (Table 2). Quantification analysis showed that the chemical family with the highest proportion across all samplings was the monoterpenes (61–81%), followed by sesquiterpenes (13–24%), oxygenated sesquiterpenes (4–13%), and oxygenated monoterpenes (0.3–1.8%) (Table 3). Monoterpenes are the most representative and simplest terpenes, and are formed from the assembly of two isoprene units (C10) in the methylerythritol phosphate (MEP) and mevalonic acid (MVA) pathways (Fig. 3). These constitute ~90% of most plant EOs, including some structural modifications such as cyclation and oxidation. The terpene content in the guayule EOs is consistent with many aromatic plants, including *Thymus pannonicus* (68%) and *Origanum vulgare var. aureum* (82%) (Popa et al., 2021).

The content of sesquiterpenes (C-15 terpenoids) in the guayule EOs (13–24%) was significantly higher than that reported for other plants such as *Lavandula x intermedia* L. (4%) or *Lavandula angustifolia* L. (5%) (Popa et al., 2021). All sesquiterpenes have a common C15 skeleton derived from the linear precursor farnesyl pyrophosphate. The structural relationships with other non-volatile compounds in guayule that share the same biosynthetic route are described in Fig. 3, and give rise to the main components of guayule resins, the guayulins and argentatins (Jara et al., 2019). This may be the reason for the high sesquiterpene content in guayule EOs.

A comparative analysis of the results with those in the literature showed that Haagen-Smit and Siu (1944) obtained EOs with a monoterpene content of 72% in accession 593, which is similar to the 69.7% obtained in the present study, but higher than the 57.9% and 22.9% reported by Scora and Kumamoto (1979) and Nik et al. (2008), respectively. In all cases, α-pinene is the main compound, although clear differences were observed between the studies: Haagen-Smit and Siu (1944) reported a content of 60%, Scora and Kumamoto (1979) 16.7%, and Nik et al. (2008) 6.6%. In the present study, the mean content of α-pinene in the 15 accessions was 26.9%, followed by sabinene (12.8%) and limonene (9.5%).

Table 2
Volatile compounds identified in guayule essential oils and their classification according to their chemical structure.

Nº	Tr (min)	Mass Fragmentation Pattern	Common name	IUPAC Name	CAS	Clasification in families*
C1	4.84	93.06(100), 91.05 (74.84), 136.12 (4.97)	Santolina triene	2,5-Dimethyl-3-vinylhexa-1,4-diene	2153–66–4	Monoterpene ($C_{10}H_{16}$)
C2	5.45	93.06 (100), 91.05 (95.99), 136.12 (2.57)	α-thujene	(1 S)-2-Methyl-5-(1-methylethyl) bicyclo (3.1.0) hex-2-ene	563–34–8	Monoterpene ($C_{10}H_{16}$)
C3	5.68	93.06 (100), 91.05 (63.72), 136.12 (1.3)	α-pinene	(1 *S*,5 *S*)-2,6,6-Trimethylbicyclo [3.1.1] hept-2-ene	80–56–8	Monoterpene ($C_{10}H_{16}$)
C4	6.2	93.06 (100), 121.10 (47.07), 136.12 (3.32)	Camphene	2,2-Dimethyl-3-methylidenebicyclo [2.2.1] heptane	79–92–5	Monoterpene ($C_{10}H_{16}$)
C5	7.12	93.06 (100), 91.05 (61.75), 136.12 (5.12)	2-thujene	Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)-	28634–89–1	Monoterpene ($C_{10}H_{16}$)
C6	7.24	93.06 (100), 91.05 (61.36), 136.12 (5.34)	Sabinene	4-methylene-1-(1-methylethyl) bicyclo [3.1.0] hexane	3387–41–5	Monoterpene ($C_{10}H_{16}$)
C7	7.87	93.06 (100), 69.06 (37.36), 136.12 (1.16)	β-myrcene	7-Methyl-3-methylene-1,6-octadiene	123–35–3	Monoterpene ($C_{10}H_{16}$)
C8	8.14	93.06 (100), 91.05 (80.09), 136.12 (1.37)	β-E-ocimene	(3*E*)-3,7-Dimethyl-1,3,6-octatriene	3779–61–1	Monoterpene ($C_{10}H_{16}$)
C9	8.54	93.06 (100), 91,05 (55.02), 136.12 (7.24)	β-terpinene	4-Methylene-1-(1-methylethyl) cyclohexene	99–84–3	Monoterpene ($C_{10}H_{16}$)
C10	9.99	67.05 (100), 93.07 (84.01), 136.12 (5.98)	Limonene	1-Metil-4-(1-methylethenyl)-cyclohexene	138–86–3	Monoterpene ($C_{10}H_{16}$)
C11	11.25	93.06 (100), 91.05 (77.42), 136.12 (1.34)	3-carene	3,7,7-Trimethylbicyclo [4.1.0] hept-3-ene	13466–78–9	Monoterpene ($C_{10}H_{16}$)
C12	15.51	69.06 (100), 79.05 (30.5), 150.14 (3.03)	2-methy-2-bornene	1,2,7,7-Tetramethylbicyclo [2.2.1] hept-2-ene	72540–93–3	Monoterpene ($C_{11}H_{18}$)
C13	16.63	91.05 (100), 92.06 (87.73), 150.12 (12.42)	Sabinol	(1 S,3 R,5 S)-4-methylidene-1-propan-2-ylbicyclo[3.1.0] hexan-3-ol	471–16–9	Oxy. Monoterpene ($C_{10}H_{16}O$)
C14	17.04	91.05 (100), 109.06 (48.58), 152.23 (1.5)	Verbenol	4,6,6-Trimethylbicyclo [3.1.1] hept-3-en-2-ol	477–67–6	Oxy. Monoterpene ($C_{10}H_{16}O$)
C15	19.89	79.05 (100), 91.05 (48.48), 150.21 (5.66)	Myrtenol	(6,6-dimethyl-2-bicyclo[3.1.1]hept-2-enyl)methanol	515–00–4	Oxy. Monoterpene ($C_{10}H_{14}O$)
C16	20.52	107.08 (100), 91.05 (96.05), 152.12 (23.82)	Vervenone	4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-one	80–57–9	Oxy. Monoterpene ($C_{10}H_{14}O$)
C17	22.25	85.06 (100), 119.08 (43.86), 236.35 (1.73)	Myrtenyl isovalerate	6,6-Dimethylbicyclo [3.1.1] hept-2-en-2-yl-methyl 3-methylbutanoate	33900–84–4	Oxy. Monoterpene ($C_{15}H_{24}O_2$)
C18	24.1	95.08 (100), 93.06 (53.38), 196.28 (4.96)	Bornyl acetate	[(1 S,2 R,4 S)-1,7,7-trimethyl-2-bicyclo[2.2.1]heptanyl] acetate	5655–61–8	Oxy. Monoterpene ($C_{12}H_{20}O$)
C19	24.42	93.06 (100), 69.06 (78.88), 182.13 (6.56)	Linalyl formate	3,7-dimethyl-1,6-octadien-3-ol	78–70–6	Oxy. Monoterpene ($C_{11}H_{18}O_2$)
C20	26.35	91.05 (100), 105.06 (85.05), 204.18 (2.66)	α-guaiene	(1 S,4 S,7 R)-1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethenyl)-azulene	3691–12–1	Sesquiterpene ($C_{15}H_{24}$)
C21	27.63	119.08 (100), 105. 06 (98.68), 204.18 (5.24)	α-cubebene	(1 *R*,5 *S*,6 *R*,7 *S*,10 *R*)-4,10-dimethyl-7-propan-2-yl-tricyclo [4.4.0.0^{1,5}] dec-3-ene	17699–14–8	Sesquiterpene ($C_{15}H_{24}$)
C22	27.74	119.08 (46.14), 161.13 (42.74), 204.18 (100)	Modephene	(1 R,2 R,5 S)-2,6,6,8-Tetramethyltricyclo [3.3.3.01,5] undec-7-ene	68269–87–4	Sesquiterpene ($C_{15}H_{24}$)
C23	28.01	162.14 (100), 147.11 (76.3), 204.18 (3.32)	α-isocomene	(1 R,2 R,5 S,8 S)-2,5,6,8-tetramethyltricyclo [6.3.0.01,5] undec-6-ene	65372–78–3	Sesquiterpene ($C_{15}H_{24}$)
C24	28.17	161.13 (100), 143.08 (98.45), 204.18 (9.78)	β-copaene	Tricyclo [4.4.0.0(2,7)] decane, 1-methyl-3-methylene-8-(1-methylethyl)	18252–44–3	Sesquiterpene ($C_{15}H_{24}$)
C25	28.7	108.09 (100), 91.05 (86.65), 204.18 (8.41)	β-isocomene	1 R,2 R,5 S,8 S)-2,5,8-trimethyl-6-methylidenetricyclo [6.3.0.01,5]undecane	71596–72–0	Sesquiterpene ($C_{15}H_{24}$)
C26	29.19	91.05 (100), 93.06 (77.64), 204.18 (2.7)	Caryophyllene	trans-(1 R,9 S)-8-Methylene-4,11,11-trimethylbicyclo [7.2.0] undec-4-ene	87–44–5	Sesquiterpene ($C_{15}H_{24}$)
C27	30.36	93.07 (100), 91.05 (29.29), 204.18 (2.72)	Humulene	2,6,6,9-Tetramethyl-1,4–8-cycloundecatriene	6753–98–6	Sesquiterpene ($C_{15}H_{24}$)
C28	30.6	91.05 (100), 105. 07 (90.43), 204.18 (18.54)	Aromandrene	1,1,7-trimethyl-4-methylidene-2,3,4a,5,6,7,7a,7b-octahydro-1aH-cyclopropa[*e*]azulene	25246–27–9	Sesquiterpene ($C_{15}H_{24}$)
C29	31.29	105.07 (100), 119.08 (95.38), 204.18 (12.72)	Germacrene D	(1E,6E,8 S)-1-methyl-5-methylidene-8-propan-2-ylcyclodeca-1,6-diene	23986–74–5	Sesquiterpene ($C_{15}H_{24}$)
C30	31.8	121.09 (100), 93.06 (86.55), 204.18 (5.8)	Bicyclogermacrene	(2E,6E)-3,7,11,11-tetramethylbicyclo[8.1.0]undeca-2,6-diene	24703–35–3	Sesquiterpene ($C_{15}H_{24}$)
C31	32.66	161.13 (100), 119.08 (97.3), 204.18 (34.39)	δ-cadinene	(1 S,8aR)-4,7-dimethyl-1-propan-2-yl-1,2,3,5,6,8a-hexahydronaphthalene	483–76–1	Sesquiterpene ($C_{15}H_{24}$)
C32	33.45	93.07 (100), 59.04 (80.22), 222.19 (2.62)	Elemol	2-[(1 R,3 S,4 S)-4-ethenyl-4-methyl-3-prop-1-en-2-ylcyclohexyl]propan-2-ol	639–99–6	Oxy. Sesquiterpene ($C_{15}H_{26}O$)

(*continued on next page*)

Table 2 (*continued*)

Nº	Tr (min)	Mass Fragmentation Pattern	Common name	IUPAC Name	CAS	Clasification in families*
C33	33.89	93.06 (100), 69.06 (79.00), 222.19 (2.12)	Nerolidol	3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol	7212–44–4	Oxy. Sesquiterpene ($C_{15}H_{26}O$)
C34	34.26	81.06 (100), 105.06 (60.68), 204.18 (9.99)	γ-muurolene	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1-α., 4a-.α., 8a.α.)	30021–74–0	Sesquiterpene ($C_{15}H_{24}$)
C35	34.32	91.05 (100), 105.06 (94.84), 220.18 (26.82)	Spathulenol	(1aR,4aR,7 S,7aR,7bR)-1,1,7-trimethyl-4-methylidene-1a,2,3,4a,5,6,7a,7b-octahydrocyclopropa[*h*]azulen-7-ol	6750–60–3	Oxy. Sesquiterpene ($C_{15}H_{24}O$)
C36	34.51	91.05 (100), 79.05 (97.89), 220.35 (3.19)	Caryophyllene oxide	(1 *R*,4 *R*,6 *R*,10 *S*)-9-Methylene-4,12,12-trimethyl-5-oxatricyclo [8.2.0.0^{4,6}] dodecane	1139–30–6	Oxy. Sesquiterpene ($C_{15}H_{24}O$)
C37	34.76	105.06 (100), 93.06 (93.38), 222. 19 (13.03)	Viridiflorol	(1aR,4 S,4aS,7 R,7aS,7bS)-1,1,4,7-tetramethyl-2,3,4a,5,6,7,7a,7b-octahydro-1aH-cyclopropa[*e*]azulen-4-ol	552–02–3	Oxy. Sesquiterpene ($C_{15}H_{24}O$)
C38	35.93	161.13 (100), 189.16 (96.96), 222.19 (54.49)	γ-eudesmol	2-[(2 R,4aR)-4a,8-dimethyl-2,3,4,5,6,7-hexahydro-1 H-naphthalen-2-yl]propan-2-ol	1209–71–8	Oxy. Sesquiterpene ($C_{15}H_{26}O$)
C39	36.16	59.04 (100), 91.05 (94.48), 220.18 (5.02)	α-copaen-11-ol	Tricyclo [4.4.0.0(2,7)] dec-8-ene-3-methanol, α., α.,6,8-tetramethyl	41370–56–3	Oxy. Sesquiterpene ($C_{15}H_{24}O$)
C40	36.47	59.04 (100), 149.13 (60.77), 222.19 (3.63)	β-eudesmol	(2 R,4aR,8aS)-Decahydro-8-methylene-α, α, 4a-trimethyl-2-naphthylmethanol	473–15–4	Oxy. Sesquiterpene ($C_{15}H_{26}O$)

In the case of sesquiterpenes (Table 3), the content found in the present study was considerably higher than that reported by Haagen--Smit and Siu (1944) (9.3%), but lower than the 39.5% and 41.5% reported by Scora and Kumamoto (1979) and Nik et al. (2008), respectively. Consistent with the findings of Nik et al. (2008), the major compounds of this family in the CAL-7 accession were germacrene D and bicyclogermacrene. These compounds also share a biosynthetic pathway with the non-volatile sesquiterpenes present in the resin, the guayulins.

The compounds with the lowest proportion in samples included the oxygenated monoterpenes and sesquiterpenes, representing 0.3–1.8% and 4.6–13.0% of the compounds, respectively. In the case of oxygenated monoterpenes, Haagen-Smit and Siu (1944) reported a higher content (5.8%) but they made no reference to the main compound found. Among the 15 accessions studied here, verbenol was the main compound found, representing 50–95% of the family, followed by myrtenal and verbenone. With regard to oxygenated sesquiterpenes, a higher content was quantified in the present study when compared with Haagen-Smit and Siu (1944) in accession 593, but a greater difference was observed (34.3%) in relation to CAL-7 (Nik et al., 2008), although the main compounds within this fraction were the same (eudesmol isomers and elemol).

Finally, the highest monoterpene fraction was found in accession AZ-6, R1101 had the highest sesquiterpene content, and R1103 and A48118 had the highest content of oxygenated terpenes and sesquiterpenes, respectively.

The EO profile appeared to be unique for each accession, allowing their differentiation. According to the PCA results, 26 of the 40 identified volatiles (shown in Table 2) could explain 81.4% of the differences between accessions (Fig. 4). When the discriminant analysis was performed with the selected volatiles, 97.8% of the samples were correctly classified within accessions (Fig. 4**a**), which clustered into differentiated groups. The finding that one of these groups contained the three hybrid accessions from guayule x mariola (R1100, R11001, and R1103), and also that the pure accession CFS17–2005 appeared outlying (which has been genetically differentiated from the other pure accessions studied in the bibliography by Ilut et al., 2017), suggested that the EO composition could be related to the genetic origin. The accessions were then grouped according to the classification of the classification of Ilut et al. (2017) (Fig. 4**b**), which separated the six groups with 94.4% of the original samples correctly classified. The mariola hybrids G3 and G4 were completely distinct from the remaining samples, whereas the pure varieties G1 and G2 and G2 slightly overlapped with non-mariola plants, but always below 8.3% (Fig. 4**b**). This separation was achieved with five canonical discriminant functions, explaining 74.2% of the total variance in the first function and 88.6% when the second function was also considered. The volatiles that contributed most to the differentiation, in order of importance, were: δ-cadinene (C31), camphene (C4), 2-thujene (C5), β-E-ocimene (C8), caryophyllene (C26), and a mixture of monoterpenes and sesquiterpenes without oxygenation.

A more detailed analysis revealed that the six genetic groups proposed by Ilut et al. (2017) were different from each other in terms of EO profile across the samplings (S1–S3) and could be separated with 100% of the original samples correctly classified (Fig. 5). However, different patterns were observed in the separation of the groups due to the distinct temporal evolution of the compounds. At sampling S1 (June 2023) (Fig. 5**a**), EOs from the hybrid accessions (mariola and non-mariola) together with the G4 group of Ilut (CFS17–2005 pure accession) were clearly separated from the other 8 pure accessions from G1 to G3. Such separation between groups could be explained by only two discriminant functions, which accounted for 91.2% of the total variance **(**Fig. 5**d)**. Within the first function, the sesquiterpene α-cubebene (C21) was found in only 3 guayule accessions, with the highest content found in CFS17–2005 (G4 group). Sabinol (C13) and linalyl formate (C19) were found in only one accession each (CAL7 and CAL2), while δ-cadinene (C31), as well as the sesquiterpene structure, was a common compound in all accessions, with the lowest content detected in CFS17–2005 (G4 group) and the mariola hybrids group (Table 4). The monoterpene β-E-ocimene (C8) was only found in the hybrid groups in this sampling (S1). Volatiles with a higher contribution to the second function, but different from the first function, included several sesquiterpenes: aromandrene (C28), which was only found in one mariola accession (R1101), followed by α-cubebene (C21) and caryophyllene (C26), with the lowest content in G3 and G4, and humulene C27, with the highest content in G1 and the lowest in G3 **(**Fig. 5**d)**.

In the S2 sampling, one month later (July 2023), it was observed that mariola and G4 groups remained distinct, but the non-mariola groups moved closer to G1 and G2, and G3, clearly separated from the other pure guayule accessions (Fig. 5**b**). As observed for S1, the group discrimination could be explained by two discriminant functions accounting for 94.4% of the total variance (Fig. 5**d**). Within the first

Table 3
Composition of guayule essential oils in terms of their chemical families and the most abundant volatiles across all samplings.

Guayule Accession	Monoterpenes (%)				Oxygenated monoterpenes (%)					Sesquiterpenes (%)				Oxygenated sesquiterpenes (%)			
	$\sum_m$	C3	C6	C10	$\sum_m$	C14	C15	C16	$\sum_m$	C26	C30	C29	$\sum_m$	C26	C38	C32
N565	70.4ab	31.9ab	13.5ab	8.9bc	0.7ab	0.6b	0.6a	0.6a	18.0ab	2.7cde	3.7abc	5.3bcde	8.9abc	3.3c	2.8cdef	2.0ab
CL1	69.2ab	31.7ab	13.0ab	8.6bc	1.0ab	0.6ab	0.4a	0.5a	19.3ab	2.5cde	4.0abc	5.6bcdef	8.8abc	3.3abc	2.0defg	2.0ab
A48118	61.4b	22.1ab	11.5b	10.1ab	0.6ab	0.4ab	0.3a	0.1a	21.1ab	1.1abc	5.4abc	5.4 f	13.0ab	3.8ab	5.0ab	2.6a
CAL-7	72.4ab	25.2ab	13.9ab	9.5abc	1.1ab	0.7ab	0.5a	0.4a	16.4b	3.2de	2.8bc	4.0bcd	8.4abc	2.4abc	3.4bcde	1.6abc
I1600	70.4ab	30.5ab	11.7b	10.2ab	0.4b	0.4ab	0.3a	nd	16.3b	1.7cde	3.8c	3.9def	11.3a	3.4abc	5.6a	1.7ab
AZ-6	80.5a	36.4a	15.7a	10.5ab	1.0ab	0.7ab	0.5a	0.6a	13.5b	1.6cde	3.1bc	4.4def	4.6c	1.9abc	1.5defg	1.6bc
CFS18–2005	69.1ab	30.4ab	13.3ab	8.7bc	0.5ab	0.5ab	0.3a	0.3a	18.0ab	1.8cde	4.0abc	4.6def	10.7ab	3.5abc	4.2abc	1.1ab
593	69.7ab	24.2ab	12.3ab	11.5a	0.3b	0.5ab	0.4a	nd	18.5ab	1.0bcde	4.3abc	6.3 f	7.9bc	2.4abc	2.3cdefg	1.7abc
CFS17–2005	70.6ab	26.5ab	12.0b	10.5ab	0.7ab	0.5ab	0.3a	nd	17.8ab	1.4bcd	4.6abc	6.2ef	8.1c	1.7ab	1.0fg	2.9bc
R1100	72.9ab	26.3ab	11.8b	8.3bc	0.7ab	0.5ab	0.5a	0.3a	17.5ab	3.8de	2.7abc	6.5ab	6.0c	1.1ab	1.1fg	3.0c
R1101	62.7ab	22.7ab	10.9b	8.3bc	1.1ab	0.5ab	0.3a	0.3a	23.7a	2.2a	7.8a	7.3cdef	7.3c	2.0abc	1.1fg	2.2bc
R1103	67.9ab	25.8ab	11.2b	7.8c	1.8a	0.8a	0.5a	0.4a	18.4ab	3.6cde	3.8ab	6.8abc	6.6c	1.5a	1.0fg	3.0c
AZ-2	70.5ab	22.1ab	13.6ab	8.8bc	0.3b	0.3ab	0.2a	nd	18.1ab	4.7e	2.1bc	4.4a	9.5abc	2.6abc	3.6abcd	2.0abc
CAL-1	73.0ab	29.1ab	13.3ab	9.8abc	0.5ab	0.4ab	0.2a	0.2a	16.8ab	2.3bcde	4.4abc	4.6bcdef	7.8bc	2.0bc	1.3efg	1.5abc
CAL-2	70.4ab	19.6b	13.9ab	11.5a	0.9ab	0.4ab	0.4a	0.3a	19.6ab	2.6ab	6.6abc	4.7bcdef	6.5c	1.2abc	0.6 g	0.8c
Bibliographic data																
593[(1)]	72.6	60	-	10	5.8	-	-	-	9.3	-	-	-	6.3	-	-	4
Unknown[(2)]	57.9	16.7	6.5	5.9	-	-	-	-	39.5	-	-	-	-	-	-	-
CAL-7[(3)]	22.9	6.6	2.2	-	-	-	-	-	41.5	12.1	16.5	4.6	34.3	9.5	1.5	12.4

[(1)] Haagen-Smit and Siu (1944); [(2)] Scora and Kumamoto (1979); [(3)] Nik et al. (2008). $\sum$, summatory of all compounds; C3, α-pinene; C6, Sabinene; C10, Limonene; C14, Verbenol; C15, Myrtenal; C16, Verbenone; C29, D-Germacrene; C30, Bicyclogermacrene; C26, Caryophyllene; C40, β-eudesmol; C38, γ-eudesmol; C32, Elemol. Different letters within the same column indicate significant differences between accessions at 95% level of confidence. "nd" means not detected.

function, the volatiles with the highest contribution were C30>C1>C19>C6. The sesquiterpene bicyclogermacrene (C30) was again the volatile with greatest contribution, with the highest content found in the mariola hybrid R1101, followed by CAL-2 (non-mariola). The other three relevant compounds (C1, C19, C6) were monoterpenes. Santolina triene (C1) was mainly present in the hybrid accessions, and was also found in CAL-7 (G2) in a lower abundance. Linalyl formate (C19) appeared in two hybrids, in R1101 (mariola) as a new compound in S2, while in CAL-2 (non-mariola) it decreased considerably compared with the previous sampling (Table 4). Sabinene (C6) was present in all groups, being most abundant in G1 and non-mariola accessions. For the second function, the volatiles with a higher contribution were the monoterpenes C3 (α-pinene), which showed a high content in G1 and G2 groups although the highest value was found in the hybrid CAL-1; followed by C10 (limonene) with the highest content in accession 593 (G3 group); and C2 (α-thujene) for which the mariola accessions showed the lowest content. For bornyl acetate (C18), the non-mariola hybrids were distinguished by their lower content.

By the end of the summer (S3, August 2023), all Ilut groups were separated, although the pure guayule accessions (G1 to G4) and non-mariola hybrids remained much closer while the mariola hybrids were the most different (Fig. 5**c**). Two discriminant functions could explain this separation, accounting for 95.4% of the total variance (Fig. 5**d**). The volatile with the highest weight on the canonical coefficients for the first function was the monoterpene α-guainene (C20), quantified in all accessions, although the lowest content was found in the mariola accessions. Other monoterpenes such as C4, C6, and C5 contributed to a lesser extent to the discrimination in both functions (Fig. 5**d**), although C6 and C4 were relevant in the second function, showing different levels of content between groups. The monoterpene C18 and the sesquiterpene C27 mainly contributed to discriminate the non-mariola hybrids, which had significantly lower levels in this last sampling (Table 4). Finally, the monoterpene C2 showed different levels between groups, being more abundant in the pure accessions.

In general, the sesquiterpenes were more important in discriminating accessions in the June sampling, while monoterpenes were more important in the July and August samplings. Other generalizations that demonstrate the characteristic behavior of hybrid accessions across the three samplings are shown in Table 4. For example, santolina triene (C1) was only significantly present in hybrid accessions (except for CAL-1 in S2). Likewise, the terpene β-E-ocimene (C8) was present in mariola hybrids and occasionally in the non-mariola hybrids AZ-2 or CAL-1 in S1. Another monoterpene, linalyl formate (C19), was characteristic of all hybrid groups in S3, and only appeared in all samplings in accession CAL-2. Finally, the oxygenated sesquiterpene viridiflorol (C37) was always detected in the non-mariola hybrid CAL-2, and only in S1 and S3 in the hybrids CAL-1 (non-mariola) and R1101 (mariola). These compounds were not apparent in pure accessions except for C1 in CAL-7 in the three sampling with a very low content in S1 and S2, and C8 and C19 in CAL-7 in S3.

For pure guayule plants, the EOs of all accessions included in group G1 were characterized by their high content of the monoterpenes C3, C4, C6, C10, C12, and C18, the sesquiterpenes C27, C30, C31, and C32, and the oxygenated sesquiterpenes C38 and C40, in all samplings. The high content of humulene (C27) was the main characteristic with regards to the other pure accessions and hybrids. Moreover, they did not contain the monoterpenes C1, C18, and C19 and the oxygenated sesquiterpene C37 (Table 4).

The G2 group was more heterogeneous as it was larger and was characterized by the highest content of the oxygenated sesquiterpene γ-eudesmol (C38) and medium-high levels of most other compounds. AZ-6 was prominent in this group, as C38 showed the lowest values and disappeared in S3. CAL-7 was the only pure accession containing the monoterpene C1, the only accession among all the pure and hybrids with C13 in S1, and the only one in this group with C21 across all samplings. Although CAL-7 is a tetraploid variety of guayule released by the

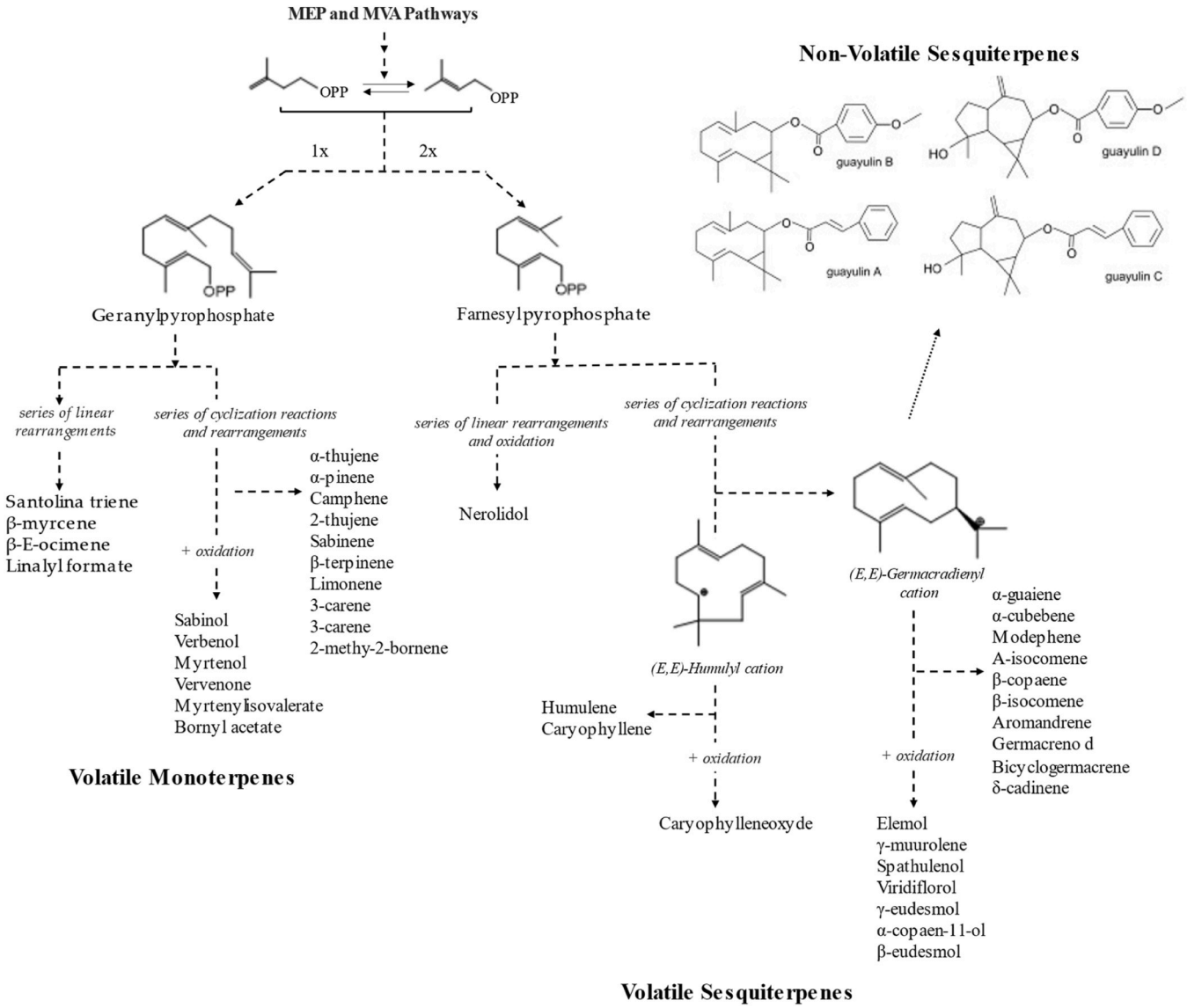


Fig. 3. Monoterpene and sesquiterpenes found in guayule essential oils, their biosynthetic pathways, and their relationship to some of the major compounds in guayule resin. Elaborated from references (Eslahi et al., 2017; Xu and Dickschat, 2020; Zhang et al., 2016; Zhou and Pichersky, 2020).

University of California in 1985, its genetic origin is unknown as it was obtained from a random sampling of 800 plants in a 4 ha cultivation area (Estilai, 1986). It is therefore difficult to hypothesize the origin of this different behavior. Finally, A48118 was prominent for its high content of the sesquiterpenes C23, C28, C30 and C35 (Table 4).

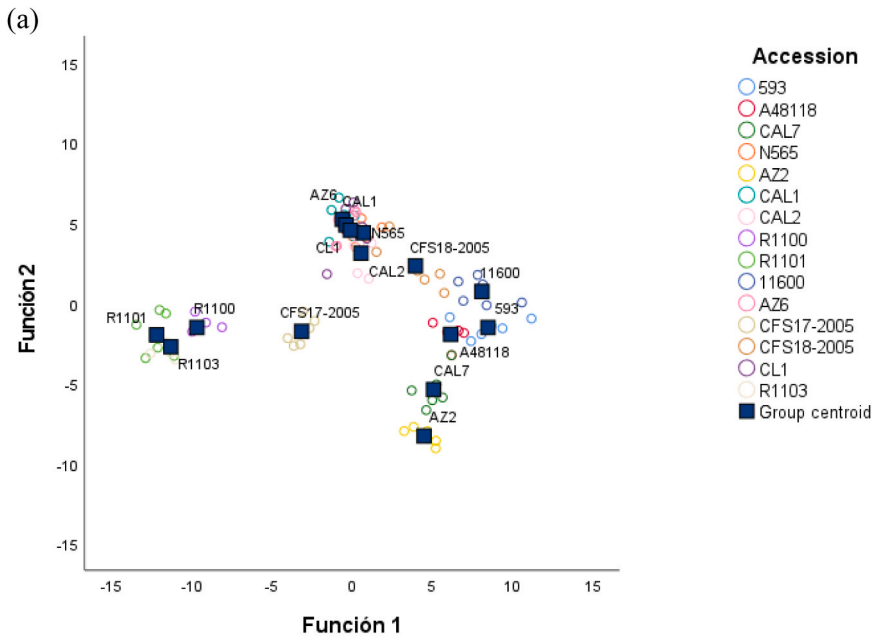
Accession 593, belonging to group G3 and one of the oldest guayule accessions extensively used for obtaining more productive accessions during the ERP project (1942–1946) (Ilut et al., 2017), contained the highest levels of C5 and C9 in all samplings, the highest level of C31 in S2 and S3 and highest levels of C10. Finally, the G4 group was characterized as having the highest levels of C21 in S1 and S2 decreasing in S3, high levels of C32 and low levels of C31 (Table 4).

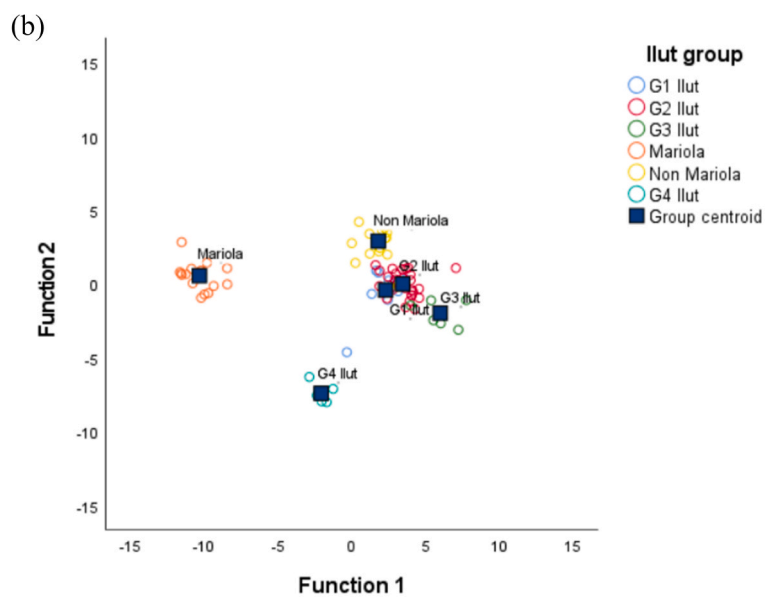
3.3. Industrial essential oil extraction

The industrial-scale distillation (60–80 mm) produced 1200 g of EOs, representing a yield of a 0.5% (dry basis) for higher size biomass, while for the smaller-size material (20 mm) an extraction yield of 0.6% dry basis (1943 g) was obtained. These yields were unexpectedly higher than those obtained in the small-scale profiling study performed for the same period (June 2023) with the exception of the AZ-2 hybrid (0.5%). Typically, laboratory-scale extraction offers a higher yield due to the limitations of homogeneity and steam circulation in industrial distillation, but this was surprisingly not the case. Once again, this high yield on an industrial scale bodes well for the exploitation of this plant in the EO industry.

In terms of the volatile profile (Fig. 6**a**), there were no significant changes between the two industrial-scale batches or when compared with the equivalent laboratory-scale profile, as terpene compounds were the most abundant, especially α-pinene (C3, 30.4–38.1%), sabinene (C6,13.2–14.6%), and limonene (C10, 6.3–7.1%), and in case of sesquiterpenes, the content of β-caryophyllene (C26, 1.2–3.5%) and germacrene D (C29, 1.17–3.61%) was notable.

Although when both samples were compared, it was observed differences on their quantification (Fig. 6**b**), while large-size biomass extraction showed a great content on volatile sesquiterpenes (C20 to C40), the small-size biomass extraction shows a higher content on terpenes specially in santoline triene (C1), α-pinene (C3), and α-isocomene (C23).



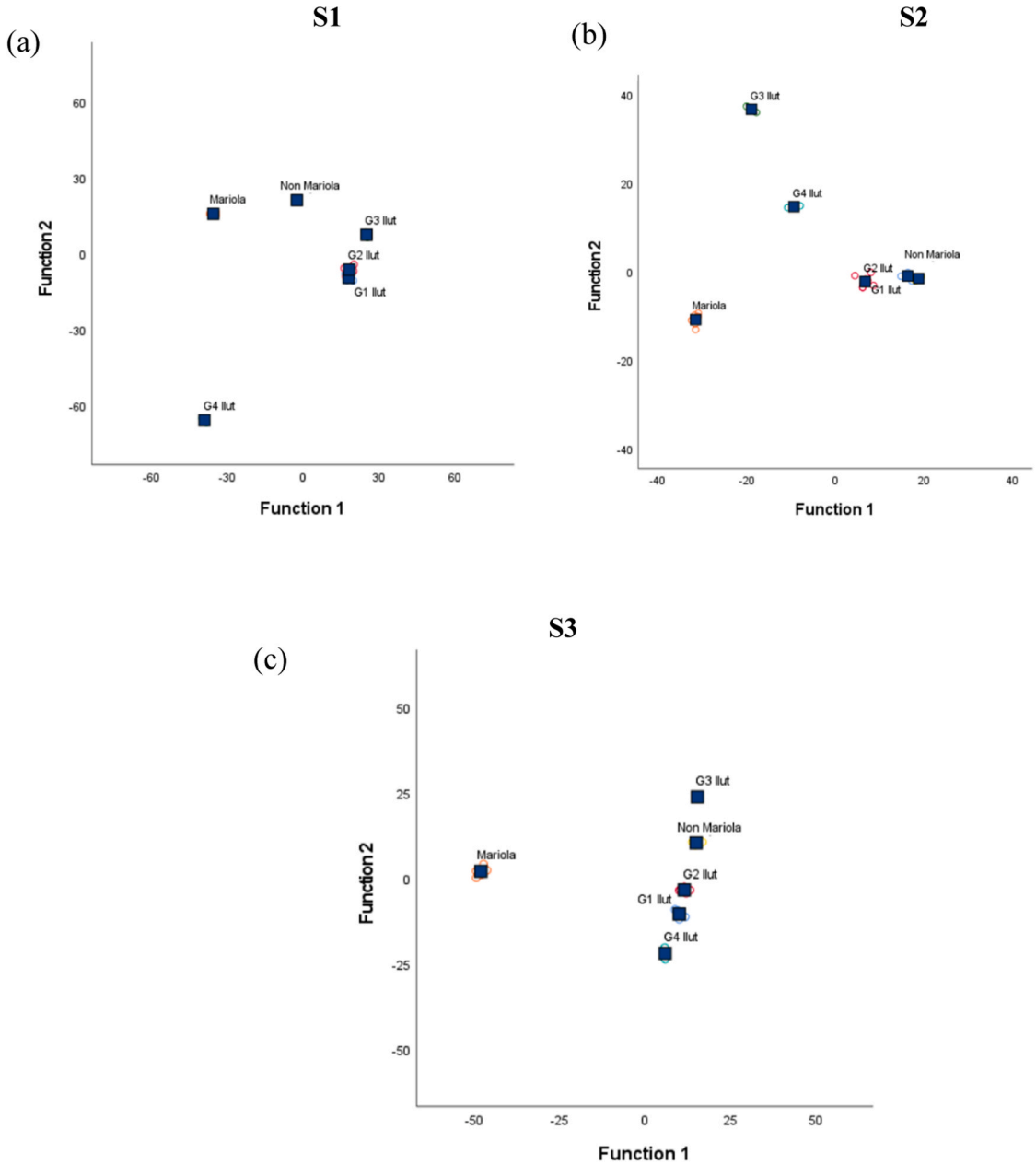


Predicted belonging to groups (%)						
Original group	G1 Ilut	G2 Ilut	G3 Ilut	Mariola	Non Mariola	G4 Ilut
G1 Ilut	91.7	8.3				
G2 Ilut	6.7	90.0			3.3	
G3 Ilut			100.0			
Mariola				100.0		
Non Mariola		5.6			94.4	
G4 Ilut						100.0

Fig. 4. Discriminant analysis of (a) individual accessions (b) accessions grouped by clusters according to Ilut et al. (2017).

4. Conclusions

The EOs obtained from guayule accessions are comparable with other aromatic plants commonly used by industry such as *Rosmarinus officinalis* and *Salvia officinalis*, demonstrating the economic potential of guayule EOs. The yield of EOs were significantly different between accessions, with the most notable differences observed between pure guayule accessions and hybrids. AZ-2, CFS18–2005, CAL-7, and CFS17–2005 showed the highest yields, reaching up to 0.9%. At an industrial-scale, the yield increased when small ground material was distillated. The yield of EOs also differed across the flowering period, which could be crucial for optimizing harvest times. The general



(d)

Sampling		Function 1	Function 2	Accumulated Variance
S1	Variance	49.8%	41.4%	91.2 %
	Volatiles*	C21>C31>C8>C26	C28>C21>C26>C27	
S2	Variance	68.9%	25.5%	94.4%
	Volatiles*	C30>C1>C19>C6	C3>C10>C2>C18	
S3	Variance	80.2%	15.2%	95.4%
	Volatiles*	C20>C4>C6>C5	C6>C4>C18>C27>C2	

* Volatiles, with the highest contribution for each discriminant function.

Fig. 5. Canonical discriminant analysis of 15 essential oils from guayule, grouped according to the Ilut classification (Ilut et al., 2017), in terms of sampling times (a) S1-June 2023, (b) S2-July 2023, (c) S3-August 2023 and (d) the variance percentage explained by the two main functions together with the volatiles high the highest contribution to the discriminant standardized canonical coefficients.

Table 4
Volatile content of essential oils (% of chromatogram area) of 15 guayule accessions in the different samplings.

a) S1

Ilut	Accession	C1	C2	C3	C4	C5	C6	C8	C9	C10	C12	C13	C18	C19	C20	C21	C23	C26	C27	C28	C30	C31	C32	C35	C37	C38	C40
G1	N565	0.00	0.00	32.19	3.12	1.76	12.47	0.00	0.00	9.09	0.18	0.00	8.00	0.00	0.52	0.00	0.51	2.62	2.00	0.00	3.95	1.52	2.50	0.00	0.00	2.64	3.74
G1	CL1	0.00	0.00	35.44	3.06	1.90	12.69	0.00	0.00	8.13	0.22	0.00	6.97	0.00	0.00	0.00	1.33	2.63	2.00	0.00	3.92	1.53	1.79	0.00	0.00	1.93	3.30
G2	A48118	0.00	0.31	27.70	1.59	8.21	11.29	0.00	0.78	10.86	0.00	0.00	4.01	0.00	0.26	0.00	1.78	0.77	0.48	0.00	5.15	1.51	2.77	0.47	0.00	3.61	3.23
G2	CAL 7	2.98	0.00	26.62	1.02	5.68	12.53	0.00	0.47	8.84	0.17	0.36	2.75	0.00	0.33	0.35	1.53	4.53	0.63	0.00	3.22	1.75	1.55	0.00	0.00	3.59	2.60
G2	11600	0.00	0.00	34.19	1.59	4.53	11.30	0.00	0.40	10.05	0.00	0.00	4.49	0.00	0.00	0.00	1.53	2.07	1.49	0.00	4.64	1.96	1.76	0.00	0.00	5.32	1.53
G2	AZ 6	0.00	0.00	37.62	3.57	3.15	12.83	0.00	0.00	9.29	0.21	0.00	6.50	0.00	0.00	0.00	1.26	1.96	1.46	0.00	3.95	1.19	1.67	0.00	0.00	1.56	2.52
G2	CFS18–2005	0.00	0.20	35.47	2.56	3.89	13.09	0.00	0.40	7.92	0.00	0.00	6.35	0.00	0.18	0.00	1.60	1.80	1.40	0.00	3.48	1.09	2.46	0.00	0.00	4.97	3.25
G3	593	0.00	0.21	30.56	1.85	9.79	12.61	0.00	0.74	11.79	0.00	0.00	4.19	0.00	0.25	0.00	1.59	0.69	0.42	0.00	4.78	1.45	1.91	0.00	0.00	3.14	2.62
G4	CFS17–2005	0.00	0.16	28.78	1.60	7.59	11.05	0.00	0.52	10.53	0.41	0.00	6.49	0.00	0.19	0.50	1.43	1.22	0.83	0.00	5.31	0.46	3.04	0.17	0.00	0.90	1.56
HM	R1100	6.64	0.00	30.38	2.06	4.34	10.62	2.55	0.00	7.75	0.16	0.00	6.21	0.00	0.00	0.00	0.64	4.14	1.46	0.00	2.72	0.81	3.16	0.00	0.65	0.71	0.83
HM	R1101	3.30	0.00	25.48	0.98	3.15	9.71	1.76	0.00	8.44	0.33	0.00	5.11	0.00	0.00	0.00	1.06	1.98	1.81	0.30	9.70	0.78	1.89	0.48	0.00	0.83	1.83
HM	R1103	5.34	0.00	32.62	2.84	3.27	10.59	1.95	0.00	7.33	0.00	0.00	7.05	0.00	0.00	0.00	0.93	3.66	1.06	0.00	2.74	0.00	2.70	0.17	0.00	0.30	0.94
HNM	AZ-2	9.34	0.16	25.97	0.98	6.03	12.97	0.42	0.35	8.10	0.30	0.00	2.92	0.00	0.14	0.35	1.14	4.16	0.52	0.00	2.07	1.76	1.38	0.00	0.00	2.68	1.94
HNM	CAL-1	3.89	0.00	30.56	2.78	5.22	10.74	1.78	0.37	9.87	0.20	0.00	6.86	0.00	0.00	0.00	0.80	2.10	0.73	0.00	4.53	1.59	1.57	0.17	1.52	1.59	2.07
HNM	CAL- 2	9.24	0.12	22.65	1.08	9.32	12.34	0.00	0.70	10.46	0.00	0.00	1.98	0.30	0.15	0.00	1.10	2.41	0.57	0.00	7.94	1.53	0.51	0.41	1.39	0.25	0.93

b) S2

Ilut	Accession	C1	C2	C3	C4	C5	C6	C8	C9	C10	C12	C13	C18	C19	C20	C21	C23	C26	C27	C28	C30	C31	C32	C35	C37	C38	C40
G1	N565	0.00	0.54	42.66	4.35	3.03	15.49	0.00	0.22	9.15	0.28	0.00	5.46	0.00	0.00	0.00	1.25	1.41	1.29	0.00	2.35	1.02	0.83	0.00	0.00	0.90	1.69
G1	CL1	0.00	0.40	37.01	3.53	2.96	13.98	0.00	0.00	8.54	0.54	0.00	5.25	0.00	0.43	0.00	1.93	2.36	1.68	0.00	3.38	1.29	1.73	0.00	0.00	1.72	3.08
G2	A48118	0.00	0.18	24.02	1.08	6.60	11.73	0.00	0.69	9.98	0.00	0.00	6.09	0.00	1.18	0.32	4.03	1.17	0.80	0.36	4.57	1.45	2.62	0.31	0.00	4.93	3.32
G2	CAL 7	0.73	0.50	30.62	2.10	9.76	13.91	0.00	0.54	9.23	0.00	0.00	4.46	0.00	0.52	0.17	2.18	1.53	0.43	0.18	2.87	0.78	2.00	0.31	0.00	3.48	2.39
G2	11600	0.00	0.62	36.50	3.34	8.68	11.85	0.00	0.81	10.30	0.00	0.00	6.67	0.00	0.00	0.00	0.84	1.54	1.02	0.00	2.30	0.70	0.90	0.00	0.00	5.18	2.55
G2	AZ 6	0.00	0.40	37.00	3.76	2.65	14.04	0.00	0.00	8.70	0.20	0.00	5.45	0.00	0.20	0.00	1.60	1.80	1.29	0.00	3.52	1.14	2.14	0.21	0.00	1.38	2.85
G2	CFS18–2005	0.00	0.54	32.84	2.19	6.94	13.35	0.00	0.66	9.33	0.00	0.00	4.90	0.00	0.71	0.19	2.52	1.12	0.79	0.00	4.24	0.99	1.39	0.00	0.00	4.65	2.89
G3	593	0.00	0.38	26.90	2.16	10.37	12.26	0.00	0.92	12.80	0.39	0.00	3.85	0.00	0.36	0.00	1.60	1.05	0.57	0.00	3.40	2.28	1.54	0.36	0.00	1.93	2.17
G4	CFS17–2005	0.00	0.44	30.41	2.25	8.39	12.21	0.00	0.65	10.68	0.46	0.00	5.47	0.00	0.35	0.42	1.45	1.29	0.87	0.00	3.70	0.43	2.30	0.00	0.00	0.80	1.41
HM	R1100	7.55	0.16	28.59	2.49	3.78	12.07	3.55	0.00	7.87	0.00	0.00	6.40	0.00	0.37	0.00	1.51	4.30	1.46	0.00	2.35	0.25	2.43	0.00	0.00	0.65	0.92
HM	R1101	6.02	0.14	25.55	1.00	4.00	11.68	3.31	0.00	8.24	0.71	0.00	3.13	0.32	0.33	0.00	1.45	1.61	1.66	0.48	7.70	0.76	1.63	0.67	0.00	1.18	1.96
HM	R1103	5.07	0.00	27.54	2.35	3.47	11.49	2.76	0.00	7.57	0.00	0.00	6.54	0.00	0.20	0.00	1.37	4.66	1.45	0.00	2.27	0.00	3.09	0.27	0.00	0.69	1.24
HNM	AZ-2	4.70	0.16	23.67	1.03	5.76	13.70	0.35	0.32	8.67	0.21	0.00	2.91	0.00	0.36	0.36	1.29	5.34	0.78	0.00	1.93	1.90	2.56	0.00	0.00	3.31	2.45
HNM	CAL-1	0.00	0.58	43.05	4.50	3.06	14.98	0.00	0.00	8.02	0.19	0.00	4.53	0.00	0.00	0.00	1.28	1.84	1.38	0.00	2.46	0.99	1.77	0.00	0.00	1.56	2.50
HNM	CAL- 2	9.11	0.31	22.27	1.15	9.18	14.00	0.00	0.73	11.26	0.00	0.00	1.39	0.12	0.41	0.00	1.70	2.33	0.54	0.00	4.90	1.29	1.21	0.49	1.69	0.85	1.40

b) S3

Ilut	Accession	C1	C2	C3	C4	C5	C6	C8	C9	C10	C12	C13	C18	C19	C20	C21	C23	C26	C27	C28	C30	C31	C32	C35	C37	C38	C40
G1	N565	0.00	0.30	21.01	3.27	2.87	12.64	0.00	0.29	8.46	0.78	0.00	5.07	0.00	0.49	0.00	1.90	2.84	2.37	0.47	4.70	1.97	2.60	0.27	0.00	3.38	4.57
G1	CL1	0.00	0.39	22.51	2.90	4.39	12.46	0.00	0.48	9.14	0.96	0.15	4.31	0.00	0.47	0.10	1.72	2.48	1.89	0.37	4.68	1.49	2.45	0.23	0.00	2.50	3.60
G2	A48118	0.00	0.31	14.58	1.38	6.01	11.64	0.00	1.23	9.50	0.44	0.00	3.87	0.00	1.10	0.30	3.13	1.37	0.95	0.49	6.40	1.68	2.39	0.52	0.00	6.38	4.77
G2	CAL 7	5.38	0.67	18.31	1.82	9.23	15.14	0.39	0.65	10.30	0.27	0.00	2.10	0.12	0.29	0.16	1.24	3.45	0.77	0.13	2.21	1.32	1.31	0.00	0.00	3.16	2.28
G2	11600	0.00	0.39	20.87	1.62	6.33	12.03	0.00	0.96	10.39	0.42	0.26	3.40	0.00	0.76	0.00	2.48	1.59	1.13	0.31	4.39	1.88	2.43	0.29	0.00	6.39	4.49
G2	AZ 6	0.00	0.74	34.58	6.16	5.55	20.31	0.00	0.47	13.66	0.94	0.00	3.40	0.00	0.00	0.00	0.76	1.06	0.61	0.00	1.96	0.48	0.43	0.00	0.00	0.00	0.50
G2	CFS18–2005	0.00	0.37	22.88	3.46	3.15	13.62	0.00	0.47	8.91	0.80	0.00	4.71	0.00	0.50	0.00	1.84	2.55	2.01	0.39	4.36	1.85	2.49	0.14	0.00	3.06	4.35
G3	593	0.00	0.36	15.06	2.23	8.83	12.14	0.00	1.30	10.03	0.62	0.00	3.97	0.00	0.51	0.10	2.00	1.38	0.86	0.20	4.61	2.63	1.77	0.49	0.00	1.76	2.49
G4	CFS17–2005	0.00	0.41	20.43	1.83	7.03	12.76	0.00	0.73	10.31	0.91	0.34	3.25	0.00	0.46	0.10	1.73	1.83	1.31	0.33	4.90	0.70	3.34	0.43	0.00	1.55	2.31
HM	R1100	5.54	0.32	19.80	2.42	5.19	12.64	5.79	0.64	9.35	0.64	0.23	4.06	0.34	0.22	0.00	0.83	3.11	1.33	0.10	3.10	0.84	3.31	0.18	0.79	1.99	1.70
HM	R1101	4.78	0.28	17.04	1.90	4.30	11.40	4.87	0.26	8.21	0.52	0.23	4.22	0.52	0.31	0.00	1.21	3.10	1.68	0.32	6.00	0.60	3.21	0.56	0.21	1.22	2.17
HM	R1103	4.06	0.29	17.25	1.82	4.45	11.49	4.35	0.26	8.55	0.56	0.28	3.86	0.41	0.23	0.00	0.95	2.59	1.61	0.27	6.43	0.65	3.41	0.75	0.00	1.64	2.43
HNM	AZ-2	3.66	0.30	16.78	0.99	5.90	14.25	0.60	0.55	9.70	0.50	0.00	2.21	0.28	0.40	0.12	1.06	4.77	0.83	0.09	2.35	1.78	1.81	0.00	0.00	4.73	3.49
HNM	CAL-1	4.94	0.33	13.53	0.39	8.77	14.13	0.00	1.16	11.51	0.33	0.15	0.31	0.27	0.30	0.00	1.21	2.93	0.59	0.25	6.33	2.25	1.24	0.68	3.85	0.73	1.66
HNM	CAL- 2	3.91	0.26	13.82	0.54	9.21	15.34	0.00	1.06	12.77	0.00	0.19	0.50	0.19	0.28	0.00	1.16	2.94	0.52	0.20	7.00	2.09	0.81	0.93	3.28	0.39	1.24

Metric parameters are shown in grey scale. The grey color represents a gradual intensity, where darker colors are assigned to larger values and lighter colors to smaller values. Each table from top to bottom matches each sampling before and after respectively. The Ilut groups are assigned 1–4; HM refers to hybrids mariola and HNM hybrids non-mariola. Values are represented as a percentage. C1, Santolina triene; C2, α-thujene; C3, α-pinene; C4, Camphene; C5, 2-thujene; C6, Sabinene; C8, β-E-Ocimene; C9, β-terpinene; C10, Limonene; C12, 2-methyl-2-bornene; C13, Sabinol; C18, Bornyl Acetate; C19, Linalyl Formate; C20, α-guainene; C21, α-cubene; C23, α-isocomene; C26, Caryophyllene; C27, Humelene; C28, Aromandrene; C30, Bicyclogermacrene; C31, δ-cadinene; C32, Elemol; C35, Spathulenol; C37, Viridiflorol; C38, γ-eudesmol; C40, β-eudesmol.

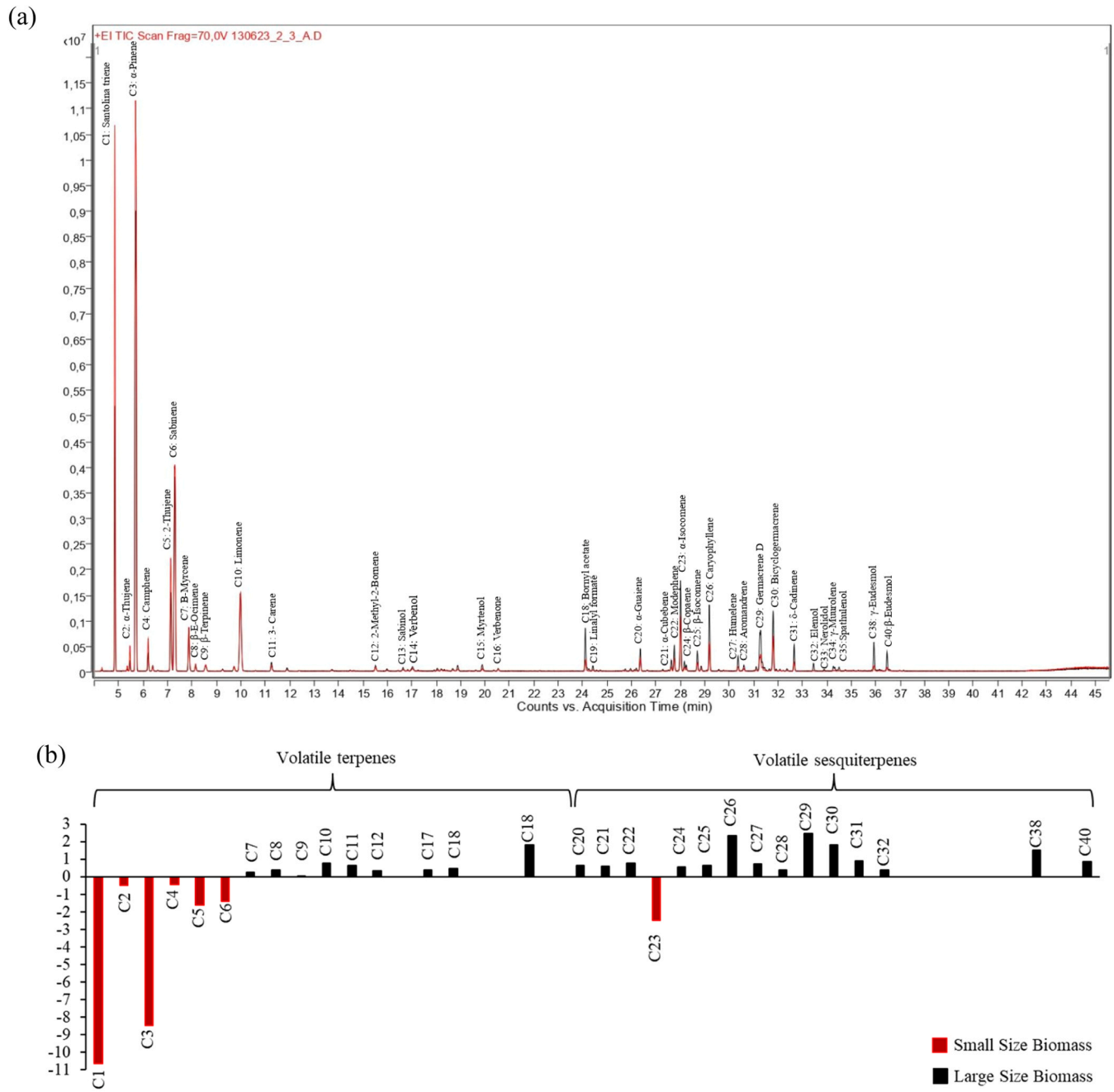


Fig. 6. (a) Essential oil volatile profile of guayule at S1 sampling, small and large size biomass industrially extracted, (b) Volatile content difference (% area) between both industrial essential oils.

tendency was an increase from June to July, although some accessions exhibited a decline in yield from July to August, and others showed an increase.

Of the guayule EOs, terpenes were the most abundant volatiles, followed by sesquiterpenes, oxygenated sesquiterpenes, and oxygenated terpenes. The EO profile was characteristic and related to genetic origin and harvesting date, allowing for differentiation between groups of accessions. Sesquiterpenes were more important for differentiation at the beginning of the season and monoterpenes were more useful in July and August. Some of the characteristic compounds in hybrids included the monoterpenes santolina triene (C1), β-E-ocimene (C8), and linalyl formate (C19), and the oxygenated sesquiterpene viridiflorol (C37).

Industrial exploitation of 1.5 Mg fresh guayule biomass ranged between 0.5% and 0.6% extraction yield depending on starting material, and showing a similar volatile profile as laboratory-scale EOs.

Further studies are required to set in which guayule extraction workflow, rubber or latex, the essential oils will be included.

Funding

This research was funded by the EU call HORIZON-CL6-2022-Circ-Bio-01-02 througth MIDAS project (Contract Number: 101082070). M. Mercedes García-Martínez acknowledges a grant for the requalification of the Spanish University System Modality Margarita Salas (2022-NACIONA-11053) funded by the European Union-NextGeneration EU through the Universidad de Castilla-La Mancha.

CRediT authorship contribution statement

Amaya Zalacain: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Investigation, Conceptualization. **Manuel Carmona:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Conceptualization. **M Engracia Carrión:** Writing – original draft, Validation, Investigation, Formal analysis. **Irene Mediavilla:** Investigation. **Luis Saúl Esteban:** Investigation. **Mercedes García-Martínez:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Formal analysis. **Emilio José González-Navarro:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis.

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.

Data Availability

Data will be made available on request.

Acknowledgements

The authors wish to thank Agroservicios Guayule S.L. and Nokian Tyres Plc. for their support in this research and to Dr. Kenneth McCreath for proofreading the manuscript.

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