# RESEARCH

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# Stability of guayulins from *Parthenium argentatum*, A. Gray during post-harvesting storage for industrial exploitation

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# Abstract

**Background** Guayulins comprise a family of sesquiterpene compounds with potential industrial applications that are extracted from the resinous fraction of the guayule plant (*Parthenium argentatum*, A. Gray). If a whole industry is to be developed around guayulins, not only their activity should be assessed, but also their stability because the quality of the final products is likely influenced by the time that elapses from harvesting to processing. Thus, the aim of the present study was to evaluate the stability of guayulin compounds from harvested guayule stems or extracted resin after storage at different temperatures to find in which form, stems or resin, is the storage better.

**Results** Results showed that, once extracted, the resin could be stored at 20-25 °C for 3 months or more without significant losses of guayulin content. In the case of harvested stems, however, the findings were more complex, with guayulins A and B degrading over time and guayulins C and D showing enrichment. In addition, analysis of the thermal and thermo-oxidative degradation of the resin and guayulins showed that while guayulins A and B showed a maximum decomposition rate around 280 °C, guayulins C and D decomposed at 245 °C. Such thermal differences might be attributed to the observed oxidation of guayulin A and B standards into guayulin C and D, respectively.

**Conclusion** These findings provide, for the first time, information on the stability of guayulins after harvesting and resin extraction, which could help to the development of an integral industrial process from harvesting to commercializing.

Keywords Guayule, Guayulins, Postharvest, Resin, Stability, Thermogravimetric analysis

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# Introduction

The profitability of guayule (*Parthenium argentatum*, A. Gray) cultivation will depend not only on the efficient production and processing of natural rubber, but also on the successful commercial exploitation of the many rubber processing byproducts, being the resin where the greatest expectations are placed due to its potential applications [1, 2]. Indeed, guayule resin contains a plethora of secondary metabolites with interesting biological activity, including monoterpenes and sesquiterpenes (essential oils), triterpenoids (argentatins), sesquiterpene esters (guayulins), organic acids (cinnamic, p-anisic, stearic, palmitic, oleic, linoleic, and linolenic), and polyphenols [1, 3].

The guayulin fraction (10-15% of the resin) is comprised of isoprenoids and aromatic acid esters, the *trans*cinnamic and *p*-anisic acid esters of partheniol (guayulins A and B) and hydroxyspathulenol (guayulins C and D), respectively [4–6]. There is accumulating evidence of the effectiveness of resin extracts against termites [7, 8], agricultural pests [9] or even as wood preservatives [10, 11]. Maatooq and Hoffmann [12] also reported fungistatic activity against *Aspergillius niger*.

The yield and composition of guayule resin varies with cultivation site, accession, age, or time of harvest [13], and it has also been suggested that resin extraction conditions strongly affect this composition [14]. Typically, the isolation/extraction of resin from guayule tissue is performed by solid–liquid extraction using organic solvents, with accelerated solvent extraction (ASE) being the most common technique at the analytical level [6, 15,

16]. Three methods (flotation, sequential or simultaneous extraction) have so far been described for the bulk extraction of rubber that includes obtaining the resin fraction [2, 14].

For any industrial-scale process of guayule agronomy to be successful, the loss of quality of the product, either during harvesting, transport, or processing of the plant material, must be minimized. Little is known about the best storage conditions for the stability of guayule byproducts, and only limited studies have examined rubber/latex stability. Some authors [17, 18] studied the degradation of rubber in harvested guayule stems, concluding that they cannot be stored at field temperatures (max 36 °C) without a loss of rubber quality. In the case of latex, Cornish et al. [17] showed that harvested guayule branches could be stored at 4 °C in an antioxidant solution for at least 2 weeks without compromising latex yield, provided that dehydration does not occur. Cornish et al. [18] went a step further and analyzed the latex content and quality in guayule homogenates at a longer period (24 weeks), finding that guayule homogenate is a stable environment for at least 13-16 weeks. McMahan et al. [19] analyzed latex quality up to 28 days, proving that storage conditions affect latex quality and that moisture extends the storage time without quality loss. Only Coffelt et al. [16] studied the effect of wet and dry storage conditions for up to 24 days in harvested guayule shrubs on resin and guayulins A and B, in addition to latex and rubber. No consistent storage treatment effects were found for rubber, resin and guayulin B concentration or yield. In the case of guayulin A, however, the wet storage

treatments resulted in a generally higher content than the other storage treatments.

As the immediate processing of guayule shrubs on a commercial scale is often not feasible and manufactured products are not immediately used, if an industry is to be developed around resins and guayulins, it will be necessary to establish the most appropriate storage conditions to maintain their concentration pre- and post their extraction. The objective of the present study was therefore to evaluate the stability of guayulins in guayule stems stored at room temperature, and in extracted resin kept at different storage temperatures to assess the sustainability of the potential extraction industry. Secondly, the stability of guayulin standards submitted to thermal analysis was also studied to clarify their behavior during harvested stem and resin storage.

## **Results and discussion**

## Resin and guayulin stability in harvested stems

The harvested guayule stems were kept in the dark at room temperature until processing, and the resin fraction and guayulin content were analyzed periodically throughout the 6-month study period (Table 1). Of the three accessions studied, AZ-2 showed the highest resin content at baseline (T0; 12.27%), followed by R1040 (10.14%), and CAL-1 (9.25%). The analysis revealed no significant differences in the resin content for R1040 throughout the study. By contrast, CAL-1 exhibited a loss of resin content of 11% at 6 months, reaching a yield of 8.23%. For AZ-2, the resin content remained stable until day 42 and then decreased until the end of the experiment (day 185), yielding a 23% loss. Surprisingly, these differential effects were not observed upon quantification of guayulins, as a clear storage degradation effect was observed for all three accessions, independently of the initial content.

Contrary to what was expected, the percentage of resin yield and total guayulin content were not related. Accession R1040 showed the highest guayulin content at T0, followed by CAL-1 and AZ-2. A two-step effect was observed for accessions AZ-2 and CAL-1, whereby the total guayulin content was stable for the first 14 days, followed by a significant decrease of 30% at 42 days after harvesting, followed by a loss higher than 4% (AZ-2) and 30% (CAL-1) after 117 days, after which it remained constant. For R1040, guayulin content was reduced by 26% at 42 days after harvesting. It was not possible to continue with the analysis of R1040 throughout the study period as the sample was exhausted.

Not surprisingly, the guayulin profile depended on the accession: AZ-2 and CAL-1 had the same gA > gB > gC > gD profile, whereas the R1040 profile was gA > gC > gB > gD. In addition, R1040 had more than double the content of gA and gC than AZ-2 and CAL-1, which were similar, whereas the content of gB and gD was similar in all three accessions.

In terms of stability along the study period, CAL-1 and AZ-2 behaved similarly, with gA and gB decreasing around 90% over time, whereas gC and gD increased to about 200% and 130% for CAL-1 and AZ-2, respectively (Fig. 1). Guayulin degradation was less marked for R1040, as gA and gB decreased around 64% and gC and gD increased by 84% and 58%, respectively. Some authors have postulated that gC and gD are likely formed by the oxidation of gA and gB, respectively [5, 6], which is in accord with the present results; that is, a decrease in gA and gB concomitant with an increase in gC and gD.

In terms of evolution, significant changes were observed after 14 or 42 days of storage depending on the guayulin component studied and the accession (Fig. 1). For example, the loss of gA content started earlier in CAL-1, and the loss of gB started later in R1040. Both

**Table 1** Resin yield and total guayulin evolution over the six-month study period for stems of three guayule accessions (AZ-2, CAL-1, and R1040)

Time (days)	AZ-2		CAL-1		R1040	
	Resin yield (%)	gT (mg/g resin)	Resin yield (%)	gT (mg/g resin)	Resin yield (%)	gT (mg/g resin)
0	12.27 d	51.38 bc	9.25 c	56.30 c	10.14 a	98.72 b
14	11.14 cd	52.79 c	7.34 a	53.91 c	10.19 a	98.20 b
42	12.09 d	35.44a	9.65 c	39.34 b	10.17 a	72.87 a
73	10.22 abc	40.47 ab	7.79 a	41.50 b	10.61 a	79.38 a
117	9.80 ab	33.89 a	9.10 bc	27.39 a	10.51 a	66.18 a
150	10.60 bc	30.22 a	9.63 c	27.29 a	-	-
185	9.43 a	30.25 a	8.23 ab	25.50 a	-	_

Different letters within columns indicate significant differences at 95% level of confidence

gT total guayulins

80

70

60

50

40

30

20





→ AZ-2 → CAL-1 → R1040

Fig. 1 Individual guayulin evolution along six months for three guayule harvested stem accessions (AZ-2, CAL-1, and R1040). Different letters for each guayulin and accession indicate significant differences at the 95% confidence level.

guayulins stabilized at 117 days. Contrastingly, for gC and gD content significant changes were observed from 42 to 73 days, after which they remained stable.

In summary, plants can be stored without the need for extracting guayulins for up to 14 days after harvest for CAL-1, and 42 days for AZ-2 and R1040. The only previous study with which the results obtained can be compared is that of Coffelt et al. [16], but they did not find consistent effects of storage treatments, and although they analyzed two different guayule accessions (one of them AZ-2 as in the present study), no clear differences were established between them. The difference with AZ-2 could be explained by the short duration of the trial (up to 28 days versus our 6-month study), as in fact, we observed that significant changes in resin content started from day 72, and in guayulin content from day 42.

# Guayulin stability in extracted resin

Given the results showing the loss of total guayulin content and changes to the guayulin profile when the harvested plants were stored at room temperature prior to industrial extraction, the stability of the guayulins in the extracted resin was next examined when stored at three different temperatures (4 °C, 20 °C and 40 °C). When stored at 4 °C, the concentration of guayulins in AZ-5 remained constant across the three months of the study, both for total and for individual guayulin content (Table 2). When the resin was stored at 20 °C, total guayulin content also remained constant up to 60 days, after which time a small decrease in gA and gB content was observed with a concomitant increase in gC and gD content. As the total content of guavulins remained constant, it is likely that the increase in polar guayulins is proportional to the reduction in apolar guayulins. Finally, when the resin was stored at 40 °C, a progressive decrease in gA and gB content was observed after 5 days, accompanied by a progressive increase of gC and gD, which was more evident as time evolved. However, total guayulin content remained constant until 60 days, at which time gA and gB decreased significantly.

The results show that in terms of guayulins, the extracted resin remains stable for longer than the resin in the stored guayule plant. Specifically, gA and gB were well preserved when the resin was stored at 4 °C or 20 °C for 60 days (Table 2), but with a significant loss of 3% observed at the latter temperature.

Contrastingly, when resin was stored at 40 °C the content of gA decreased progressively after 7 days; the

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Time (Days)	Guayulin A	(mg/g resir	(د	Guayuli	in B (mg/g n	esin)	Guayulin C	. (mg/g resin)		Guayulin	D (mg/g re:	sin)	Total Guay	yulins (mg/g	resin)
	4 °C	20 °C	40 °C	4 °C	20 °C	40 °C	4 °C	20 °C	40 °C	4 °C	20 °C	40 °C	4 °C	20 °C	40 °C
0	20.34 bc	20.34 b	20.34 ef	7.14 a	7.14 abc	7.14 cde	20.35 abc	20.35 a	20.35 a	8.83 abc	8.83 a	8.83 ab	56.66 ab	56.66 ab	56.66 bc
<del>, -</del>	20.03 abc	20.17 b	20.16 def	7.04 a	6.94 a	7.09 cde	20.20 abc	20.38 a	20.31 a	8.69 ab	8.84 a	8.81 ab	55.96 ab	56.33 a	56.38 bc
2	19.83 a	20.09 ab	20.17 def	7.04 a	7.15 abc	7.18 de	20.00 ab	20.53 ab	20.33 a	8.69 ab	8.85 a	8.99 ab	55.56 a	56.63 ab	56.67 bc
m	20.18 abc	20.10 ab	20.02 def	7.12 a	7.14 abc	6.97 cd	20.45 abc	20.52 ab	20.09 a	8.97 bc	8.91 a	8.70 a	56.73 ab	56.66 ab	55.78 bc
5	20.12 abc	20.18 b	19.97 de	7.05 a	7.12 abc	7.06 cde	19.98 a	20.40 a	20.51 ab	8.77 abc	8.87 a	9.01 b	55.92 ab	56.57 ab	56.55 bc
7	20.02 abc	20.34 b	19.80 d	7.03 a	7.14 abc	6.96 cd	20.21 abc	20.64 abc	20.42 ab	8.67 ab	9.03 ab	9.00 ab	55.93 ab	57.14 ab	56.18 bc
13	20.37 bc	20.29 b	19.70 d	7.07 a	7.17 abc	7.00 cde	20.61 c	20.70 abc	21.01 bc	8.83 abc	9.08 ab	9.35 c	56.89 ab	57.24 ab	57.07 с
23	20.47 c	20.25 b	19.10 c	7.21 a	7.16 abc	6.91 c	20.58 abc	20.85 abc	21.25 c	8.92 abc	9.11 ab	9.44 c	57.18 b	57.36 ab	56.70 bc
40	20.33 bc	20.26 b	18.20 b	7.15 a	7.16 abc	6.64 b	20.54 abc	20.85 abc	21.30 c	8.91 abc	9.09 ab	9.45 c	56.94 ab	57.36 ab	55.59 b
60	20.40 bc	20.11 ab	17.78 b	7.18 a	7.18 abc	6.63 b	20.43 abc	21.20 c	21.49 c	8.95 abc	9.31 b	9.65 c	56.96 ab	57.79 b	56.93 bc
06	20.40 bc	19.65 a	15.53 a	7.17 a	6.96 ab	5.89 a	20.51 abc	21.13 bc	21.28 с	9.01 c	9.27 b	9.61 c	57.08 b	57.01 ab	52.30 a
Different letters	for each guayu	lin and tempe	stature indicate	significant	differences at	t the 95% con	fidence level								

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content of gB was also preserved better at 4 °C and 20 °C without differences between the two temperatures and decreased progressively at 40 °C from 23 days onwards. Thus, gB appeared slightly more stable than gA. As expected, the content of gC started increasing at 40 °C from 23 to 60 days. At this time gC became more sensitive to temperature, with differences in its content at the three temperatures tested. The lower content was usually found at lower temperatures. The same behavior was observed for gD, although in this case it increased earlier, from 7 to 60 days. Thus, concerning the storage time and temperature, it could be concluded that guayulins can be well preserved for 90 days at 4 °C, 60 days at 20 °C, and 13 days at 40 °C.

This is the first time that the stability of guayulins is assessed once the resin is extracted, so it is not possible to establish a comparison with previous research.

#### Thermal Behavior of guayulin standards

In both stability tests, either with the harvested stem or with the resin, it was evident that the stability of gA and gB is poorer than gC and gD, because as the former decreased the latter increased. As mentioned earlier, some authors [4-6] have suggested a relationship between them, such that gA and gB might oxidize to gC and gD, respectively. The transformation of one into the other was not equimolar. When considering the moles of gA+gC per gram of resin with respect to the original ones, a consistent loss of aromatic acid functionality was observed (Fig. 2). The overall levels of cinnamate and p-anisate decrease, approaching a net 40-50% loss over the period of the study. In other words, the formation of new gC and gD does not keep up with the oxidation or other degradation of gA and gB. These results are similar to those obtained by other authors [6]. The use of high temperature (>150 °C) to remove the solvent increased the proportion of gC and gD, but with an overall reduction in the total sesquiterpene ester content.



Fig. 2 Relative concentrations of total resin chromophores over time

To test how this process can be explained, guayule resin (AZ-5) and gA, gB, gC and gD standards were subjected to thermal analysis from 25 to 450 °C. The guayule resin response on the TGA curve (Fig. 3) revealed a sample weight loss of 25% up to 130 °C, likely due to the evaporation of the acetone used for extraction and the guayule essential oils volatile at that temperature [2].

This is supported by the endothermic peaks on the DTA curve around that temperature where the sample absorbed a great amount of energy. A second effect was observed between 140 °C and 160 °C, whereby the sample released some energy, indicating a possible sample oxidation, and no mass loss was observed. As the temperature increased, there was a progressive loss of mass until 425 °C, at which point there was a new endothermic peak with complete sample degradation.

To explain what occurs around 150 °C, the four guayulin standards (gA, gB, gC and gD) isolated in our laboratory were submitted to the same thermal analysis process (25 to 450 °C), which revealed interesting information about their thermal behavior and stability. The gA standard had a crystalline appearance and melted at 130 °C. It showed the same oxidation pattern



Fig. 3 Thermogravimetric analysis of guayule resin



at 140 °C as that of the raw resin, with no mass loss, and started to decompose from 210 °C reaching a maximum at 247 °C (Fig. 4a). Oxidative and non-oxidative reactions that become prominent above about 200 °C, may be Cope-type rearrangements such as those reported by Takeda et al. [20] for bicyclogermacrene, the parent sesquiterpene hydrocarbon of partheniol. The standard was also submitted to DSC analysis from 25 to 150 °C (40 min), followed by a cool-down to 25 °C, and then a reheat to 150 °C (Fig. 4b). The DSC plot revealed an endothermic peak at 130 °C corresponding to the melting point of gA, which demonstrates that it is crystalline.

Nevertheless, after heating to 150 °C the sample did not crystallize during cooling and exhibited a gel-like consistency, which was recovered and dissolved in ethanol for analysis by HPLC–DAD (Fig. 5). Results showed that the purity of the gA standard decreased from 98 to 79% but, more importantly, gC appeared in the chromatogram with a purity of 14%. Another assay was performed in which the gA standard was subjected to 150 °C for 24 h and then analyzed chromatographically. It was also observed that gA transformed into gC, which corroborates the suggestions of some studies [4–6], probably following the same oxidative rearrangement that takes place to convert bicyclogermacrene to spathulenol [21].



Fig. 4 Guayulin A thermogravimetric analysis a TG/DTA response in air and nitrogen atmosphere, b scanning calorimetry (DSC) analysis



Fig. 5 HPLC–DAD analysis of guayulin A standard (gA). a Control, b after thermogravimetric analysis, c gA heated at 150 °C for 24 h

The gC standard had a gel-like appearance, not crystalline as gA, that needed to be suspended in ethanol prior to the TG analysis. The thermal analysis revealed a large endothermic peak around 100 °C corresponding to the solvent evaporation. The gC standard appeared stable until 280 °C, at which point the decomposition rate was maximum (derivative thermogravimetric data not shown; DTG). The gC standard was heated to

150 °C during 24 h and then analyzed by chromatography, revealing that the gC standard was stable (Fig. 6a).

The gB standard was also tested in the same manner, which revealed its transformation into gD (Fig. 7), corroborating the suggested oxidation [5, 6]. The temperature at which the gB standard showed the maximum decomposition rate was 245 °C (DTG data), very similar to the gA decomposition. In the case of the gD standard, it.

Although the stability tests of guayulins were not performed at temperatures as high as 150 °C, as they will not be usual storage temperatures, it is likely that after the necessary time, the conversion of gA and gB into gC and gD can occur.

# Conclusions

Guayulin stability is highly influenced by storage both in plant material and in the resin. The stability of gA and gB is less than that of gC and gD, as while the former



Fig. 6 HPLC–DAD analysis of a guayulin C standard (gC) and b guayulin D standard (gD) heated at 150 °C for 24 h



Fig. 7 HPLC–DAD analysis of guayulin B standard (gB). a Control, b gB heated at 150 °C for 24 h

decreased, the latter increased over storage time, both in harvested stems and in extracted resin. However, guayulins are more stable in the extracted resin than in harvested stems. In harvested stems, both the resin and guayulin content decrease over time, as well as the guayulin profile changes, from the very beginning of the study, accession dependent. In extracted resin, guayulin concentration is quite stable, especially when kept at lower temperatures (4 °C and 20–25 °C) for at least 3 months. Even if the resin is kept at 40 °C, guayulin loss is not significant until 2 months, although it is seen an important change in guayulin profile.

By thermal degradation analysis, it was observed that the gA standard with a crystalline aspect clearly oxidize into gC (gel-like appearance) at 150 °C. The changes in guayulin profiles in harvested stems and extracted resin are thus likely produced by oxidative processes that are occur at higher rates at high temperatures.

These findings will be very useful in the development of an integrated industrial method that starts from the cultivation of the plant to the commercialization and final use of the guayulins, which would help to make guayule profitable.

## **Materials and methods**

# Guayule samples

Stems of four guayule accessions (CAL-1, AZ-2, R1040, and AZ-5) were sampled from an experimental plot in Santa Cruz de la Zarza (Toledo, Spain) in April 2021. Plants were dried for 48 h at 60 °C to achieve a moisture content < 12%. The leaves were then manually removed from the stems, which were then cut into pieces of about 1 cm in length using a manual cutter and ground in a two-step process immediately before starting the study: in the first step, a particle size of 2 mm was obtained using a hammer grinder (Mader 57075, Mealhada, Portugal); in the second step, the material was grounded to a particle size of 0.5 mm using a centrifuge grinder (Retsch ZM 1000, Haan, Germany).

# **Resin extraction**

# Stability analysis of guayulins in harvested stems

Resin from the ground stems of the CAL-1, AZ-2 hybrids and the pure R1040 accession stored (20 g) in Falcon tubes sealed with parafilm at room temperature was periodically extracted and analyzed throughout the 6-month study period using the ASE E-914 extractor (BUCHI, Postfach, Switzerland), as described by Rozalén et al. [15]. Briefly, a representative sample of  $1\pm 0.005$  g of guayule stem obtained by homogenization of the 20 g ground sample was extracted with acetone using the following conditions to obtain the resin fraction: pressure 100 bar, temperature 40 °C, and three extraction cycles

of 10/20/30 min, with heat-up 1 min, discharge 3 min, flush with solvent 2 min, and flush with nitrogen 5 min. Resin extracts were collected and evaporated in a parallel solvent evaporation system (Multivapor BUCHI P-6; Postfach, Switzerland) at 150 mbar and 50 °C. After evaporation, the pre-weighed flasks containing the resin were stored for 60 min in a desiccator before final weighing. The resin percentage was then determined gravimetrically considering the dry weight. Each sample was extracted twice and analyzed later by HPLC–DAD.

## Stability study of guayulins from guayule resin

A bulk resin extraction was performed using the AZ-5 accession by mixing a sample of  $500 \pm 0.01$  g of guayule with 2.5 L of acetone in a flask. The mixture was heated using a flask-heating mantle (6000-mL flask capacity, model 313-500; JP Selecta, Barcelona, Spain) for 45 min to achieve boiling [14]. The extraction was carried out using reflux to prevent solvent loss from evaporation. After extraction, the resin was vacuum filtrated to remove any remaining rubber or other smaller solid residues, and then decanted overnight. Acetone evaporation was performed in a Laborota 4000 Evaporator (Heidolph Instruments, Schwabach, Germany) at 210 mbar and 50 °C. The percentage of resin was then determined gravimetrically as above mentioned.

Two samples of 20 mL of resin per treatment were kept in opaque glass bottles (30 mL) with screw caps and sealed with parafilm without atmosphere modification and storage at the following different temperatures in the dark:  $4 \,^{\circ}$ C, 20  $^{\circ}$ C (room temperature) and 40  $^{\circ}$ C. Samples were periodically analyzed for three months to evaluate resin and guayulins stability by HPLC–DAD.

#### Guayulin quantification by HPLC-DAD

Guayulin analysis was performed according to Rozalén et al. [22]. Briefly, 20 mg of resin dissolved in ethanol at 10 mg/mL and filtered (0.22  $\mu$ m) were injected into an Agilent 1200 high-performance liquid chromatography (HPLC) system (Agilent Technologies, Palo Alto, CA) equipped with a diode array detector (DAD) (Agilent Technologies, G1315D). Separation was performed on a reverse-phase ACE Excel 3 C18-PentaFluorPhenyl (PFP) column ( $150 \times 4.6$  mm, 3 µm particle size) protected with an ACE Excel HPLC Pre-column Filter (0.5 µm particle size) (both from Advanced Chromatography Technologies Ltd., Reading, Berkshire, UK). The solvents were Milli Q-grade water (solvent A) and acetonitrile (solvent B). The elution gradient for solvent B was as follows: 0 min, 60%; 10 min, 60%; 20 min, 80% and hold 5 min; 35 min, 100% and hold 2 min. Guayulins were identified by their characteristic UV absorption spectra (maximum at 256 nm for guayulins B and D, maximum at 276–278 nm for guayulins A and C) and retention time [17]. Agilent ChemStation software (version B.03.01) was used for the quantification of the guayulins using a guayulin A standard five level calibration curve (2.5–250 mg/ L,  $r^2$ =0.9997).

Guayulins standards were isolated in the laboratory due to the lack of commercial standards. Resin was fractionated using flash chromatography (VersaFlash Station System I equipped with a Versaflash cartridge, 23 mm×110 mm, containing Spherical C18 bonded silica 30 g, 20–45  $\mu$ m) with acetonitrile:water (80:20) as solvent, and a flow rate of 20 cm<sup>3</sup> min.<sup>-1</sup>. The corresponding fraction for each guayulin was evaporated under vacuum and crystallized from hexane/chloroform as described by Martínez et al. [5]. Molecular weight and mass fragmentation patterns were confirmed by high-performance liquid chromatography coupled to mass spectrometry (LC–MS) (data not shown). [5]

## Thermogravimetric analysis (TGA)

Approximately 20 mg of guayule resin, and guayulin A, B, C and D standards were sampled for TGA using a TGA/differential thermal analysis (DTA) platform (TG/DTA 6200, model SII apparatus, Seiko Instruments Inc., Tokyo, Japan) and subjected to progressive heating at 10 °C/min from 30 to 500 °C. Tests were carried out under synthetic air atmosphere consisting of pure oxygen (20%), pure nitrogen (80%) and no impurities, with a flow of 50 mL/min. Data were gathered every 0.1 s.

The guayulin A standard was also used for differential scanning calorimetry (DSC) analysis using settings of 150 °C for 40 min, cooling down to 25 °C, and reheating to 150 °C, to evaluate its crystallization behavior.

# **Data analysis**

Statistical analysis was performed with IBM SPSS Statistics for Windows (Version 25.0. Armonk, NY: IBM Corp., 2017). Analysis of the variance (ANOVA) and Tukey's test were used to compare the evolution in time (0, 14, 42, 73, 117, 150, and 185 days) of both resin and guayulins (individual gA, gB, gC and gD, and total gT) in stems of three accessions (CAL-1, AZ-2, and R1040) stored at room temperature. Multivariate analysis of the variance (MANOVA) was used to analyze guayulin stability in the extracted resin of accession AZ-5. Significant differences at 95% confidence interval were established by comparing means in all cases.

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#### Author contributions

GL and MMG-M. carried out the experiments and made the first figures and text draft. JC was in charge of thermal analysis. MEC. made the statistical analysis. AZ designed the methodology, validated the results and revised the manuscript. MC supervised the experiments and was in charge of final review and editing of text and figures.

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#### Availability of data and materials

The datasets used during the current study are available from the corresponding author on reasonable request.

#### Declarations

**Ethics approval and consent to participate** Not applicable.

#### **Consent for publication**

Not applicable.

#### **Competing interests**

The authors declare that they have no competing interests.

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