

Research Article

Chemical Analysis of Solid Tree Resins of *Protium*
Heptaphyllum and *Protium* *Icicariba*Lumír Ondřej Hanuš^{1*}; Yoav Giladi²; Maria Luiza
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Abstract

Solid tree resins of *Protium heptaphyllum* and *Protium icicariba* were studied and analyzed volatile compounds compared. The absence of sabinene in the resin of the tree *Protium icicariba* is the main distinguishing feature.

Keywords: *Protium heptaphyllum*; *Protium icicariba*; Resin; Volatile compound; Terpenes; GC/MS

Introduction

Protium icicariba, commonly known as "Almesca" or "Icica," is a tropical tree native to South America, particularly found in the Amazon Rainforest. It is renowned for its resin, which has been traditionally used by indigenous communities for its medicinal properties and as an incense ingredient.

Protium heptaphyllum, often referred to as "Breu Branco", "Mel Branco", "Almesca" or "Almécaga," is another tree species found in the Amazon Rainforest, and the Atlantic rainforest. It is well-known for producing a fragrant resin called "Breu" or "White Breu," which is used in traditional healing practices and as incense in spiritual rituals.

Both *Protium icicariba* and *Protium heptaphyllum* play significant roles in the cultural and traditional practices of indigenous peoples in their respective regions. Their resins have a long history of use for spiritual and medicinal purposes and continue to be valued for their aromatic and therapeutic qualities.

Almesca, also known as Breu Branco, is a fascinating resin (Figure 1) extracted from trees of the genus *Protium*, which are found in the Amazon rainforest, predominantly within Brazil. This resin has been an integral part of indigenous cultures for centuries, serving various traditional uses ranging from medicinal applications to spiritual practices. Almesca (Breu Branco) is particularly valued for its distinctive, pleasant aroma and its wide range of beneficial properties.

Extraction and Uses

The resin is traditionally harvested by making incisions in the tree's bark, allowing the sap to exude and harden into a resinous mass. This method of extraction is sustainable, as it does not harm the tree when done correctly, and allows for continuous harvesting from the same individual over many years (Figure 1).



Figure 1: Resin secreted from the bark of *Protium heptaphyllum*.

Almescar resin is known for its antiseptic, anti-inflammatory, analgesic, and gastroprotective properties [1-3], making it a valuable resource in traditional medicine for treating wounds, skin infections, and various other health issues. It is also used in aromatherapy due to its calming and grounding scent.

Environmental and Economic Impact

The sustainable harvesting of Almescar resin can have positive environmental and economic impacts. It provides a renewable resource that encourages the preservation of the Amazon rainforest and its biodiversity. Economically, it supports local communities by creating income opportunities that are aligned with the conservation of their natural environment. There's a growing interest in non-timber forest products like Almescar resin, as they offer a way to value the standing forest, providing an alternative to logging and other destructive practices.

Scientific Research

Protium heptaphyllum was for the first time published by Marchand [4] in 1873 and *Protium icariba* by the same author [5] in 1867. Research into Almescar and other Amazonian resins is ongoing, with scientists exploring their potential applications in modern medicine and industry.

In this study we collected resin samples (Figure 2–5) of *Protium heptaphyllum* and *Protium icariba* from different sources (Figure 6). We analyzed their chemical compositions and made a comparison of the results.

Materials And Methods

Plant Materials

(Figure 2 to Figure 6)

Sample Preparation

120 mg of tree resin was used for analysis. Headspace gas



Figure 2: Almescar Sample 1 Botanical name: *Protium heptaphyllum*
Originated from: Caraíva - Bahia, Brazil
Sample type: fresh resin.



Figure 3: Almescar Sample 2 Botanical name: *Protium heptaphyllum*
Originated from: Jambreiro-Bahia Brazil.



Figure 4: Almescar Sample 3 Botanical name: *Protium icariba* Originated from: caraíva Bahia Brazil Sample type: fresh resin.



Figure 5: Almescar Sample 4 Botanical name: *Protium icariba*
Originated Belém- Pará Brazil Sample type: dry after curing.

chromatography/ mass spectroscopy analysis was used for the indirect determination of volatile constituents in solid samples by analyzing the associated vapor phase. Analytical method validation - selectivity, specificity, accuracy, precision, linearity, range, limit of detection, limit of quantification, ruggedness, and robustness are beyond the scope of this manuscript and will be published elsewhere.

General Experimental Procedures

Experimental conditions: For the GC/MS analysis, we employed



Figure 6: Illustration of the map of Brazil showing: location A where samples 1-3 were collected and location B where sample 4 was collected.

an Agilent 7890B GC combined with an Agilent 5977B MSD and a PAL 3 (RSI 85) chromatograph. The column used was HP-5MS UI, 30 m \times 0.25 mm with a film thickness of 0.25 μ m, provided by Agilent Technologies, Inc. The analytical conditions were set with the column initially held at 35°C for 5 min. Subsequently, the temperature was programmed to rise from 35°C to 150°C at a rate of 5°C/min, then increasing by 15°C/min to 250°C, with a hold time of 90 min. The specific settings were as follows: Inlet temperature at 250°C, detector temperature at 280°C, split injection ratio of 1:5, initial temperature at 100°C, and initial time set to 4.0 min. Helium was used as the carrier gas with a flow rate of 1 mL/min. Incubation time: 6 min; Incubation temperature: 80°C.

Identification

For compound detection and identification, we referenced various standards, retention times, and retention indices (Chromatogram 1,2, Table 1; Chromatogram 3,4, Table 2). Additionally, we consulted multiple libraries: NIST/EPA/NIH Mass Spectral Library 2017, Wiley Registry of Mass Spectral Data 11th Edition, FFNSC3, © 2015, and the Adams EO library, Mass Spectral Library, containing 2205 compounds. The identification of volatile compounds in the samples was based on a comparison of their mass spectra and retention times, along with their Kovats retention indices, either to those of injected standards or by referencing the National Institute of Standards and Technology's Mass Spectral Library database.

Commercially available standards for α -pinene, camphene, β -pinene, myrcene, Δ^3 -carene, α -terpinene, *p*-cymene, limonene, 1,8-cineole, α -ocimene, trans- β -ocimene, γ -terpinene, terpinolene, linalool, isopulegol, geraniol, β -caryophyllene, α -humulene, cis-nerolidol, trans-nerolidol, caryophyllene oxide, guaiol, and α -bisabolol were obtained from Restek (Bellefonte, PA, USA).

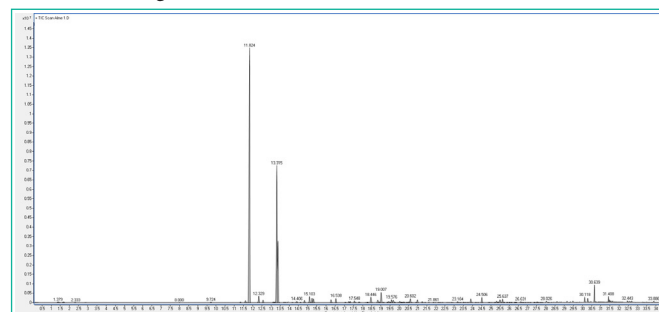
Results

All samples contained high amounts of pinene (α and β). Both samples of *P. heptaphyllum* contained high amounts of sabinene while

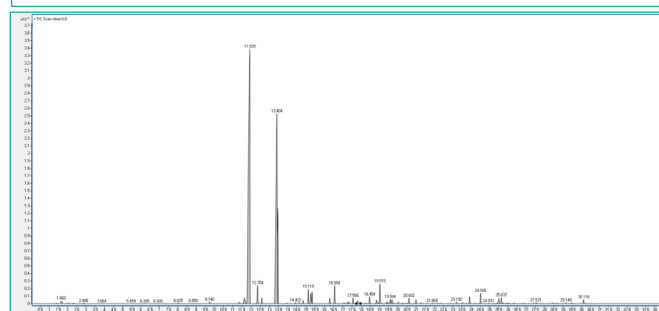
the two samples of *P. icariba* did not revealed any sabinene. Sample 4 of *P. icariba* contained a unique chemical composition with high amounts of *p*-cymene and α - and β -phellandrene.

As we did not have all the main compounds as standards, it was impossible to quantify all these volatiles exactly. Percentage compositions (in Tables as % were obtained from electronic integration measurements without considering relative response factors).

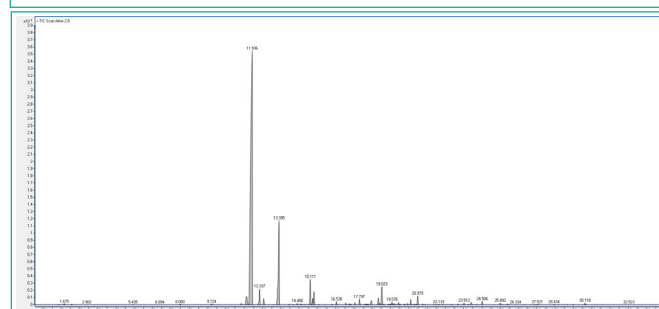
(Chromatogram 1 to 4 & Table 1 & 2)



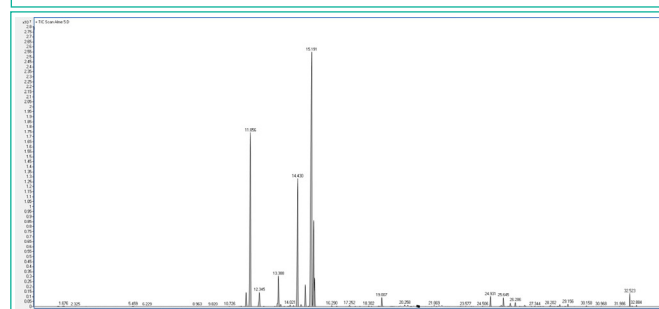
Chromatogram 1: GC/MS chromatogram of the sample 1 (*Protium heptaphyllum*).



Chromatogram 2: GC/MS chromatogram of the sample 2 (*Protium heptaphyllum*).



Chromatogram 3: GC/MS chromatogram of the sample 3 (*Protium icariba*).



Chromatogram 4: GC/MS chromatogram of the sample 4 (*Protium icariba*).

Table 1: *Protium heptaphyllum* (Aubl.) Marchand - concentrations of content compounds (% of total).

No	RT	Compound	1 %	2 %
1	2.301	2-methyl-3-butene-2-ol		0.06
2	3.864	3-ethyl-2,2-dimethyloxirane		0.03
3	6.205	3-methyl-2-butenal		0.02
4	6.758	hexenal		0.02
5	8.025	3,3,5,5-tetramethylcyclopentene	0.03	0.07
6	11.368	tricyclene	0.12	0.17
7	11.52	cumene		0.02
8	11.68	α -thujene	0.35	0.79
9	12.025	α -pinene	44.62	45.68
10	12.37	camphene	1.08	1.54
11	12.594	dehydrosabinene	0.4	0.47
12	13.428	sabinene	21.43	28.05
13	13.484	β -pinene	8.78	6.28
14	13.901	6-methyl-5-hepten-2-one		0.01
15	14.205	trans-p-menth-2-ene		0.02
16	14.422	α -phellandrene		0.12
17	14.614	α -methyl-anisole	0.04	0.09
18	14.847	α -terpinene	0.32	0.26
19	15.175	p-cymene	0.83	1.12
20	15.288	limonene	0.67	0.81
21	15.352	1,8-cineole	0.56	0.93
22	16.29	γ -terpinene	0.43	0.42
23	16.546	cis-sabinene hydrate	0.59	1.43
24	17.332	2-(2,2,4-trimethyl-1-cyclopent-3-enyl)acetaldehyde	0.17	0.13
25	17.644	linalool	0.01	0.02
26	17.813	3-thujen-2-ol		0.25
27	18.157	β -thujone	0.01	0.01
28	18.246	dehydrosabinaketone		0.04
29	18.454	α -campholenal	0.91	0.63
30	18.839	trans-pinocarveol	0.47	0.36
31	19.015	camphor	1.66	1.83
32	19.36	3-thujen-2-one	0.09	0.07
33	19.424	sabina ketone	0.2	0.16
34	19.512	pinocamphone	0.04	0.03
35	19.584	pinocarvone		0.35
36	19.673	endo-borneol	0.52	0.37
37	20.049	terpinen-4-ol	0.31	0.13
38	20.434	α -terpineol	0.05	0.03
39	20.61	myrtenal	0.62	0.43
40	20.987	verbenone	0.4	0.32
41	21.26	trans-carveol	0.13	0.08
42	21.861	cumin aldehyde	0.04	0.03
43	21.989	carvone	0.01	0.01
44	22.142	2,5-dimethoxytoluene		0.03
45	23.192	bornyl acetate	0.16	0.15
46	23.616	undecanal	0.03	
47	24.603	δ -elemene		0.01
48	24.931	α -cubebene		0.03
49	25.3	butoxyethoxyethyl acetate	0.28	
50	25.42	cyclosativene		0.03
51	25.637	copaene	0.51	0.49
52	25.741	daucene		0.06
53	25.885	α -bourbonene	0.06	0.07
54	26.005	β -cubebene	0.06	
55	26.054	β -elemene		
56	26.286	cyperene		0.02
57	26.126	tetradecane	0.04	
58	26.374	dodecanal	0.1	
59	26.631	β -ylangene		0.01
60	26.968	sibirene		0.01
61	27.368	guaia-6,9-diene	0.07	0.04
62	27.833	9-epi-(E)-caryophyllene		0.02
63	28.026	dodecanol	0.22	
64	28.202	γ -muurolene		0.02
65	28.282	α -amorphene		0.02
66	28.443	β -selinene	0.05	0.06
67	28.531	δ -selinene	0.02	0.02
68	28.603	pentadecane	0.11	
69	28.627	α -selinene		0.02
70	28.715	α -muurolene	0.01	0.03
71	28.996	γ -cadinene	0.01	0.03
72	29.148	δ -cadinene	0.11	0.08
73	29.268	dauca-4(11),8-diene		
74	29.468	cis-dracunculifolol	0.14	
75	30.639	isopropyl laurate	1.6	
76	31.408	lauryl acrylate	0.49	
77	31.473	heptadecane	0.25	
78	32.443	octadecane	0.13	
79	33.493	14-methyl-pentadecanoic acid methyl ester	0.04	
			90.80%	94.90%

Table 2: *Protium icariba* (DC.) Marchand - concentrations of content compounds (% of total) in samples 3 and 4.

No	RT	Compound	3 %	4 %	RI
1	5.459	toluene	0.04	0.07	763
2	6.229	3-methyl-2-butenal		0.01	782
3	8	3,3,5,5-tetramethylcyclopentene	0.08		857
4	10.726	bornylene		0.02	906
5	11.247	5,5-dimethyl-1-vinylbicyclo[2.1.1]hexane		0.02	921
6	11.351	tricyclene	0.19	0.07	925
7	11.512	cumene		0.02	927
8	11.616	α -thujene	1.79	1.56	929
9	11.856	α -pinene	66.61	20.43	937
10	12.345	camphene	1.89	1.74	952
11	12.586	dehydrosabinene	0.64	0.05	956
12	13.388	β -pinene	12.25	3.22	979
13	13.7	trans-p-menth-2-ene	0.04	0.04	981
14	13.901	6-methyl-5-hepten-2-one		0.04	986
15	14.277	Δ^2 -carene		0	1001
16	14.43	α -phellandrene		13.05	1005
17	14.606	Δ^3 -carene	0.13	0.24	1011
18	14.847	α -terpinene		2.06	1017
19	15.007	p-ment-1-ene		0.05	1025
20	15.191	p-cymene	2.67	39.5	1025
21	15.239	limonene	0.68		1030
22	15.296	β -phellandrene		7.73	1031
23	15.352	1,8-cineole	1.35	1.99	1032
24	15.64	cis- β -ocimene		0	1038
25	16.29	γ -terpinene	0.06	0.09	1060
26	16.554	cis-sabinene hydrate	0.32	0.06	1070
27	17.252	terpinolene		0.17	1088
28	17.324	2-(2,2,4-trimethyl-1-cyclopent-3-enyl)acetaldehyde	0.08		
29	17.821	3-thujen-2-ol	0.61	0.03	1107
30	18.149	β -thujone	0.11		1114
31	18.462	α -campholenal	0.6	0.03	1125
32	18.823	trans-pinocarveol	0.75		1139
33	18.911	verbenol	0.16		1140
34	19.007	camphor		0.89	1142
35	19.32	menthone		0	1154
36	19.52	pinocamphone	0.08	0.02	1160
37	19.592	pinocarvone	0.29	0.02	1164
38	19.68	borneol	0.19	0.01	1166
39	19.937	cis-pinocamphone	0.22	0.01	1173
40	20.033	terpinen-4-ol	0.07	0.03	1177
41	20.258	p-cymen-8-ol	0.07	0.14	1183
42	20.426	α -terpineol	0.12	0.09	1189
43	20.61	myrtenal	0.56	0.05	1193
44	20.795	homomyrtenol	0.06		1194
45	20.995	verbenone	0.88	0.02	1205
46	21.252	trans-carveol	0.07		1217
47	21.725	thymol methyl ether		0.01	1235
48	21.869	cumin aldehyde	0.03	0.07	1239
49	21.981	carvone	0.03		1242
50	21.989	carvacrol methyl ether		0.03	1244
51	22.101	carvotanacetone		0.03	1246
52	22.158	thymoquinone		0.02	1250
53	22.133	2,5-dimethoxytoluene	0.05		1251
54	22.286	piperitone		0.07	1253
55	22.615	3,5-dimethoxytoluene		0.01	1274
56	23.184	bornyl acetate	0.08		1285
57	23.288	p-cymen-7-ol		0.03	1289
58	23.577	carvacrol		0.03	1299
59	24.603	δ -elemene		0.01	1338
60	24.931	α -cubebene		0.87	1351
61	25.428	cyclosativene		0.01	1368
62	25.525	ylangene		0.15	1372
63	25.645	copaene	0.03	0.79	1376
64	25.741	daucene		0	1381
65	25.893	α -bourbonene	0.09	0.03	1384
66	26.014	β -cubebene		0.38	1389
67	26.286	cyperene		0.39	1399
68	26.535	α -gurjunene	0.01	0.05	1409
69	26.879	γ -maaliene	0.02		1435
70	27.16	α -bergamotene		0.03	1435
71	27.248	α -guaiene		0.01	1439
72	27.368	guaia-6,9-diene	0.04		1450
73	27.649	α -humulene		0.03	1454
74	28.202	γ -muurolene		0.13	1477
75	28.314	α -curcumene		0.02	1483
76	28.442	β -selinene	0.04	0.03	1486
77	28.523	δ -selinene	0.01		1490
78	28.627	α -selinene	0.02		1494
79	28.715	α -muurolene	0.02	0.16	1499
80	28.996	γ -cadinene	0.02	0.05	1513
81	29.156	δ -cadinene	0.03	0.18	1524
82	29.316	cadina-1,4-diene		0	1533
83	29.509	α -calacorene		0.01	1542
84	29.837	β -calacorene		0	1563
85	30.158	caryophyllene oxide		0.06	1581
86	30.527	humulene epoxide I		0.01	1604
87	30.968	δ -cadinol		0.02	1645
			94.15%	97.50%	

Table 3: The main compounds and the main differences in samples of *P. heptaphyllum* and *P. icicariba*.

	<i>P. heptaphyllum</i> sample 1 (%)	<i>P. heptaphyllum</i> sample 2 (%)	<i>P. icicariba</i> sample 3 (%)	<i>P. icicariba</i> sample 4 (%)	RI
α -thujene	0.35	0.79	1.79	1.56	929
α -pinene	44.62	45.68	66.61	20.43	937
camphene	1.08	1.54	1.89	1.74	952
sabinene	21.43	28.05	0	0	974
β -pinene	8.78	6.28	12.25	3.22	979
α -phellandrene	0	0.12	0	13.05	1005
p-cymene	0.83	1.12	2.67	39.5	1025
limonene	0.67	0.81	0.68	0	1030
β -phellandrene	0	0	0	7.73	1031
1,8-cineole	0.56	0.93	1.35	1.99	1032
cis-sabinene hydrate	0.59	1.43	0.32	0.06	1070
terpinolene	0	0	0	0.17	1088
camphor	1.66	1.83	0	0.89	1142
isopropyl laurate	1.6	0	0	0	1618

Discussion

Previous studies suggest that the outcomes of resin analysis are influenced by multiple factors, including the freshness of the sample, preservation methods, processing techniques (e.g., steam distillation, hydrodistillation, organic solvent extraction), seasonal variation, and the geographic origin of the collected samples. These variables significantly impact the chemical composition of the resin. In resin produced by *Protium heptaphyllum*, the predominant terpenes and terpenoids identified in previous studies include limonene [6-8], terpinolene [9-14], p-cymene [13,15-17], and p-cymen-8-ol [7,9,11-13], with α -pinene [10,13-15], 1,8-cineole [3,7,8,18], α -phellandrene [8,10,13,18], and sabinene [3,7-10,15,18] observed in smaller concentrations. *Protium icicariba* has only been analyzed in three scientific papers. Siani et al. [19] analyzed the essential oils from the oleoresin in three different years.

The main compounds were p-cymene (20-40%), p-cymen-8-ol (10-26%), terpinolene (5.8-31.0%), limonene (5.8-8.0%), α -pinene (5.6-7.7%), and sabinene (1.6-2.3%). Pereira et al. [20] studied dehiscent/indehiscent ripe fruits and found as the main compounds E- β -ocimene (31.07/28.14%), α -fenchene (27.67/12.83%), limonene (16.10/21.29%), and α -phellandrene (8.86/12.50%). da Paz Lima et al. [21] found in *Protium icicariba* luteol, and α - and β -amyrin.

(Table 3)

The evaluation and the ranking of the most prominent substances in all four samples is compared in Table 3. All four samples contained very high amounts of α -pinene and high amounts of β -pinene. Both samples of *P. heptaphyllum* contained high amounts of sabinene while the two *P. icicariba* samples contained no sabinene at all. *P. icicariba* sample 4 contained a unique chemical composition with high amounts of p-cymene and α - and β -phellandrene. Some compounds reported in previous studies, such as limonene [6-8] terpinolene [9-14], and p-cymen-8-ol [7,9,11-13], were found in negligible concentrations or were not detected in our samples. This discrepancy may stem from factors beyond sample processing, such as genetic variability, environmental conditions, or differences in collection and preservation methods.

The research of these substances in *P. heptaphyllum* and *P. icicariba* deserves greater attention due to the frequent use of their resins in indigenous cultures. Further research is essential to fully explore their chemical composition and potential applications.

Author Statements

Conflict of Interest Statement

No potential conflict of interest was reported by the author.

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