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UNVEILING PINE RESIN CHEMISTRY

DESVENDANDO A QUÍMICA DA RESINA DE PINHEIRO

REVELANDO LA QUÍMICA DE LA RESINA DE PINO

https://doi.org/10.56238/sevened2025.036-145

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ABSTRACT

Pine resins, mostly the ones provided by Pinus elliottii, are known to present interesting activities in biological systems and are commonly treated as promising source of active compounds. Due to our interest in Natural Product chemistry and in diterpenes, also known to be pine resins components, we developed this work about the chemical composition of those resins. The essential oil composition showed the presence mostly of □- and □- pinene, □-phelandrene and □-cimene. The non-volatile fractions showed dehydroabietic acid as the primary component and revealed other abietane diterpenes were less abundant. This study revealed that these resins are surely good sources of dehydroabietic acid for further chemical studies with this natural product.

Keywords: *Pinus elliottii*. Pine Resins. Diterpenes. Chemical Composition. Monoterpenes.

RESUMO

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As resinas de pinheiro, principalmente aquelas provenientes de Pinus elliottii, são conhecidas por apresentar atividades interessantes em sistemas biológicos e são comumente tratadas como fontes promissoras de compostos ativos. Em função do nosso interesse na Química de Produtos Naturais e nos diterpenos, também conhecidos como componentes das resinas de pinheiro, desenvolvemos este trabalho sobre a composição química dessas resinas. A composição do óleo essencial mostrou predominantemente a presença de α- e β-pineno, β-felandreno e p-cimeno. As frações não voláteis apresentaram o ácido desidroabiético como componente principal e revelaram que outros diterpenos do tipo abietano estão presentes em menor abundância. Este estudo demonstrou que essas resinas são, de fato, boas fontes de ácido desidroabiético para futuros estudos químicos com esse produto natural.

Palavras-chave: *Pinus elliottii*. Resinas de Pinheiro. Diterpenos. Composição Química. Monoterpenos.

RESUMEN
Las resinas de pino, principalmente las provenientes de Pinus elliottii, son conocidas por presentar actividades interesantes en sistemas biológicos y son comúnmente consideradas como fuentes prometedoras de compuestos activos. Debido a nuestro interés en la Química de Productos Naturales y en los diterpenos, también conocidos como componentes de las resinas de pino, desarrollamos este trabajo sobre la composición química de dichas resinas. La composición del aceite esencial mostró predominantemente la presencia de α- y β-pineno, β-felandreno y p-cimeno. Las fracciones no volátiles presentaron al ácido deshidroabiético como componente principal y revelaron que otros diterpenos del tipo abietano se encuentran en menor abundancia. Este estudio demostró que estas resinas son, sin duda, buenas fuentes de ácido deshidroabiético para futuros estudios químicos con este producto natural.

Palabras clave: *Pinus elliottii*. Resinas de Pino. Diterpenos. Composición Química. Monoterpenos.

1 ABOUT PINE RESINS

Pinus is a coniferous genus in the family Pinaceae, characterized by rapid growth, tolerance to low temperatures, and the ability to develop in nutrient-poor soils (Figure 1) (Correa *et al.,* 2018). In general, species of this genus are widely distributed and occur in several countries, including China, the United States, and India, while in Brazil they are predominantly found in subtropical regions (Gonçalves *et al.*, 2018).

Figure 1

Conifer of the genus Pinus

Source: http://www.mundoreal.xyz/arvores-brasileira-o-pinus-elliottii/grande-pinus-elliottii/

A notable feature of *Pinus* species is the production of a resin, a viscous yellow to brown liquid with a strong odor (Figure 2). This exudate comprises a volatile fraction, known as turpentine, which contains monoterpenes and sesquiterpenes, and a non-volatile fraction, commonly referred to as resin or pitch, in which diterpenes are predominant. Upon attack by pathogens or herbivores, these trees activate a chemical defense mechanism based on the secretion of this resin, which contributes to inhibiting or limiting the action of invaders through a diverse repertoire of specialized metabolites (Keeling; Bohmann, 2006).

Figure 2

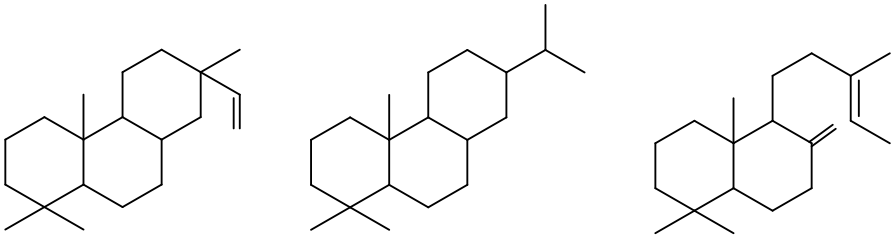
Pinus resin collection

Source: https://tecflora.com.br/2018/03/23/resinagem-revista-campo-e-negocios/

Several phytochemical studies have reported that *Pinus* resins contain a mixture of monoterpenes, tricyclic diterpenic acids, mainly from the abietane and pimarane classes, and bicyclic diterpenes from related structural classes, as illustrated in Figure 3. In some cases, sesquiterpenes are also detected among the resin constituents (Rezzi *et al.,* 2002; Silva *et al.,* 2014). Among the diterpenes obtained from *Pinus* genus, dehydroabietic acid is one of main substances due to its promising antibacterial, insecticidal, antifungal, anti-aging, antiprotozoal, and anti-inflammatory properties (Soares *et al.*, 2019; Berger *et al.*, 2017; Fonseca *et al*., 2004; Gao *et al*., 2015; Kim *et al.*, 2015; Dembitsky *et al.*, 2021). It is a tricyclic aromatic diterpenoid characterized by three fused rings, three chiral carbon centers, and a reactive carboxylic acid functional group (Lv *et al.*, 2025).

Figure 3

Main chemical skeletons found in isolated diterpenes of the genus Pinus



Pinus resins constitute a valuable source of natural products with diverse applications and significant economic relevance. They are employed in cosmetic fragrances, as flavoring additives in foods, and as intermediates in the synthesis of fragrance chemicals, among other industrial uses (Leandro *et al.,* 2014).

In addition, *Pinus* resins exhibit a broad spectrum of biological activities, including anti-inflammatory and antifungal effects (Boeck *et al.*, 2005), as well as antimicrobial (Tanaka *et al.*, 2008; Fallarelo *et al.*, 2013) and anti-allergic properties (Gonçalves *et al.*, 2018). Considering the structural diversity of diterpenes present in these resins and this documented bioactivity profile, *Pinus* resins represent a promising source of potentially active compounds that may play an important role in this study, particularly in the search for molecules with relevant biological properties.

3 EXTRACTION AND PURIFICATION OF *Pinus* RESIN CHEMICALS

3.1 PLANT MATERIAL AND EXPERIMENTAL ANALYSES

Several methods have been described in the literature for the isolation of metabolites from *Pinus* resins. In the following sections of this chapter, the logical sequence and the results obtained by our research group in studies of Pinus oleoresins will be presented. Some *Pinus* resin sources are available from commercial suppliers. In Brazil, these resins can be obtained from the company Tecflora, which provides material from different *Pinus* species, such as:

1. *Pinus elliotti* from Buri, São Paulo-SP;
2. *Pinus caribaea* x *Pinus elliotti,* from Buri, São Paulo-SP;
3. *Pinus caribaeai,* from Cajuri, Minas Gerais-MG.

The metabolites were separated using vacuum liquid chromatography (VLC) and classical column chromatography (CCC) in glass columns, depending on the amount of sample available, employing silica gel 60, 70–230 mesh (0.063–0.200 mm), and silica gel 60H, 70–230 mesh (0.040–0.063 mm) (Merck®) as stationary phases. The ^{1}H nuclear magnetic resonance (NMR) spectra were acquired on a Bruker® AVANCE DRX 400 spectrometer operating at 400 MHz. All experiments were conducted in the Department of Chemistry of the Faculty of Sciences, Philosophy and Letters of Ribeirão Preto, at University of São Paulo (USP).

The identification methods included irradiation under ultraviolet (UV) light and comparative thin-layer chromatography (TLC) on Merck® aluminum plates coated with silica gel 60 GF254 (0.25 mm), with visualization using vanillin sulfuric acid reagent followed by heating. High-performance liquid chromatography (HPLC) analyses were performed on a Shimadzu Prominence system (CBM-20A/LC-6AD) equipped with a manual injector, DGU-20A5 degasser, SPD-20A diode array detector (DAD), and a microcomputer running LCsolution software for data acquisition and processing. The chromatographic separations were carried out on a Shimadzu C18 reversed-phase column (4.6 mm × 250 mm, 5 µm) with a 20 µL injection loop, using acetonitrile/water (ACN/H_2O, 9:1) as the mobile phase at a flow rate of 1.0 mL min^{-1}. HPLC analyses employed HPLC-grade reagents from J. T. Baker® and ultrapure water obtained from a Milli-Q® system. The solvents used for isolation, purification, and as reaction media were ethyl acetate (EtOAc), hexane (Hex), dichloromethane (DCM), methanol (MeOH), and ethanol (EtOH), all analytical grade (P.A.).

3.2 COMPARATIVE STUDY OF *PINUS* RESINS BY HPLC

The resins were coded as follows: RP-1 (resin from sample 1), RP-2 (resin from sample 2), and RP-3 (resin from sample 3). These resins consist of a white solid mass impregnated with a light-yellow oily fraction. Samples from the three *Pinus* resins were analyzed by HPLC to identify and compare their major constituents in both the oily fraction and the solid mass; notably, RP-3, being a highly dry resin, lacked the oily fraction. Thus, the solid masses and oily fractions described below were evaluated.

- Resin 1 (mass): RP-1M; Resin 1 (oil): RP-1O.
- Resin 2 (mass): RP-2M; Resin 2 (oil): RP-2O.
- Resin 3 (mass): RP-3M; (does not contain the "oil" part).

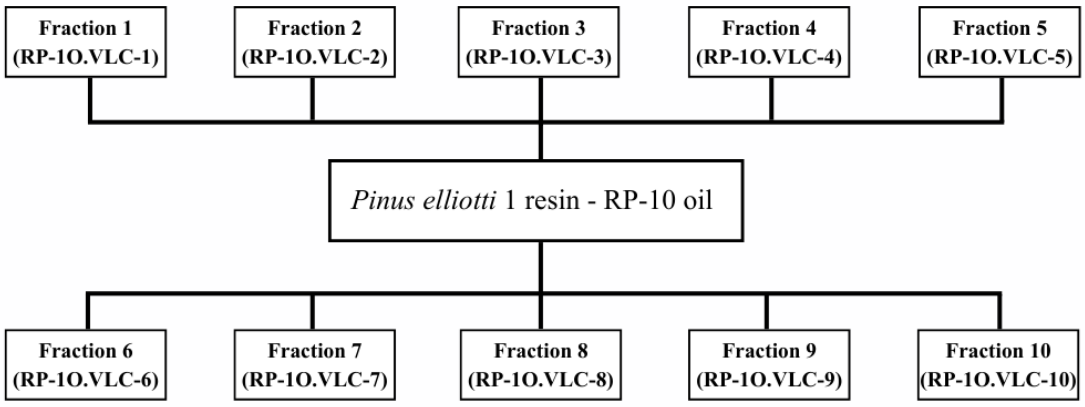
3.3 ISOLATION AND PURIFICATION OF DEHYDROABIETIC ACID (DI) FROM *PINUS ELLIOTTII* RESIN

The fractionation of RP-1O was done by VLC. In this case, it was carried out only with the "oil" part and the following fractions were generated, which were thus coded, as shown in figure 4.

- Fraction 1 - Hex: RP-1O.
- Fraction 2 - 9:1 (Hex/EtOAc): RP-1O.
- Fraction 3 - 8:2 (Hex/EtOAc): RP-1O.
- Fraction 4 - 7:3 (Hex/EtOAc): RP-1O.
- Fraction 5 - 6:4 (Hex/EtOAc): RP-1O.
- Fraction 6 - 1:1 (Hex/EtOAc): RP-1O.CLV-6.
- Fraction 7 - 4:6 (Hex/EtOAc): RP-1O.
- Fraction 8 - 3:7 (Hex/EtOAc): RP-1O.
- Fraction 9 - EtOAc: RP-1. LVC -9.
- Fraction 10 - EtOH: RP-1O. LVC -10.

Figure 4

Flowchart of the coding of fractions obtained by VLC from Pinus elliottii resin



The isolation and purification of the chemical constituents from *Pinus elliottii* resin were performed starting from 50.0 g of crude material. The procedure began with a VLC separation (Pelletier *et al.*, 1986), using 240.0 g of silica gel 60 and 240.0 g of silica gel 60H as stationary phases. The column was packed under vacuum with hexane as the initial solvent, and the sample was adsorbed onto 77.6 g of silica gel 60 prior to loading. During

the chromatographic run, ten fractions were collected, using an elution gradient of increasing polarity as summarized in Table 1.

Table 1

Elution scheme used in RP-1O fractionation

Solvent used	Volume collected (mL)	Fraction	Mass obtained (g)
Hex	500	RP-1O.VLC-1	0.018
Hex/EtOAc 9:1	500	RP-1O.VLC-2	0.720
Hex/EtOAc 8:2	500	RP-1O.VLC-3	0.960
Hex/EtOAc 7:3	500	RP-1O.VLC-4	41.450
Hex/EtOAc 6:4	500	RP-1O.VLC-5	0.550
Hex/EtOAc 1:1	500	RP-1O.VLC-6	1.430
Hex/EtOAc 4:6	500	RP-1O.VLC-7	2.000
Hex/EtOAc 3:7	500	RP-1O.VLC-8	0.990
EtOAc	500	RP-1O.VLC-9	0.460
EtOH	500	RP-1O.VLC-10	0.340

Hex: hexane; EtOAc: ethyl acetate; EtOH: ethanol

All fractions obtained from this procedure were analyzed through CTLC using as mobile phase a mixture of Hex/EtOAc (8:2). In this analysis, it was possible to observe that the fractions RP-1O.CLV-3 and RP-1O.CLV-4 had a similar chemical profile and therefore were selected to continue the isolation.

3.4 FRACTION RP-1O.VLC-3

With a portion of this fraction (0.96 g), a CCC separation was carried out using 25.0 g of silica gel 60 and 1.0 g of silica gel 60H, with a mixture of hexane/ethyl acetate (8.5:1.5) as the mobile phase. A total of 64 subfractions were collected, and the elution scheme followed that shown in Table 2.

Table 2

Elution scheme used in the fractionation RP-1O.VLC-3

Fraction	Mass obtained (mg)
RP-1O.VLC-3 (6-11)	41.4
RP-1O.VLC-3 (12)	6.6
RP-1O.VLC-3 (13-14)	190.4
RP-1O.VLC-3 (15-18)	207.0
RP-1O.VLC-3 (19-25)	111.1
RP-1O.VLC-3 (26-40)	5.1
RP-1O.VLC-3 (41-63)	17.9
MeOH	375.2

MeOH: methanol

All subfractions were pooled and analyzed by CTLC using a mixture of hexane/ethyl acetate (8:2) as the mobile phase. Analysis of the chromatographic profile indicated that subfraction RP-1O.CLV-3 (19–25) contained a major spot. This substance was labeled DI (111.1 mg) (Figure 5), and its chemical structure was elucidated by ^{1}H NMR spectroscopy and compared with data reported in the literature.

3.5 Fraction RP-1.VLC-4

With a part of this fraction (5.0 g), a VLC was performed again using 150.0 g of silica gel 60H as the stationary phase, and the column was packed under vacuum with hexane, with the sample adsorbed onto 11.0 g of silica gel 60. A total of ten fractions were collected, and the elution scheme with an increasing polarity gradient followed that shown in Table 3.

Table 3

Elution scheme used in the fractionation RP-1O.VLC-4

Solvent used	Volume collected (mL)	Fraction	Mass obtained (g)
Hex	250	RP-1O.VLC-4 (1)	0.0028
Hex/EtOAc (97:3)	250	RP-1O.VLC-4 (2)	0.0044
Hex/EtOAc (47:3)	250	RP-1O.VLC-4 (3)	0.0013

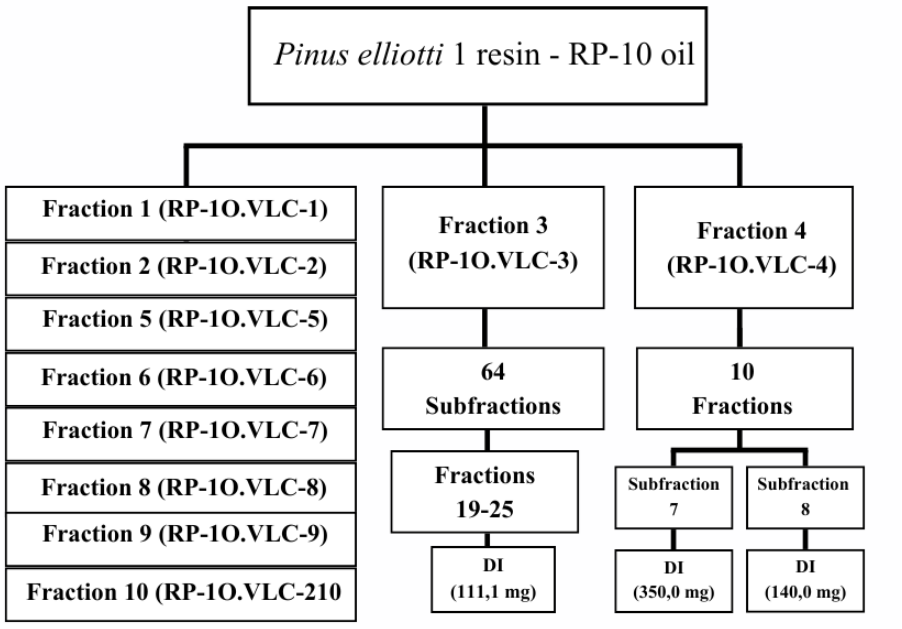
Hex/EtOAc (91:9)	250	RP-1O.VLC-4 (4)	1.1900
Hex/EtOAc (22:3)	250	RP-1O.VLC-4 (5)	1.9300
Hex/EtOAc (17:3)	250	RP-1O.VLC-4 (6)	0.9200
Hex/EtOAc (41:9)	250	RP-1O.VLC-4 (7)	0.3500
Hex/EtOAc (4:1)	250	RP-1O.VLC-4 (8)	0.1400
EtOAc	250	RP-1O.VLC-4 (9)	0.6800
MeOH	250	RP-1O.VLC-4 (10)	0.4600

Hex: hexane; EtOAc: ethyl acetate; MeOH: ethanol

All fractions were analyzed by CTLC using a mixture of hexane/ethyl acetate (8.5:1.5) as the mobile phase. Analysis of the chromatographic profiles showed that subfractions RP-1O.VLC-4 (7) and RP-1O.VLC-4 (8) exhibited a major spot with a similar chemical profile. This substance was designated DI (490.0 mg) (Figure 5), and its chemical structure was elucidated by ^{1}H NMR spectroscopy and compared with data reported in the literature.

Figure 5

Flowchart of the isolation and purification of dehydroabietic acid from Pinus elliottii resin



4 RESULTS

4.1 COMPARATIVE STUDY OF *PINUS* RESINS

4.1.1 Comparison of chromatograms obtained by HPLC

Samples from the three *Pinus* resins were subjected to HPLC analysis (Figures 6–10) to compare their main constituents in both the more solid portion of the resin (“mass”, M) and the more liquid portion (“oil”, O). Resin 3 (RP-3) did not exhibit a significant liquid fraction.

Figure 6

Chromatogram obtained by HPLC at λ = 201nm of the fraction RP-1M

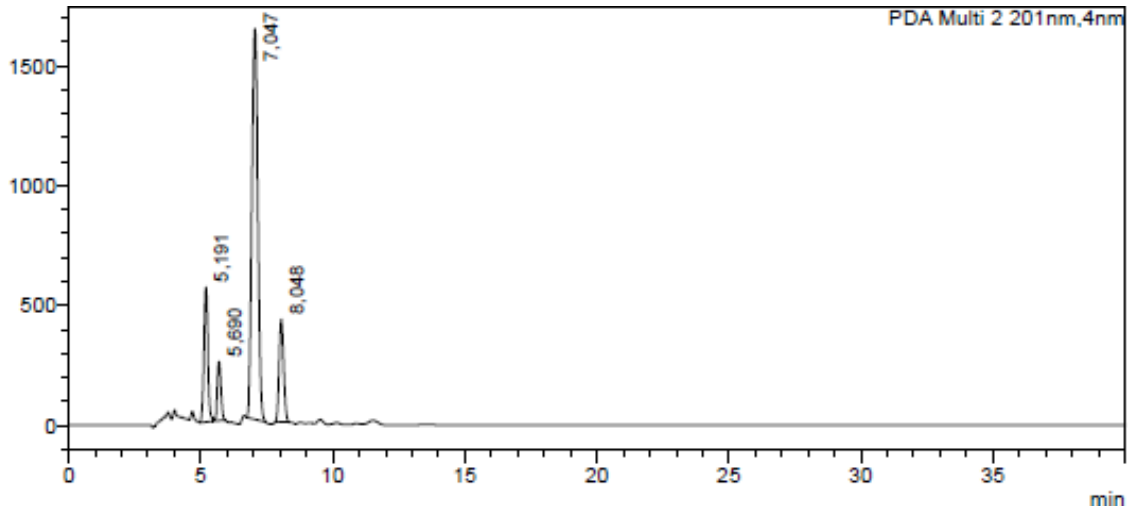


Figure 7

Chromatogram obtained by HPLC at λ = 201nm of the RP-1O fraction

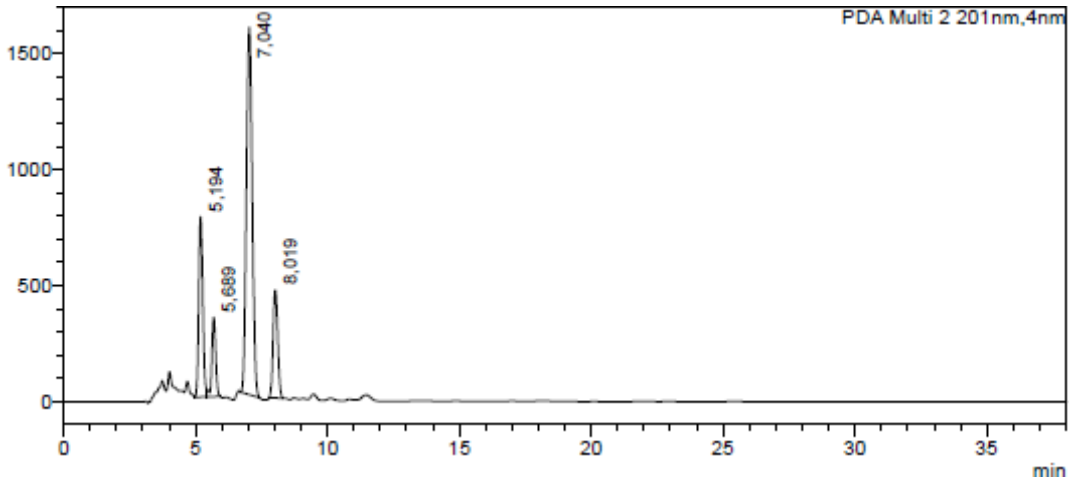


Figure 8

Chromatogram obtained by HPLC at λ = 201nm of the fraction RP-2M

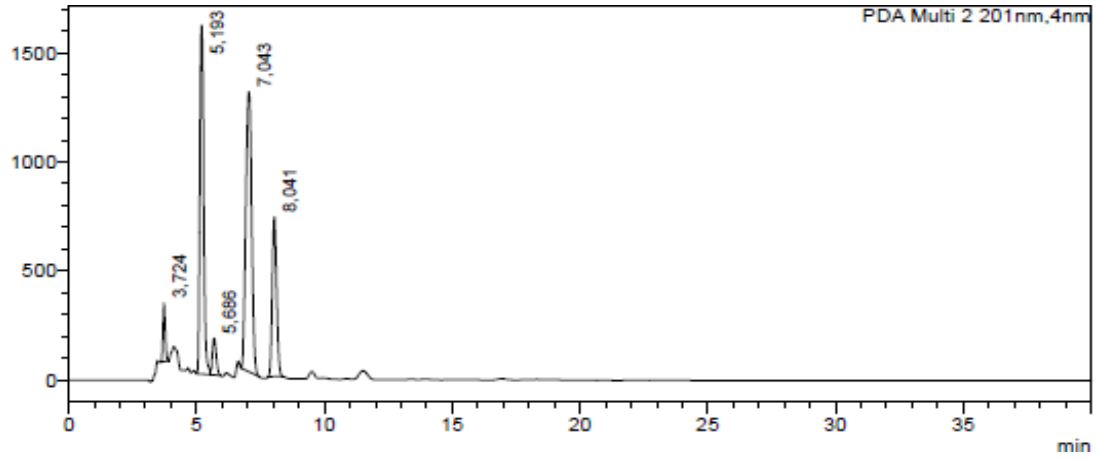


Figure 9

Chromatogram obtained by HPLC at λ = 201nm of the RP-2O fraction

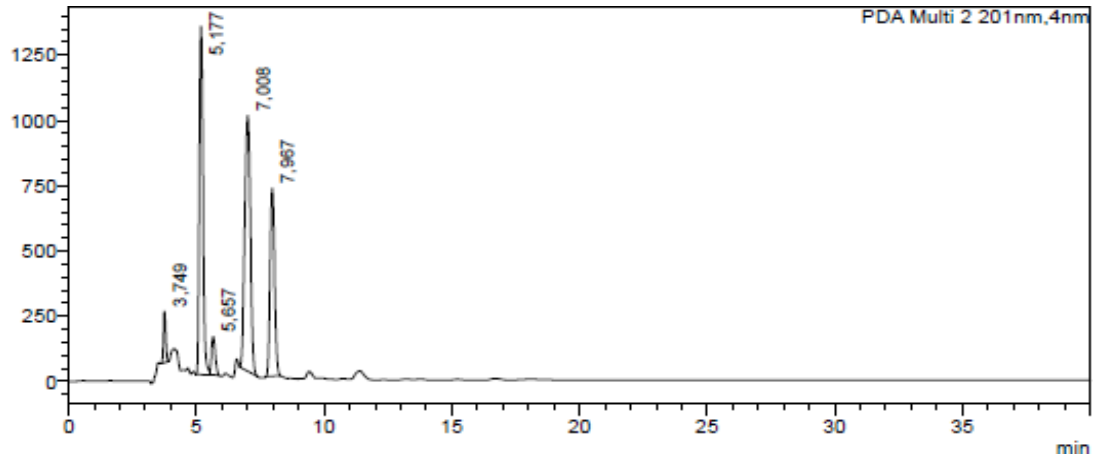
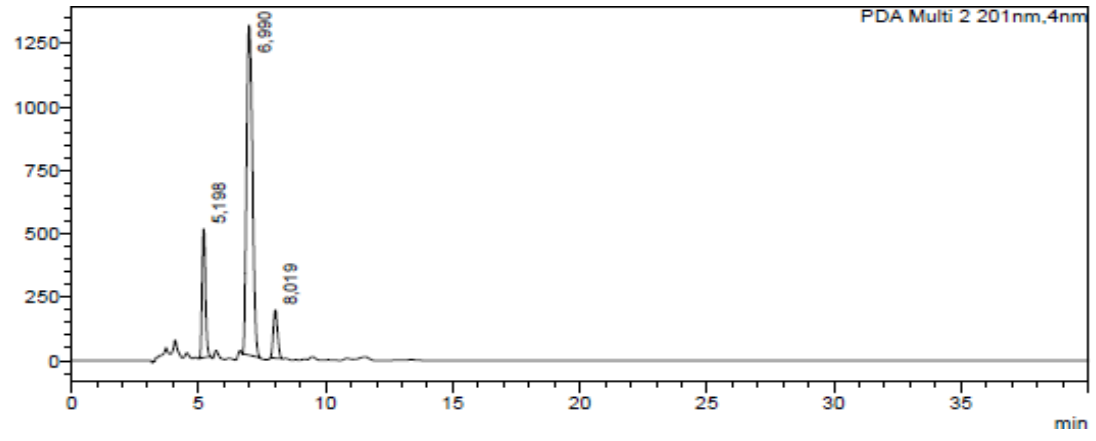


Figure 10

Chromatogram obtained by HPLC at λ = 201nm of the fraction RP-3M



The first conclusion that can be drawn from this analysis is that there is no significant difference between the “mass” and “oil” portions of the same resin. The chromatographic profiles of RP-1M and RP-1O are practically identical, as are those of RP-2M and RP-2O. Therefore, separating and using the “mass” and “oil” portions independently does not provide any analytical advantage.

Notable differences were observed among resins 1, 2, and 3. Although peaks at retention times of 5.2 min, 7.0 min, and 8.0 min were present in all chromatograms, their relative intensities varied across the resins, indicating compositional differences. For example, the peak at 5.2 min predominated in resin 2 but not in resins 1 and 3, which featured the 7.0 min peak as their major component; resin 3 exhibited an even simpler profile, with only two additional minor components.

4.1.2 Comparison of the essential oils of each resin

Essential oils were also obtained from the same samples analyzed by HPLC in the previous section, namely RP-1M, RP-1O, RP-2M, RP-2O, and RP-3M. The first parameter evaluated was the yield of each extraction process, as summarized in Table 4.

Table 4

Yields of the processes for obtaining essential oils

Sample of *Pinus*	Mass used (g)	Mass of essential oil (g)	Yeld (%)
RP-1M	34.519	4.0989	11.9
RP-1O	21.7038	3.7303	17.2
RP-2M	34.4530	4.1226	12.0
RP-2O	23.5235	4.9997	21.3
RP-3M	24.5657	1.6279	6.6

The portions designated as “oils” afforded higher yields than the corresponding “mass” portions. Both RP-1M and RP-2M provided approximately 12% essential oil, whereas RP-1O and RP-2O gave yields between 17% and 21%, and RP-3M, which was much drier than the others, afforded a lower yield of 6.6% essential oil.

The oils were subsequently analyzed by gas chromatography coupled to mass spectrometry (GC–MS). In this technique, the oil components are first separated by gas chromatography and then introduced into the mass spectrometer, where they are fragmented; the resulting mass spectra are compared with an instrument library that assigns the identity of each component based on similarity of fragmentation patterns, which is well established for many monoterpenes commonly found in essential oils.

For each oil, a table of identified components was constructed, including the percentage peak area of each signal in the chromatogram. The peak area is proportional to the relative amount of each constituent and can be used for comparative evaluation of the compositions, which are complex; therefore, the discussion focuses on the major components listed in Table 5. The main components tabulated have their structures depicted on Figure 11.

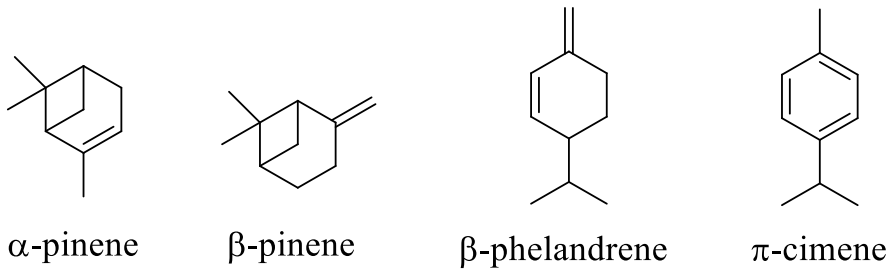
Table 5

Simplified composition of essential oils - percentage of comparison of peak areas in CG-MS analysis

Sample	**α-pinene**	**β-pinene**	**β-phellandrene**	***p*-cimene**
RP-1M	51.2	30.2	5.8	6.2
RP-1O	46.7	30.0	6.3	7.1
RP-2M	66.5	20.2	9.3	-----
RP-2O	50.4	28.8	12.6	-----
RP-3M	73.9	4.9	16.5	-----

Figure 11

Structures of the main components of essential oils



As expected for *Pinus* resins, the main component in all samples is α-pinene, with β-pinene occurring as the second most abundant constituent in all cases except resin 3. β-phellandrene, which is generally the third most abundant component, becomes the second major constituent in resin 3, representing the main distinguishing feature of this species (*caribaeae*). The distinguishing characteristic of *P. elliottii* is the presence of *p*-cymene, which was not identified in any of the other samples, whereas the hybrid (*elliottii* × *caribaeae*) exhibits feature of both parents: it lacks *p*-cymene, as in *caribaeae*, but shows an α and β-pinene ratio like that of *elliottii*.

These compositional differences suggest that there may be meaningful variation in the biological activity of both the resins and their essential oils, and that such variation can be exploited to obtain different major constituents for studies on natural diterpene derivatives. Consequently, resin 1 was selected for further investigation, as its composition has already been partially described in the literature.

4.2 ^{1}H NMR ANALYSIS OF THE ISOLATED SUBSTANCE

According to the literature, dehydroabietic acid (DI), a diterpene of the abietane class, is the major component of resins from *P. elliottii*. The research group, which focuses on structural modification of diterpenes, is particularly interested in developing methodologies to obtain this skeleton in high yield from a commercial source; therefore, fractionation of the resin was undertaken, although the absence of authentic standards for the isolation of individual constituents made this task more challenging.

To confirm the identity of DI, the ^{1}H NMR spectrum of the isolated substance was recorded in $CDCl_3$ and compared with data reported in the literature (Van Beek *et al.*, 2007). Based on this comparison, the isolated compound was unambiguously identified as dehydroabietic acid obtained from *Pinus elliottii* resin.

4.2.1 Identification of Dehydroabietic Acid (DI)

The signals observed in the spectrum shown in Figure 12 and their chemical shifts are consistent with those expected for the target compound. In the ^{1}H NMR spectrum, a singlet at 1.17 ppm, integrating for three protons, can be assigned to the methyl hydrogen at C-20 (H-20), which is characteristic of this structure.

Additional characteristic resonances of this diterpene include the aromatic protons H-11, H-12, and H-14: H-11 appears as a doublet (*d*) at *δ* 6.94 ppm (1H), H-12 as a doublet

of doublets (*dd*) at *δ* 6.77 ppm (1H), and H-14 at *δ* 6.67 ppm (1H). These downfield signals, located in the *δ* 6.50–8.00 ppm region, are consistent with aromatic hydrogens and are in excellent agreement with the proposed structure.

In the ^{1}H NMR spectrum of this compound, less downfield signals corresponding to the methyl group at C-19 (H-19) were also observed, appearing as a singlet (*s*) at *δ* 1.38 ppm and integrating for three protons, together with a doublet (*d*) at *δ* 1.18 ppm integrating for six protons, attributable to the methyl groups at C-16 and C-17 (H-16 and H-17).

In addition to the methyl resonances of H-16, H-17, H-19, and H-20, broad, unresolved signals were detected between *δ* 0.70 and 2.00 ppm, corresponding to the methylene protons present in the DI skeleton; these resonances appear in an overlapping region of the ^{1}H NMR spectrum, which hinders more detailed analysis.

Some other signals, such as H-5 and H-15 are also shown in Table 6, and because they are methylene carbon hydrogen, they are in a position that differentiates them from the others. Therefore, using the data of this work and compared with the data already obtained in the literature, it was possible to verify through these commented signals the attribution performed for the substance of interest (Van Beek *et al.*, 2007) (Table 6). Will also be shown below some data of the DI isolated from *Pinus elliottii* resin, such as: Data from DI: M.F. = $C_{20}H_{28}O_2$ e M.W. = 300.43 g mol^{-1}.

Table 6

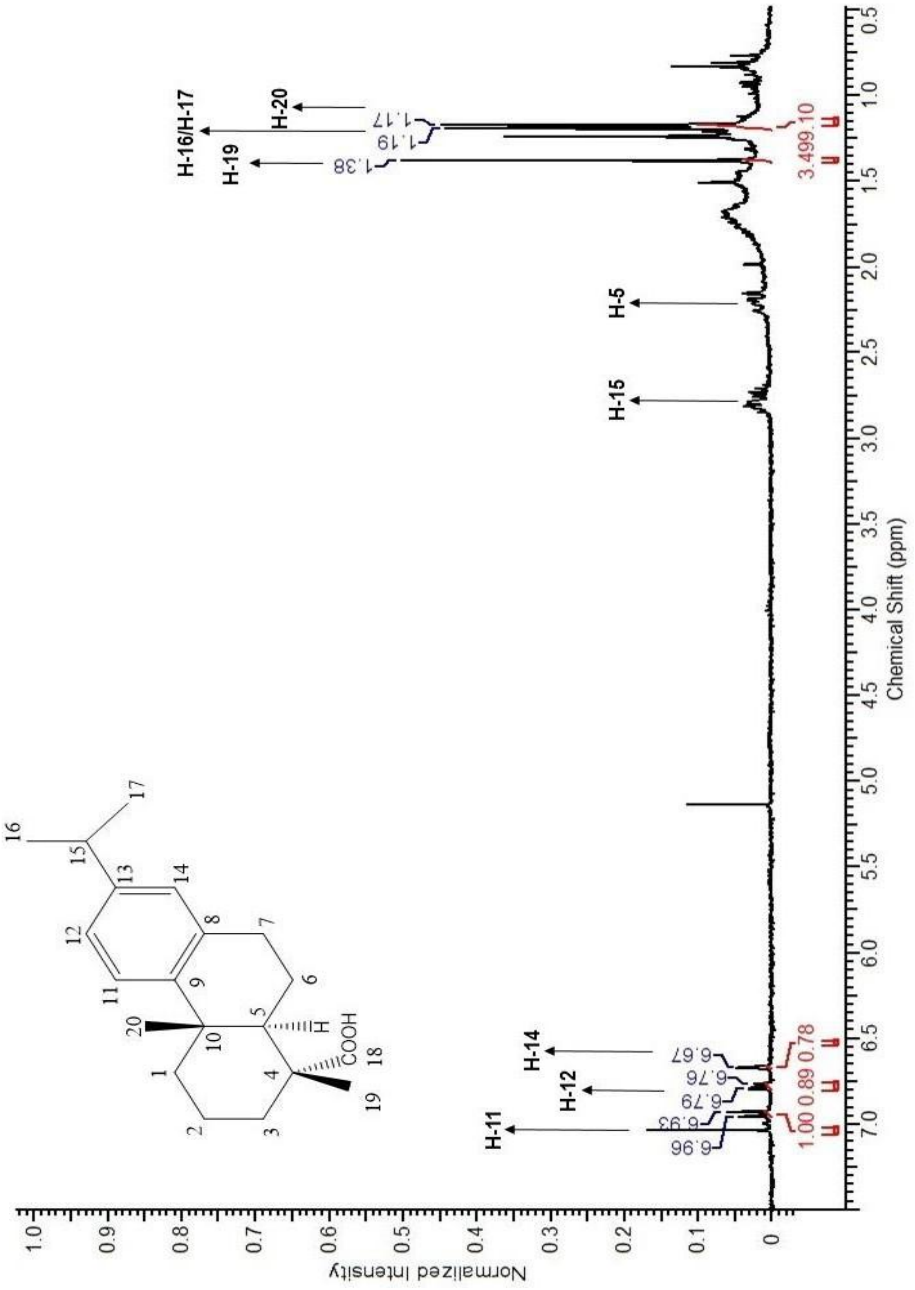
^{1}H NMR data of the substance DI and chemical displacement

Attribution	DI (δ - literature)	DI (δ - experimental)
H-5	2.24	2.24
H-11	7.18	6.94
H-12	7.00	6.77
H-14	6.89	6.67
H-15	2.83	2.84
H-16	1.23	1.18
H-17	1.23	1.18
H-19	1.29	1.38

H-20	1.22	1.17

Figure 12

^{1}H NMR spectrum of dehydroabietic acid, 400 MHz, $CDCl_3$



5 CONCLUSIONS AND PERSPECTIVES

Resins from the genus *Pinus* are an important source of secondary metabolites, particularly terpenoids. Comparative GC-MS analyses showed that the “mass” and “oil” portions of each *Pinus* resin exhibit essentially identical profiles, whereas the three resins

differ markedly in the relative abundances of α-pinene, β-pinene, β-phellandrene, and *p*-cymene, enabling the use of simple chemical markers to distinguish the species and their hybrid. This study also demonstrated that the “oil” fractions yield more essential oil than the corresponding “mass” fractions and that the combination of VLC, CCC, and CTLC constitutes an effective strategy to isolate dehydroabietic acid (DI) from *P. elliottii* in preparative quantities, with ^{1}H NMR data providing robust confirmation of its molecular structure.

These findings support the use of *Pinus* resins as practical commercial sources of DI and other terpenoids and establish a workflow that can be scaled to larger batches and extended to additional *Pinus* species to access a broader range of abietane and pimarane diterpenes. Furthermore, the application of systematic bioassays to chemically profiled resins, together with the integration of quantitative NMR, is expected to facilitate the development and evaluation of semi-synthetic abietane libraries for diverse applications, including agrochemicals, medicinal agents, and other bioactive products.

REFERENCES

Berger, M.; C. J. Andreu-Fernández; A. Pérez-Peña; T. Gutiérrez; J. M. Padrón; A. M. Amorós; and J. Marco-Contelles. (2017). Synthesis and antimicrobial evaluation of novel analogues of dehydroabietic acid prepared by C–H activation. *European Journal of Medicinal Chemistry, 126*, 937–943.

Boeck, P., Sá, M. M., Souza, B. S. D., Cercená, R., Escalante, A. M., Zachino, S. A., & Yunes, R. A. (2005). A simple synthesis of kaurenoic esters and other derivatives and evaluation of their antifungal activity. *Journal of the Brazilian Chemical Society*, *16*, 1360-1366.

Correa, J. S., Santos, R. R., & Anaissi, F. J. (2018). Purification and Characterization of Colophony Extracted of *Pinus elliottii* (Engelm, var. elliottii). *Orbital: The Electronic Journal of Chemistry*, 200-203.

Dembitsky, V. M.; Gloriozova, T. A.; and Poroikov, V. V. (2021). Antiprotozoal and antitumor activity of natural polycyclic endoperoxides: Origin, structures and biological activity. *Molecules, 26*(3), 686.

Fallarero, A., Skogman, M., Kujala, J., Rajaratnam, M., Moreira, V. M., Yli-Kauhaluoma, J., & Vuorela, P. (2013). (+)-Dehydroabietic acid, an abietane-type diterpene, inhibits *Staphylococcus aureus* biofilms in vitro. *International Journal of Molecular Sciences*, *14*(6), 12054-12072.

Fonseca, T.; Gigante, B.; Marques, M. M.; Gilchrist, T. L. (2004). Synthesis and antiviral evaluation of benzimidazoles, quinoxalines and indoles from dehydroabietic acid. *Bioorganic & Medicinal Chemistry, 12*, 103–112.

Gao, Y.-Q.; Zeng, Y.; Liu, J.-F.; Zhang, W.-J.; Wang, J.-X.; and Chen, Y. (2015). Turning renewable resources into value-added products: Development of rosin-based insecticide candidates. *Industrial Crops and Products, 76*, 660–671.

Gonçalves, M. D., Bortoleti, B. T. S., Tomiotto-Pellissier, F., Miranda-Sapla, M. M., Assolini, J. P., Carloto, A. C. M., & Arakawa, N. S. (2018). Dehydroabietic acid isolated from *Pinus elliottii* exerts in vitro antileishmanial action by pro-oxidant effect, inducing ROS production in promastigote and downregulating Nrf2/ferritin expression in amastigote forms of *Leishmania amazonensis*. *Fitoterapia*, *128*, 224-232.

Keeling, C. I., & Bohlmann, J. (2006). Diterpene resin acids in conifers. *Phytochemistry*, *67*(22), 2415-2423.

Kim, J.; Im, C.; Cho, Y.; Hwang, S.; Kim, J.; Choi, J.; Kim, S.; and Bae, M. (2015). The natural phytochemical dehydroabietic acid is an anti-aging reagent that mediates the direct activation of SIRT1. *Molecular and Cellular Endocrinology, 412*, 216–225.

Leandro, L. F., Cardoso, M. J. O., Silva, S. D. C., Souza, M. G. M., Veneziani, R. C. S., Ambrosio, S. R., & Martins, C. H. G. (2014). Antibacterial activity of *Pinus elliottii* and its major compound, dehydroabietic acid, against multidrug-resistant strains. *Journal of Medical Microbiology*, *63*(12), 1649-1653.

Lv, Y.-F.; Zhang, X.-Y.; Li, J.-Q.; Wang, L.-N.; Zhao, H.-T.; Chen, Y.; and Liu, Z.-P. (2025). Synthesis and anti-inflammatory activity of novel 1,2,3-triazole derivatives from 7-oxodehydroabietic acid. *BMC Chemistry, 19*, 88.

Pelletier, S. W., Chokshi, H. P., & Desai, H. K. (1986). Separation of diterpenoid alkaloid mixtures using vacuum liquid chromatography. *Journal of Natural Products*, *49*(5), 892-900.

Rezzi, S., Bighelli, A., Castola, V., & Casanova, J. (2002). Direct Identification and Quantitative Determination of Acidic and Neutral Diterpenes Using 13C-NMR Spectroscopy. Application to the Analysis of Oleoresin of Pinus nigra. *Applied Spectroscopy*, *56*(3), 312-317.

Silva, S. D. C., Souza, M. G. M., Cardoso, M. J. O., Moraes, T. S., Ambrósio, S. R., Veneziani, R. C. S., & Martins, C. H. G. (2014). Antibacterial activity of *Pinus elliottii* against anaerobic bacteria present in primary endodontic infections. *Anaerobe*, *30*, 146-152.

Soares, A. C. F.; Matos, P. M.; Silva, K. F.; Martins, C. H. G.; Veneziani, R. C. S.; Ambrósio, S. R.; Dias, H. J.; Santos, R. A. and Heleno, V. C. G. (2019). Antimicrobial potential of natural and semi-synthetic *ent*-kaurane and *ent*-pimarane diterpenes against clinically isolated Gram-positive multidrug-resistant bacteria. *Journal of the Brazilian Chemical Society, 30*, 333–341.

Tanaka, R., Tokuda, H., & Ezaki, Y. (2008). Cancer chemopreventive activity of “rosin” constituents of *Pinus* spez. and their derivatives in two-stage mouse skin carcinogenesis test. *Phytomedicine*, *15*(11), 985-992.

Van Beek, T. A., Claassen, F. W., Dorado, J., Godejohann, M., Sierra-Alvarez, R., & Wijnberg, J. B. (2007). Fungal biotransformation products of dehydroabietic acid. *Journal of Natural Products*, *70*(2), 154-159.