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Characterization of essential oils from odoriferous resins of Amazonian species of *Protium* Burm. f.

Caracterização dos óleos essenciais de resinas odoríferas de espécies amazônicas de *Protium* Burm. f.

Caracterización de aceites esenciales a partir de resinas odoríferas de especies amazónicas de *Protium* Burm. f.

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Abstract
The odoriferous resins produced by the trees of *Protium* spp (Burseraceae) are known in the Amazonian region as "breus" where they have been used to caulk canoes, as a mosquito repellent, and also have potential use in perfumery. Most species in this region have had the essential oils of their resins characterized, thus, the objective of this study was to evaluate for the first time the volatile constituents from the resin of *Protium gallosum* Daly, *P. paniculatum* Engl., *P. pillosum* (Cuatrec.) Daly and *P. paniculatum var. riedelianum* (Engl.) Daly. The samples from the individuals that had been previously identified were collected at the Adolpho Ducke Forest Reserve and subjected to hydrodistillation in a Clevenger apparatus, and the essential oils obtained were analyzed using GC-FID and GC-MS. The essential oil of *P. gallosum* showed high percentages of oxygenated monoterpenes (43.61%) with a predominance of terpin-4-ol (25.15%), and *P. paniculatum* consisted exclusively of monoterpenes that were predominantly *p*-cymene (43.52%; hydrocarbon monoterpene) and the 1,8-cineole (16.83%; oxygenated monoterpene). Most of the monoterpenes identified from *P. pillosum* and *P. paniculatum var. riedelianum* were hydrocarbons with a predominance of α-pinene, with 37.74% and 50.93%, respectively. 1,8-Cineole (21.89%) was also identified in the essential oil of *P. pillosum*. The results obtained this study add chemical knowledge to Burseraceae resins from a biological reserve of the Central Amazon.
Keywords: *Protium gallosum*; *Protium paniculatum*; *Protium pillosum*; *Protium paniculatum var. riedelianum*.

Resumo
As resinas odoríferas produzidas pelas árvores de *Protium* spp (Burseraceae) são conhecidas na região amazônica como "breus" onde têm sido usadas para calafetar canoas, como repelente de mosquitos, e também têm potencial uso em perfumaria. A maioria das espécies desta região teve os óleos essenciais de suas resinas caracterizados, assim o objetivo deste estudo foi a avaliação pela primeira vez dos constituintes voláteis da resina de *Protium gallosum* Daly, *P. paniculatum* Engl., *P. pilosum* (Cuatrec.) Daly e *P. paniculatum var. riedelianum* (Engl.) Daly. As amostras dos

indivíduos previamente identificados foram coletadas na Reserva Florestal Adolpho Ducke e submetidas à hidrodestilação em aparelho de Clevenger e os óleos essenciais obtidos foram analisados por CG-DIC e CG-EM. O óleo essencial de *P. gallosum* apresentou altas porcentagens de monoterpenos oxigenados (43,61%) com predominância de terpin-4-ol (25,15%), *P. paniculatum* consistiu exclusivamente de monoterpenos predominantemente o *p*-cimeno (43,52%; monoterpeno hidrocarboneto) e o 1,8-cineol (16,83%; monoterpeno oxigenado). A maioria dos monoterpenos identificados de *P. pilosum* e *P. paniculatum var. riedelianum* foram hidrocarbonetos com predominância de α-pineno, com 37,74% e 50,93%, respectivamente. 1,8-Cineol (21,89%) também foi identificado no óleo essencial de *P. pilosum*. Os resultados obtidos neste estudo agregam conhecimento químico às resinas de Burseraceae de uma reserva biológica da Amazônia Central.
Palavras-chave: *Protium gallosum*; *Protium paniculatum*; *Protium pillosum*; *Protium paniculatum var. riedelianum*.

Resumen
Las resinas odoríferas producidas por los árboles de *Protium* spp (Burseraceae) son conocidas en la región amazónica como "breus" donde se utilizan para calafateo de canoas, como repelente de mosquitos y también tienen potencial en perfumería. La mayoría de las especies de esta región tenían caracterizados sus aceites esenciales a partir de sus resinas, así, el objetivo de este estudio fue evaluar por primera vez los constituyentes volátiles de la resina de *Protium gallosum* Daly, *P. paniculatum* Engl., *P. pilosum* (Cuatrec.) Daly y *P. paniculatum var. riedelianum* (ingl.) Daly. Las muestras de individuos previamente identificados fueron colectadas en la Reserva Forestal Adolpho Ducke y sometidas a hidrodestilación en un aparato Clevenger y los aceites esenciales obtenidos fueron analizados por GC-FID y GC-MS. El aceite esencial de *P. gallosum* presentó altos porcentajes de monoterpenos oxigenados (43,61 %) con predominio de terpin-4-ol (25,15 %), *P. paniculatum* consistía exclusivamente en monoterpenos con predominio de p-cimeno (43,52%; monoterpeno hidrocarbonado) y 1,8-cineol (16,83%; monoterpeno oxigenado). La mayoría de los monoterpenos identificados de *P. pilosum* y *P. paniculatum var. riedelianum* fueron hidrocarburos con predominio de α-pineno, con 37,74% y 50,93%, respectivamente. También se identificó 1,8-Cineol (21,89%) en el aceite esencial de P. pilosum. Los resultados obtenidos en este estudio agregan conocimiento químico a las resinas de Burseraceae de una reserva biológica en la Amazonía Central.
Palabras clave: *Protium gallosum*; *Protium paniculatum*; *Protium pillosum*; *Protium paniculatum var. riedelianum*.

1. Introduction

The odoriferous resins produced by the trees of *Protium* spp (Burseraceae) are known in the Amazonian region as "breus" and have been used to caulk canoes, as a mosquito repellent, and also have potential use in perfumery. Most species in this region have had the essential oils of their resins characterized, and many studies have been done with resins collected in the Adolpho Ducke Forest Reserve located to northeast of the city of Manaus, AM, Brazil (latitude 02º 55′ S, longitude 59º 59′ W) since this forest fragment has had its flora identified and mapped, which favors the studies of their species (Ribeiro et al., 1999). The volatile compositions of the resins are rich in monoterpenes as *P. strumosum*, which consists of high concentrations of limonene (75.5%) and *p*-cymene (31.5%); *P. altsonii* showed *trans*-dihydro-α-terpineol (25.8%) (Zoghbi et al. 2005); α-pinene (17.57%) and limonene (46.11%) predominated in *P. aracouchini* (Lima et al., 2021). High levels of *p*-cymene (35.8 and 38.9%) and limonene (90.93%) were also found in the resin of *P. hebetatum* whose production is stimulated by movement of insects on its surface (Pinto et al., 2010) and *P. spruceanum* (Lima et al. 2014), respectively. In the essential oils of the resin from *P. hebetatum*, produced via chemical induction, α-pinene (28.9-23.7%), *o*-cymene (18.03-31.16%) and limonene (31.89-14.95%) were identified, with limonene being predominant (Lima et al., 2016).

Studies conducted by Ramos et al. (2000), with six species of *Protium*, showed that the essential oils of the resin had variable percentages of *p*-cymene (11.3-90%). Essential oils that are rich with high levels of α-pinene (42.9%) and *p*-cymene (33.3%) have shown potential against *Leishmania* (Santana et al., 2020). The resin from *Protium* with sesquiterpene as the predominant constituent are rare and *Protium decandrum* has been found to be rich in *trans*-α-bergamotene (47.7%) (Carvalho et al., 2010). However, there is a lack of chemical studies on the resin from *Protium gallosum* Daly, *P. paniculatum* Engl., *P. pillosum* (Cuatrec.) Daly and *P. paniculatum var. riedelianum* (Engl.) Daly. Thus, this is the first study related to evaluation of the volatile constituents in the resin of these four species.

2. Methodology

The samples of the resins were collected from specimens of *Protium gallosum. P. paniculatum. P. pillosum* and *P. paniculatum var. riedelianum* located in the Adolpho Ducke Forest Reserve. The essential oils were obtained using hydrodistillation extraction in a Clevenger apparatus over the course of 4 hours.

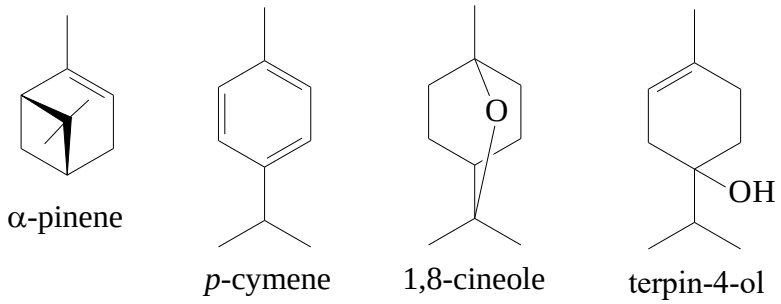
The sample oils were analyzed in a GC-MS (QP5000, Shimadzu), operating using electron impact (70 eV), with a DB-5 capillary column (30 m × 0.25 mm × 0.25 μm). The operating conditions were as follows: carrier gas was helium (flow 10 $mL.min^{-1}$); temperature programmed at 60-240 ºC (3 $ºC.min^{-1}$); injection size of 1.0 μL; sample injection temperature at 250 ºC; detector temperature 290 ºC; split 1:20. The volatile components were identified by comparing their mass spectrum to those of the GC-MS database (NIST 62.lib), the literature (McLafferty and Stauffer 1989) and retention indices (Adams, 2007). Quantitative analysis was performed using GC-FID (GC 2010, Shimadzu) under the same conditions as the GC-MS method.

3. Results and Discussion

The essential oils obtained via hydrodistillation provided the following high yields: 6.4% from *P. gallosum* and 6.1% from *P. pillosum*, and the yields of essential oil from *P. paniculatum* and *P. paniculatum* var. *riedelianum* were 1.6 and 1.2% respectively. The chemical compositions of the essential oils are compiled in Table 1, and the volatile compounds are mainly monoterpenes and sesquiterpenes, but the levels of sesquiterpenes of the samples of essential oils are low (<25%) as shown in Table 2. The essential oil of *P. gallosum* showed high percentages of oxygenated monoterpenes (43.61%) with a predominance of terpin-4-ol (25.15%), and *P. paniculatum* consists exclusively of monoterpenes, with the hydrocarbon, *p*-cymene (43.52%; hydrocarbon monoterpenes) and 1,8-cineole (16.83%; oxygenated monoterpenes) predominating. The majority of the monoterpenes identified in *P. pillosum* and *P. paniculatum* were hydrocarbons with predominance of α-pinene, with 37.74% and 50.93%, respectively. 1,8-Cineole (21.89%) was also identified in the essential oil of *P. pillosum*. Figure 1 illustrates the molecules of the predominant constituents of essential oils.

The monoterpene hydrocarbons α-pinene (13.7-61.8%) and *p*-cymene (11.3-90.0%) have previously been found at different levels in resin samples of *Protium* from the Amazonian region (Ramos et al., 2000; Lima et al., 2016), with the presence of *p*-cymene as a main constituent, which is associated with aged resin. Chemical studies of the resin of *P. pilosum* has not been previously performed, but the monoterpenes α-pinene (31.7%) and *p*-cymene (31.2%) were found in aerial parts of this species (Zoghbi et al., 2005). The bicyclic monoterpene α-pinene plays a crucial role in the fragrance and flavor industry since it has a fresh pine scent and a woody flavor (Vespermann et al., 2017) and have been reported by wide range of pharmacological activities (Salehi, et al., 2019, Joshi et al., 2020; Allenspach & Steuer, 2021). The scent of *p*-cymene has been described as woody and spicy (Lasekan et al., 2013). The oxygenated monoterpene terpin-4-ol was identified with high percentages in aerial parts of the Amazonian species *P. decandrum* (Carvalho et al., 2010) and 1,8-cineole from the resin of *P. heptaphyllum* from northeastern Brazil (Mobin et al., 2016; Rocha et al., 2022). Studies of the essential oils from *P. paniculatum var. riedelianum* have been performed using the leaves, which showed themselves to be rich in β-caryophyllene (30.59%) and caryophyllene oxide (20.63%) (Lima et al., 2022).

Figure 1. Main components of *Protium sp* essential oils from the resin.



Source: Authors.

Table 1. Volatiles (%) of the essential oils from *P. galosuum, P. paniculatum, P. pillosum* and *P. paniculatum var.riedelianum.*

Compounds	PGA	PPA	PPI	PPVR	KI
tryciclene			0.65		**926**
α-pinene	4.13	0.34	**37.74**	**50.93**	932
camphene			0.21	2.88	946
sabinene	1.55		0.93		970
β-pinene	1.69		5.97	5.91	974
α-phellandrene		8.27	7.71		1015
3-*p*-menthene				0.72	982
α-terpinene		5.59		0.25	1014
o-cymene	1.25		0.5	10.14	1021
p-cymene		**43.52**			1026
limonene	1,.53			7.73	1029
1,8-cineole	0.95	**16.83**	**21.89**		1027
β-phellandrene		7.06			1032
γ-terpinene	0.25	0.40	0.29	0.45	1055
δ-terpinene	0.36				1061
fenchone				0.64	1082
terpinolene	0.25			0.43	1085
p-cymenene		0.46			1087
trans-sabinene hydrate		0.56			1095
trans-sabinol	2.23			0.32	1134
camphor	1.17		0.41	3.77	1137
cis-dyhydro-α-terpineol		1.95		5.96	1141
trans-dyhydro-α-terpineol	0.83	0.78		0.31	1156
trans-pinocamphone	0.69				1160
3-thujanol	0.74		0.50		1161
terpin-4-ol	**25.15**	1.76			1172
p-cymen-8-ol		1.98			1181
α-terpineol	6.23	4.58	6.70	1.58	1187
myrtenal	1.02				1189
myrtenol	1.59				1192

verbenone	3.01				1202
carvone		0.48			1243
carvenone		1.93			1252
β-bourbonene	0.39				1382
β-elemene	0.85				1390
cis-caryophyllene			0.57		1398
trans-caryophyllene	5.91		8.02		1418
γ-elemene	1.39				1433
α-humulene	0.78		0.50		1451
γ-muurolene	1.04				1477
germacrene B	6.32				1560
caryophyllene oxide	3.42				1582
α-bisabolol	1.28			1.28	1670
n-dodecanol			0.61		1674
epi-α-bisabolol	4.48			4.48	1686
benzyl benzoato			4.45		1754
Total Identificado	**80.48**	**96.49**	**97.65**	**97.78**	

PGA = *P. gallosum*; **PPA** = *P. paniculatum;* **PPI** = *P. pillosum***; PPVR** = *P. paniculatum var.riedelianum.* Source: Authors.

Table 2. Types of compounds (%) identified in the essential oils.

Essential oils	Monoterpenes		Sesquiterpenes		Others
	Hydrocarbon	Oxygenated	Hydrocarbon	Oxygenated	
P. gallosum	11.01 (8)	43.61 (11)	16.68 (7)	9.18 (3)	
P. paniculatum	65.64 (7)	30.85 (9)			
P. pillosum	54.0 (8)	29.50 (4)	9.09 (3)		5.06 (2)
P. paniculatum var.riedelianum	79.44 (9)	12.58 (6)		5.76 (2)	

In brackets, the number of compounds found. Source: Authors.

4. Conclusion

In the analysis of the chemical profile of the volatile constituents from the resins collected from *Protium* specimens in the Adolpho Ducke Forest Reserve, high percentages of monoterpene hydrocarbons such as α-pinene from the species *P. pillosum* and *P. paniculatum* var. *riedelianum,* and *p*-cymene from the species *P. paniculatum* were detected. The high levels of the oxygenated monoterpenes 1,8-cineole from *P. paniculatum* and *P. pillosum* and terpin-4-ol from *P. gallosum* were found. As such, the results obtained through this study add to the chemical knowledge of resins from Burseraceae that is found in a biological reserve in the Central Amazon.

The chemical composition and variation of the predominant monoterpenes in *Protium* resin is evidently related to metabolism of species and/or exposure time da resin on tree trunks. The high levels of α-pinene in the essential oils of *P. pillosum* and *P. paniculatum var.riedelianum* can be indicative of fresh resins because the odor of this monoterpene can be detected in the field work by the fresh, camphor and woody aroma.

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