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Essential oil from the aerial parts of four species of *Protium* (Burseraceae) – A contribution to the aromatic flora of the Adolpho Ducke Forest Reserve, Amazonas state, Brazil

Óleos essenciais das partes aéreas de quatro espécies of *Protium* (Burseraceae) – Uma contribuição para a flora aromática da Reserva Florestal Adolpho Ducke, Amazonas, Brasil

Aceites esenciales delas partes aéreas de cuatro especies de *Protium* (Burseraceae) – Una contribución a la flora aromática de la Reserva Forestal Adolpho Ducke, Amazonas, Brasil

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Abstract
The Adolpho Ducke Forest Reserve is a fragment of the Amazon Rainforest that has the highest density of collections deposited in the INPA herbarium, and this characterized, vascular flora has been an important basis for chemical studies. This paper describes the chemical composition of the volatiles of aerial parts of the four species of *Protium* that occurr in the Ducke Reserve. The essential oils obtained by hydrodistillation were analyzed using GC-FID and GC-MS. *P. altsonii* showed high percentages of the monoterpenes α-pinene (22.05%) and limonene (17.76%) in the leaves, and linear long-chain fatty alcohols, such as n-tetradecanol (19.93%) and hexadecanol (32.56%), in the branches. The essential oil from leaves of *P. laxiflorum* provided sesquiterpenes α-copaene (18.98%), *epi*-α-cadinol (9.17%) and β-caryophyllene (9.04%) as the main compounds. In *P. pallidum*, the sesquiterpenes caryophyllene oxide (29.72%), α-cadinol (28.82%) and *epi*-α-cadinol (13.96%) were predominant in the leaves, and α-cadinol (25.50%), α-eudesmol (22.29%) and caryophyllene oxide (12.81%) predominated in the branches. β-Caryophyllene (30.59%) and caryophyllene oxide (20.63%) were the main compounds in the leaves of *P. paniculatum var. riedelianum.* The results presented herein contribute to the knowledge of the aromatic flora of the Ducke Reserve.
Keywords: Amazonia; *Protium altsonii*; *Protium laxiflorum*; *Protium pallidum*; *Protium paniculatum* var. *riedelianum.*

Resumo

A Reserva Florestal Adolpho Ducke é um fragmento de Floresta Amazônica com maior densidade de coletas depositadas no herbário do INPA, essa flora vascular caracterizada tem sido uma base importante para estudos químicos. Esse trabalho descreve a composição dos voláteis de partes aéreas de quatro espécies de *Protium* ocorrentes na Reserva Ducke. Os óleos essenciais obtidos foram analisados por CG-DIC e CG.EM. *Protium altsonii* apresentou altos percentuais dos monoterpenos α-pinene (22.05%) e limoneno (17.76%) em folhas e álcoois graxos lineares de cadeia longa como *n*-tetradecanol (19.93%) e hexadecanol (32.56%) nos galhos. Os óleos essenciais das folhas *de P. laxiflorum* forneceram os sesquiterpenos α-copaene (18.98%), *epi*-α-cadinol (9.17%) e β-caryophyllene (9.04%) como majoritários. Em *P. pallidum*, os sesquiterpenos caryophyllene oxide (29.72%), α-cadinol (28.82%) e *epi*-α-cadinol (13.96 %) foram predominantes em folhas, α-cadinol (25,5%), α-eudesmol (22,29%) e caryophyllene oxide (12,81%) nos galhos. β-Caryophyllene (30.59%) e caryophyllene oxide (20.63%) foram majoritários em folhas de *P. paniculatum var.riedelianum.* Os resultados contribuem para o conhecimento da flora aromática da Reserva Ducke.

Palavras-chave: Amazônia; *Protium altsonii*; *Protium laxiflorum*; *Protium pallidum*; *Protium paniculatum* var. *riedelianum.*

Resumen

La Reserva Forestal Adolpho Ducke es un fragmento de selva amazónica con la mayor densidad de colecciones depositadas en el herbario del INPA, esta flora vascular caracterizada ha sido una base importante para estudios químicos Este trabajo describe la composición de las partes aéreas volátiles de cuatro especies de *Protium* presentes en Reserva Ducke. Los aceites esenciales obtenidos por hidrodestilación fueron analizados por GC-FID y GC-MS. Las hojas de *Protium altsonii* mostraron altos porcentajes de los monoterpenos α-pineno (22.05%) y limoneno (17.76%) y alcoholes grasos lineales de cadena larga como n-tetradecanol (19.93%) y hexadecanol (32.56%) en las ramas. Los aceites esenciales de las hojas de *P. laxiflorum* proporcionan los sesquiterpenos α-copaeno (18.98 %), *epi*-α-cadinol (9.17 %) y β-cariofileno (9.04 %) como constituyentes principales. En *P. pallidum* predominaron en hojas los sesquiterpenos óxido de cariofileno (29.72%), α-cadinol (28.82%) y *epi*-α-cadinol (13.96%), el α-cadinol (25.5%), α-eudesmol (22.29%) y óxido de cariofileno (12.81%) en las ramas. El β-cariofileno (30.59%) y el óxido de cariofileno (20.63%) fueron mayoritarios en las hojas de *P. paniculatum var. riedelianum*. Los resultados contribuyen al conocimiento de la flora aromática de Reserva Ducke.

Palabras clave: Amazonia; *Protium altsonii*; *Protium laxiflorum*; *Protium pallidum*; *Protium paniculatum* var. *riedelianum.*

1. Introduction

The Adolpho Ducke Forest Reserve, located 26 km from Manaus, Amazonas state, is a fragment of the Amazonia Rainforest with an area of 100 km^2 and constitutes one of the places with the highest density of collections deposited in the INPA herbarium (Ribeiro et al., 1999). This characterized vascular flora has been an important basis for chemical studies, including those carried out with species of *Protium* Burm. f. (as know as "breu", from the family Burseraceae Kunth) especially related to the composition of the volatile constituents of their resins and aerial parts. However, there is a lack of knowledge regarding the chemistry of the aerial parts of *Protium altsonii* Sandwith, *Protium laxiflorum* Eng, *Protium pallidum* Cuatrec. and *Protium paniculatum var. riedelianum* (Engl.) Daly. The literature on the essential oils of *P. altissonii* refers to resins collected in the Ducke Reserve that were found to be rich in monoterpenes with a predominance of *p*-cymene (31.5%) and *trans*-dihydro-α-terpineol (25.8%) (Zoghbi et al., 2005); α-pinene (42.9-61.8%) and α-phelandrene (14.7-22.26%) (Ramos et al., 2000). The essential oils from the resin of *Protium paniculatum var. riedelianum* also found in the Ducke Reserve are dominated by monoterpenes with a predominance of *p*-menth-1-ene (31.4%) and *p*-cymene 33.8-90.0% (Ramos et al., 2000). There are no previous studies related to the volatile constituents of the species *P. laxiflorum* and *P. pallidum*. This paper describes the chemical composition of the volatile constituents of the aerial parts of the four species of *Protium* occurring in the Adolpho Ducke Forest Reserve and, as such, contributes to the knowledge of the aromatic flora of this forest reserve and to grow up researches in the Amazonia, with mention to the legacy of the work from Zoghbi et al. (2000).

2. Methodology

2.1 Plant material and oil distillation

The samples from individual, previously marked specimens of *P. altsonii* (ID 1630-09), *P. laxiflorum* (ID 1668-09), *P. pallidum* (ID 1688-09) and *Protium paniculatum* var. *riedelianum* (ID 1602-09) were collected at the Adolpho Ducke Forest Reserve located to northeast of city of Manaus (latitude 02º 55′ S, longitude 59º 59′ W). The leaves and branches were dried in an air conditioned room at 25 °C for seven days, milled and submitted to hydrodistillation in a Clevenger-type apparatus for 4 hours to produce oil yields of 0.68 and 0.50 % of *P. altsonii*, 0.16 and 0.09% of *P. pallidum*, 0.35 and 0.08 of *P. paniculatum var.riedelianum* leaves and branches, respectively, and 0.13 % of *P. laxiflorum* leaves.

2.2 Gas chromatography-mass spectrometry (GC/MS)

Analyses of volatile constituents were performed on a mass spectrometer (Shimadzu, QP5000), equipped with a DB-5 fused silica capillary column (J&W Scientific – Serial No. 8766726; 5% phenylmethylsiloxane; 30 m × 0.25 mm × 0.25 μm). The electron impact technique (70 eV) was used, with the injector temperature at 240 °C and the detector at 230 °C. The carrier gas was helium at the working rate of 1.0 mL/min. The column temperature was initially 60 °C and then was gradually increased at the rate of 3 °C/min up to 240 °C. Compounds were identified by comparing their mass spectrum to those of the GC-MS database (NIST 62.lib), existing literature (Mc Lafferty & Stauffer, 1989) and retention indices (Adams, 2007). Quantitative analysis was performed using a gas chromatograph (Shimadzu, GC 2010 GC-FID) under the same conditions as the GC-MS method.

3. Results and Discussion

Table 1 presents the chemical composition found for the essential oils of the four species of *Protium* and Table 2 shows the types of chemical constituents in each essential oil. The chemical structures of the predominant constituents of the essential oils are shown in Figure 1.

The essential oil from the leaves of *P. altsonii* showed high percentages of hydrocarbon monoterpenes and sesquiterpenes with the predominance of monoterpenes α-pinene (22.05%) and limonene (17.76%). In the essential oil from the branches of this specimen, high percentages of the long-chain linear alcohols n-tetradecanol (19.93%) and hexadecanol (32.56%) were detected. Generally, the essential oils of *Protium* species have high levels of sesquiterpenes in the aerial parts and monoterpenes in the resins; thus, the high percentage of monoterpenes, including α-pinene and limonene in *P. altsonii* leaves, as well as the high contents of linear fatty alcohols long-chain on branches, had not been previously reported in the genus. Limonene, with percentages close to those that found in *P. altsonii* leaves, was previously reported in essential oils of *P. hebetatum* resin (Lima et al., 2016).

The branches of *P. laxiflorum* produced only traces of essential oils, and the leaves provided essential oils with a low percentage of monoterpenes (2.08%). Among the sesquiterpenes, the majority were α-copaene (18.98%), *epi*-α-cadinol (9.17%) and β-caryophyllene (9.04%). α-Copaene, with levels close (15.2 -19.6%), was previously found in the aerial parts of two species of *Protium* from Ducke Reserve (Carvalho et al., 2013; Lima et al., 2021),

The presence of monoterpenes was not detected in the essential oils of *P. pallidum*, and oxygenated sesquiterpenes, such as caryophyllene oxide (29.72%), α-cadinol (28.82%) and *epi*-α-cadinol (13.96%), were predominant in the leaves; while α-cadinol (25.5%), α-eudesmol (22.29%) and caryophyllene oxide (12.81%) were predominant in the branches. A high content of oxygenated α-cadinol sesquiterpene was registred in the essential oil from branches of *P. trifoliatum* (Oliveira et al., 2018) and *epi*-α-cadinol (20.4%) in resin of *P. spruceanum* (Zoghbi et al., 2002). Caryophyllene oxide is a common sesquiterpene in

the aerial parts of *Protium;* however, the percentage that we detected in *P. pallidum* leaves is comparable to that found by Carvalho et al. (2009) in leaves of *P. elegans*.

The essential oil from leaves of *P. paniculatum var. riedelianum* presented the sesquiterpenes β-caryophyllene (30.59%) and caryophyllene oxide (20.63%) as the main constituents. β-caryophyllene, with a content between 35.9-22.8%, was previously found in the aerial parts of some *Protium* species (Carvalho et al., 2009; Carvalho et al., 2010; Siani et al., 1999). Among the essential oils analyzed, the oil obtained from the branches of this specimen showed the highest number of chemical constituents; however, with low percentages of its constituents (< 7.58%).

The literature has reported the importance of the bicyclic sesquiterpene ß-caryophyllene and its various pharmacological properties (Maffei, 2020) and Vasconcelos et al., (2021) showed the herbicidal potential of essential oils with a predominance of ß-caryophyllene and caryophyllene oxide.

Table 1. Chemical composition (%) of essential oil of *P. altsonii*, *P. laxiflorum*, *P. pallidum* e *P. paniculatum var. riedelianum.*

Constituents	PAL.1	PAL.2	PLA.1	PPA.1	PPA.2	PPVR.l	PPVR.2	RI Exp.
Tricyclene	1.16							924
α-Pinene	**22.05**	1.45				0.89	0.24	931
β-Pinene	4.79					0.51		974
o-Cymene							0.46	1020
p-Cymene	0.57		2.08					1019
Limonene	**17.76**	1.59				0.39	0.45	1025
γ-Terpinene	0.56							1063
m-Cymene							0.45	1084
α-Campholenol							1.19	1120
trans-Pinocarveol						0.56	2.46	1134
trans-Pinocamphone							1.03	1153
Pinocarvone						0.31	1,85	1155
Borneol							0.87	1160
n-Nonanol							1.45	1167
Terpin-4-ol	2.21	2.30				0.36	1.44	1172
α-Terpineol	1.33	3.50				0.36	1.56	1185
trans-Dihydro carvone							0.36	1198
Myrtenal						0.39	2.11	1189
Myrtenol						0.52	1.05	1192
Verbenone							0.44	1202
trans-Anethole							2.23	1278
δ-Elemene	0.42							1336
α-Cubebene	0.28							1351
α-Copaene	2.33	1.68	**18.98**		1.49	2.92	6.15	1375
β-Elemene	1.14			1.13	**12.78**	0.62	0.47	1390
Longifolene					0.85		0.66	1400
β-Caryophyllene	7.75	7.05	9.04	0.85		**30.59**	4.09	1417
γ-Elemene	2.43							1433
α-*trans*-Bergamotene						0.52	1.10	1434

α-Humulene	0.86	0.79		0.21		9.6	1.11	1453
Seychellene			8.57				3.28	1459
γ-Gurjunene							1.53	1474
γ-Muurolene	0.42				0.55	0.57		1477
Germacrene D	4.81					3.10	0.21	1478
β-Selinene	5.20				5.47	3.22	1.41	1483
α-Selinene	3.33				0.68	3.31	1.93	1493
α-Murolene	0.90			0.26	1.18	1.54	6.51	1498
β-Bisabolene							0.71	1506
γ-Cadinenoe			7.07		0.73		0.62	1511
cis-Calamenene					0.74		0.8	1518
δ-Cadinene	1.78					2.11	6.08	1521
α-Calacorene							1.10	1538
Germacrene B	13.33	2.78		0.87				1560
trans-Nerolidol						0.34	1.98	1560
Spathulenol		1.83		0.69	1.94	1.47		1576
Caryophyllene oxide				**29.72**	**12.81**	**20.63**	7.58	1582
Khusimone	0.71	7.36		1.05				1591
Humulene 1,2-epoxide						3.68	2.06	1606
Dill apiole			8.93					1623
1-*epi*-Cubenol			4.08	1.00	1.16		1.67	1627
γ-Eudesmol				0.45	1.15			1631
epi-α-Cadinol			**9.17**	**13.96**	5.82		2.5	1641
Cubenol	1.03	0.35						1642
α-Muurolol	0.97	0.58	3.54	4.27	1.16		5.03	1645
α-Eudesmol				5.06	**22.29**		1.19	1649
α-Cadinol				**28.82**	**25.50**	1.89	0.65	1661
N-Tetradecanol		**19.93**						1673
α-Bisabolol							7.37	1681
Hexadecanol		**32.56**						1877
Octadecanol		5.05						2080
Total Identified	**98.12**	**88.8**	**71.46**	**88.34**	**96.3**	**90.4**	**87.43**	

1-leaves; 2-branches; PAL= *P. altsonii*; PLA = *P. laxiflorum*; PPA=*P. pallidum*; *PPVR =P. paniculatum var.riedelianum*

PAL.1	**PAL.2**	**PLA.1**	**PPA.1**	**PPA.2**	**PPVR.l**	**PPVR.2**

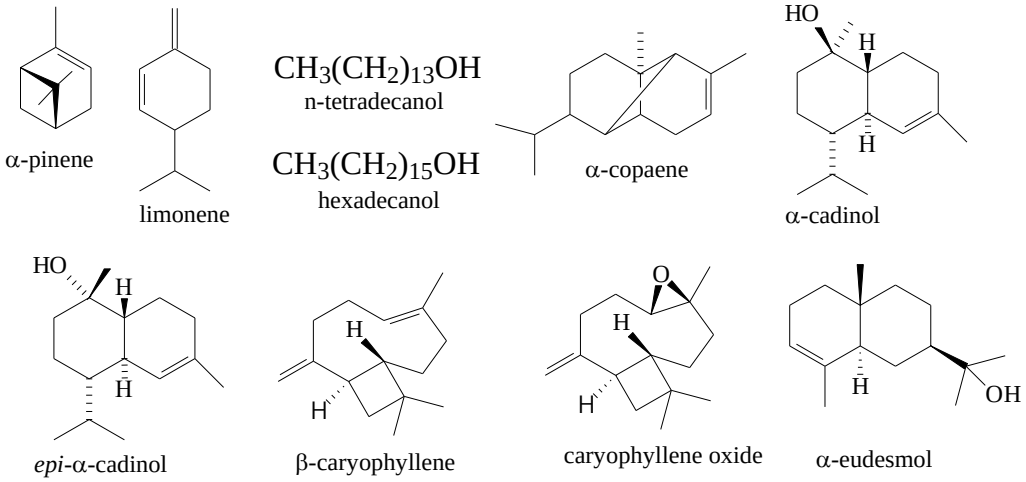
Fonte: Autores.

Table 2. Types of compounds identified in essential oil of species of *Protium* and their respective percentages.

Essential oils	Monoterpenes		Sesquiterpenes		Others
	Hydrocarbon	Oxygenated	Hydrocarbon	Oxygenated	
PAL-1	46.89 (6)	3.54 (2)	44.98 (14)	2.71 (3)	
PAL-2	3.04 (2)	5.80 (2)	12.3 (4)	10.12 (4)	57.54 (3)*
PLA-1	2.08 (1)		43.66 (4)	16.79 (3)	8.93 (1)**
PPA-1			3.32 (5)	56.2 (8)	28.82 (1)*
PPA-2			24.47 (9)	71.83 (8)	
PPVR-1	1.79 (3)	2.50 (6)	58.1 (11)	26.12 (4)	1.89 (1)
PPVR-2	1.60 (4)	15.72 (11)	38.63 (18)	22.01 (7)	9.47 (3)*

–In brackets, the number of compounds found; * long-chain fatty alcohols; **phenylpropanoid. Fonte: Autores.

Figure 1. Chemical structures of the predominant compounds of the essential oils.



Fonte: Autores.

4. Conclusion

The chemical profile of the essential oils of the aerial parts of the four species of *Protium* occurring in the Adolpho Ducke Forest Reserve consisted of monoterpenes, sesquiterpenes and linear long-chain fatty alcohols with a predominance of monoterpenes in the leaves, and linear long-chain fatty alcohols in the branches of *Protium altsonii*; hydrocarbon sesquiterpenes in the branches of *P. laxiflorum* and the leaves and branches of *P. paniculatum var. riedelinum*; and oxygenated sesquiterpenes in the leaves and branches of *P. pallidum*. These results presented herein contribute to the knowledge of the aromatic flora of the Ducke Reserve.

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