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Essential oils, chemical stimulation of resin and occurrence of galls in three Amazonian species of *Protium*

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Abstract
Protium is the most frequent genus of Burseraceae in the Adolpho Ducke Forest Reserve (Manaus, Amazonas), which is where its species were previously identified and mapped and this has favored various studies of this central Amazon forest. A number of *P. divaricatum* Engl., *P. opacum* Swart and *P. strumosum* Daly trees were selected with the objective of providing information about their chemical and biological aspects. Resin exudation was stimulated with the use of ethephon and the essential oils from the aerial parts and the resin were evaluated using GC. The occurrence of galls was also registered. The hydrocarbon sesquiterpene ß-caryophyllene was detected in high percentages in the essential oils of leaves and branches of *P. divaricatum* (52.75% and 14.74%) and *P. opacum* (39.55% and 17.86%), respectively. The predominant oxygenated sesquiterpenes were spathulenol in the leaves (19.52%) and branches (31.29%) of *P. divaricatum*, in addition to khusimone (32.36%) in the branches of *P. opacum*. Monoterpenes were identified in the essential oils of the *P. strumosum* resin, with *p*-cymene (58.97%) predominating in the naturally exuded resin, limonene (61.45%) and *p*-cimen-8-ol (53.92%) in the resin of the first and second extraction with ethephon, respectively. On the trees, galls were identified that will help in the identification of the three species.

Keywords: Breu, ß-caryophyllene, khusimone, limonene, *p*-cymene

Óleos essenciais, estimulação química de resina e ocorrência de galhas de três espécies amazônicas de *Protium*

Resumo
Protium é o gênero mais frequente de Burseraceae da Reserva Florestal Adolpho Ducke (Manaus, Amazonas), onde as suas espécies foram previamente identificadas e mapeadas favorecendo os vários estudos dessa floresta da Amazônia Central. Selecionamos algumas árvores de *P. divaricatum*, *P. opacum* e *P. strumosum* como o objetivo de fornecer informações sobre seus aspectos químicos e biológicos. Estimulamos à exsudação de resina com o uso de ethephon e avaliamos por CG os seus óleos essenciais das partes aéreas e resina. Registramos também a ocorrência de galhas. O sesquiterpeno hidrocarboneto ß-caryophyllene foi detectado em altos percentuais nos óleos essenciais de folhas e galhos de *P. divaricatum* (52,75% e14,74%) e *P. opacum* (39,55% e 17,86 %), respectivamente. Os sesquiterpenos oxigenados predominantes foram o espatulenol de folhas (19,52%) e galhos (31,29%) of *P. divaricatum* além da khusimona (32,36%) dos galhos de *P. opacum*. Nos óleos essenciais da resina de *P. strumosum* foram identificados monoterpenos, predominando o *p*-cymene (58,97%) na resina exsudada naturalmente, limonemo (61,45%) e *p*-cimen-8-ol (53,92%) na resina da primeira e segunda extração com ethephon, respectivamente. Nas árvores, foram identificadas galhas que auxiliarão na identificação das três espécies.

Palavras-chave: Breu, ß-cariofileno, khusimona, limoneno, *p*-cimeno

Introduction

Natural resins have been used since ancient times as constituents of varnishes, adhesives, in the preparation of cosmetics and for medicinal purposes, in addition to being used as incense in ritual ceremonies in temples and churches. In the Burseraceae family, some species belonging to two genera produce resinous exudates (gum-resin), such as myrrh, the exudate obtained from trees of species of *Commiphora*, frankincense produced by trees of the genus

Boswellia, which have considerable therapeutic and commercial value (Hanus *et al*., 2005, Shen & Lou, 2008). The Brazilian Burseraceae resin is mainly obtained from *Protium* species, with the Amazon basin being the primary center for diversity of the genus, due to the occurrence of 73 species, of which 42 of them are endemic in the region (Daly, 1992). Most species are locally known as “breu”, which consist of a volatile perfumed fraction (essential oil) and a non-volatile fraction, usually composed of terpenoids and steroids. “Breus” are used to caulk wooden boats, as incense in religious ceremonies, as an insect repellent, and for headache treatment, as well as having potential for use in perfumery.

The Adolpho Ducke Forest Reserve in Manaus, Amazonas state has 26 species of *Protium* that have been previously identified and mapped and which have favored the various chemical and biological studies in this forest of the central Amazon. The taxonomy of the genus is confusing and marked by several synonyms; however, the morphotypes of the galls found in the forest reserve have been of great importance for the knowledge of its diversity and also as a tool for species identification (Ribeiro *et al*., 1999). Most species are producers of resin with a pleasant odor, which is secreted by the tree trunk naturally or through injuries. More recent studies with species in this forest reserve have shown that stimulation of resin exudation using a solution of 2-chloroethylphosphonic acid (ethephon) on *P. aracouchini* (Lima *et al*., 2021) and *P. hebetatum* (Lima *et al*., 2016) increased the production of resin and the contents of its essential oils.

The chemical composition of volatiles from *Protium* species from Ducke Reserve has been investigated and has shown that essential oils extracted by hydrodistillation are characterized by producing monoterpenes and sesquiterpenes. In most species, monoterpenes predominate in the essential oils of the resins (Pinto *et al*., 2010, Lima *et al*., 2016, Santana *et al*., 2020) and sesquiterpenes are predominant in aerial parts (Carvalho *et al*., 2009, Carvalho *et al*., 2013, Pinto *et al*., 2010, Oliveira *et al*., 2018, Lima *et al*., 2022). Now, three species from the Ducke Reserve have been selected for evaluation, namely, *Protium divaricatum* Engl. [syn *Tingulonga divaricata* (Engl.) Kuntze], *Protium opacum* Swart and *P. strumosum* Daly with the objective of providing information about their chemical and biological aspects.

Materials and Methods

Fieldwork

The fieldwork was carried out in the Adolpho Ducke Forest Reserve (AM-010, km 26) with the following specimens previously mapped and identified by Douglas C. Daly (New York Botanical Gardens, USA) and the taxonomist José Eduardo L. S. Ribeiro (INPA) during the Flora Project (Ribeiro *et al*., 1999) at the Instituto Nacional de Pesquisas da Amazônia (INPA): *P. divaricatum* (No. 1323 and 2309), *P. opacum* (No. 1628 and 3442), and *P. strumosum* (No. 1670 and 1672). Resin found on the trunk of tree 1670 (*P. strumosum*) was collected, and tree 1672, as well as trees of *P. divaricatum* and *P. opacum*, which did not present resin. These were then subjected to the experiment with 2-chloroethylphosphonic acid (ethephon) to stimulate exudation. A hole of 0.5 cm in diameter was made in the trunk at a height of 1.30 m and an 2-chloroethylphosphonic acid water solution of 0.4 mg/mL was used. For the control, only distilled water was used (for each tree).

Resin from tree 1672 was collected 40 days after the experiment and, then, this tree was observed for a period of 6 months. Samples of resin were collected to obtain their essential oils. The leaves and branches of the individuals No. 2309 and 1628 were collected to obtain essential oils. Samples of branches containing foliar galls were collected and transported in plastic bags with moistened paper to the Laboratory of Plant Taxonomy at Instituto Nacional de Pesquisas da Amazônia, where the galls that had been previously separated into morphotypes were then photographed.

Extraction of essential oils and analysis of volatile constituents

The essential oils were obtained by hydrodistillation extraction in a Clevenger system over the course of 4 hours. Analyses were performed on a GC-MS (QP5000, Shimadzu), operating by electron impact (70 eV), which used a DB-5 capillary column (30 m × 0.25 mm × 0.25 μm) in the GC experiment. 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 database of the GC-MS (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.

Results and discussion

The individuals of *P. divaricatum* and *P. opacum* selected for this study did not present naturally secreted resins in their trunks, nor when stimulated by the use of ethephon. The essential oil yields obtained by hydrodistillation and the volatile constituents of the aerial parts are shown in Table 2 and 3, respectively.

The trunk of tree No. 1672 exuded 19.9 g of resin (1672 A) in 40 days after the application of the ethephon and, in following 40 days, it produced 5.45 g of resin (1672 B); however, after this second collection, there was no more exudation during the observation period (six months). In the hole without ethephon (control), there was no production of resin. The essential oil yield of the resin from the first ethephon exudation (1672 A) was about 5 times higher than that of the resin exuded naturally from the tree 1670. Similar yields of essential oils were reported in ethephon-exuded resin from *P. hebetatum* from the Adolpho Ducke Forest Reserve (Lima *et al*., 2016).

The volatile constituents of the aerial parts are shown in Table 2, and are grouped into monoterpenes and sesquiterpenes of the hydrocarbon and oxygenated type (Table 3).

Table 1. Yields (%) of essential oils by hydrodistilling of leaves, branches and resin of *Protium*.

Individual	Tree No.	Yield of essential oil (%)		
		Leaves	Branches	Resin
P. divaricatum	2309	0.31	0.08	
P. opacum	1628	0.07	0.07	
P. strumosum	1670			4.4
P. strumosum	1672			21.9 (1672 A) 9.7 (1672 B)

A - 1nd resin collected (40 days after the application of the ethephon); B - 2nd resin collected (80 days after the application of the ethephon.

Table 2. Chemical composition (%) of essential oils from aerial parts of *Protium divaricatum* and *Protium opacum* in the Adolpho Ducke Forest Reserve.

Constituent	*P. divaricatum*		*P. opacum*		RI Exp
	leaves	branches	leaves	branches	
α-pinene				1.76	932
trans-sabinol				0.94	1135
α-terpineol			0.48		1189
α-copaene	1.16	2.43	14.24	1.42	1375
ß-elemene	1.68	3.48			1392
cyperene	2.61	3.47			1398
δ-gurjunene			1,86		1410
ß-caryophyllene	52.75	14.74	39.55	17.86	1420
α-humulene	7.35	4.15	8.24	3.26	1451
seychellene			0.79		1458
δ-muurolene			1.28		1469
γ-gurjunene	0.63	0.45			1475
γ-muurolene	0.78	1.37		3.75	1478
ß-selinene	tr	0.68			1487
viridiflorene				4.29	1492
α-selinene	tr	0.63			1495
α-muurolene	0.76	1.55	1.47	1.22	1504
cis-calamenene				2.36	1521
δ-cadinene	0.52	1.53	5.44		1521
α-calacorene			0,51	0,66	1541
trans-nerolidol			0.54		1565
spathulenol	19.52	31.29		10.17	1579
khusimone			3.31	32.36	1591
1-*epi*-cubenol	0.75	0.68	0.94		1629
epi-α-cadinol	0.65	3.49	2.58	3.85	1638
cubenol	0.67	2.08	0.68		1641
α-eudesmol	tr	1.08			1649
α-murolol			2.67	6.76	1649
Total	89.83	72.65	84.58	90.66	

tr = traces of the substance (< = 0.31%).

ß-Caryophyllene was detected in high percentages in the four essential oils especially from the leaves. The importance of this bicyclic sesquiterpene hydrocarbon has been widely reported due to its various pharmacological activities, which include the treatment of metabolic and neurological disorders, chronic pain and inflammation (Fidyt *et al*., 2016; Francomano *et al*., 2019, Maffei, 2020). α-Copaene was another hydrocarbon sesquiterpene identified in high percentages in *P. opacum* leaves. The most abundant oxygenated sesquiterpenes were spatulenol, which was identified in leaves and branches of *P. divaricatum* and khusimone from branches of *P. opacum*.

The chemical composition of the essential oils of the naturally exuded resin and when stimulated by the use of ethephon consisted of nineteen monoterpenes, nine of which are hydrocarbons and the others are oxygenated monoterpenes (Table 4.) In the naturally exuded resin (1670), *p*-cymene was identified as the major volatile constituent (58.97 %). In the resin stimulated by ethephon in the first 40 days, limonene was more abundant (61.45%); however, in the resin obtained after 80 days, the limonene content was reduced (14.14%), and the monoterpene *p*-cimen-8-ol (53.92%) predominated in the chemical composition.

Table 3. Types of terpenes (%) identified in the essential oils of *P. divaricatum* and *P. opacum* in the Adolpho Ducke Forest Reserve.

Mono and sesquiterpenos	KI	*P. divaricatum*		*P. opacum*	
		leaves	branches	leaves	branches
Hydrocarbon monoterpenes	932				1.76
Oxygenated monoterpenes	1135-1189			0.48	0.94
Hydrocarbon sesquiterpenes	1375-1541	69.27	65.32	73.38	34.82
Oxygenated sesquiterpenes	1565-1649	20.92	7.33	10.72	53.14

KI = Kovats retention index; tr = traces of the substance (< = 0.31).

The chemical composition of the volatiles extracted from the resin by hydrodistillation differs from the volatiles extracted with hexane (Zuñiga *et al*., 2017). In previous studies, the monocyclic monoterpenes limonene and *p*-cymene were detected in high concentrations in the essential oils of resins from *Protium* species from the Adolpho Ducke Forest Reserve: limonene contents ranged from 14.5 to 46.11% (Lima *et al*., 2016; Lima *et al*., 2021) and *p*-cymene ranged from 33.3 to 74.6% (Pinto *et al*., 2010; Santana *et al*., 2020).

Based on previous studies related to the stimulation of resin with ethephon (Lima *et al*., 2016 and Lima *et al*., 2021), the differences between *Protium* species regarding production of resin, yield of essential oil and percentages of the predominant constituents became evident.

The resins that exude from the trunks of *Protium* solidify when exposed to air due to the evaporation and oxidation of their volatile constituents; therefore, the results of this paper suggest that the resin stimulated with ethephon in the first 40 days was the freshest and the others underwent oxidative processes, and their predominant components were *p*-cymene and *p*-cymen-8-ol.

P. divaricatum showed galls of isolated occurrence (Figure 1), green color with globoid, fusiform and curled margin morphotypes (Figure 1A-D).

Table 4. Chemical composition (%) of essential oils from *P. strumosum* naturally exuded and stimulated with ethephon resin.

Substância	1670	1672 A	1672B	IR
α-pinene	0.66	10.26		932
camphene	0.19	0.33		946
sabinene		0.71		970
β-pinene	0.49	1.77		974
α-terpinene	4.31			1013
p-cymene	58.97	0.49		1020
limonene	3.2	61.45	14.14	1028
1,8-cineole	3.1	7.54	6.79	1030
γ-terpinene	5.73			1054
terpinolene	2.58			1084
cis-limonene-oxide		1.99		1129
camphor	2.46	0.37		1138
cis-dihydro-α-terpineol	1.49			1138
borneol	0.83			1165
p-methylacetophenone	1.31	0.75	4.52	1183
p-cymen-8-ol	4.13	7.74	53.92	1186
α-terpineol	3.02	2.79		1186
carvone		1.39	7.34	1246
cis-verbenil acetate	0,89			1286
Total	93.36	97.58	86.71	

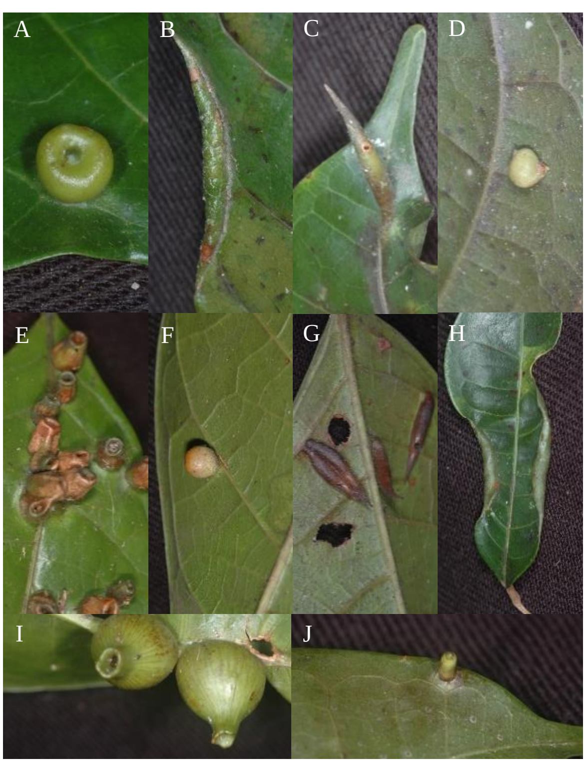

Figure 1. Gall morphotypes associated with *P. divaticatum*. Legend: globoid (A and D), curled margin (B) and conical (C); *P. opacum*: conical (E) (globoid (F), fusiform (G), curled margin (H); *P. strumosum*: ovoid (I), cylindrical (J) - Photos by MP Lima.

The galls found on *P. opacum* were grouped with brown color and isolated with green color, and their morphotypes are shown in Figure 1 (E-H). The foliar galls found on *P. strumosum* were green, and grouped or isolated with morphotypes I and J.

This study shows a difference in the aspects of galls of the three species when compared to the galls of the Ducke Reserve registered by Fernandes (2010), such as morphotypes of isolated occurrence, flattened globoid (A) and conical with pointed apex, green coloration with brown spots (C) of *P. divaricatum*, as well as the conical morphotype, grouped with a brown color (E) and fusiforme morphotype (G) of *P. opacum*. These are also the first reports of galls of *P. strumososm* with these morphological aspects.

Conclusion

Considering the importance of *Protium* resin for the Amazon, this study adds to the knowledge on aromatic flora, and contributes to the extraction of breus of the region and the reporting and characterization of gall morphotypes, which significantly contributes to the identification of the three species of the forest reserve.

In this research carried out with specimens of *Protium strumosum*, it was evidenced that chemical stimulation increases production of resin; however, the chemical composition and the variation of the predominant monoterpenes seems to be related to the exposure time of resin, whether it is exuded naturally or stimulated with ethephon.

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References

Adams, R. P. (2007). Identification of essential oil components by gas chromatography/mass spectroscopy (4ª ed.). Carol Stream, Illinois: Allured Publishing Corporation.

Carvalho, L. E., Pinto, D. S., Lima, M. P., Marques, M. O. M., & Facanali, R. (2009). The chemistry of essential oils of *Crepidospermum rhoifolium*, *Trattinnickia rhoifolia* and *Protium elegans* of the Amazon Region. *Journal of Essential Oil Bearing Plants*, *12*(1), 92-96. doi: 10.1080/0972060X.2009.10643698

Carvalho, L. E., Lima, M. P., Magalhães, L. A. M., Marques, M. O. M., & Facanali, R. (2013). Essential oils of *Protium* of the Adolpho Ducke Forest Reserve: *Protium crassipetalum*, *P. heptaphyllum* subs. *ulei*, *P. pilosissimum* and *P. polybo*tryum. *Journal of Essential Oil Bearing Plants*, *16*(4), 551-554. doi: 10.1080/0972060X. 2013.831557

Daly, C. D. (1992). New taxa and combinations in *Protium* Burm. f. Studies in neotropical Burseraceae VI. *Brittonia*, *44*(3), 280-299. doi: 10.2307/2806927

Fernandes, S. P. C. (2010). Insetos galhadores associados à família Burseraceae da reserva florestal Ducke, Manaus, Amazonas, Brasil (Dissertação de mestrado). Instituto Nacional de Pesquisas da Amazônia, Manaus, Amazonas. Recovered from https://bdtd.inpa.gov.br/bitstream/tede/2112/5/Disserta%c3%a7%c3%a3o_Sheila%20Patricia%20Carvalho%20%20Fernandes.pdf

Fidyt, K., Fiedorowicz, A., Strządała, L., & Szumny, A. (2016). β-caryophyllene and β-caryophyllene oxide-natural compounds of anticancer and analgesic properties. *Cancer Medicine*, 5(10):3007-3017. doi: 10.1002/cam4.816.

Francomano, F., Caruso, A., Barbarossa, A., Fazio, A., Torre, C.L., Ceramella, J., Mallamaci, R., Saturnino, C., Iacopetta, D. & Sinicropi, M. S. (2019). β-Caryophyllene: a sesquiterpene with countless biological properties. *Applied Sciences*, 9, 5420. doi:

10.3390/app9245420.

Hanus, L. O., Rezanka, T. Dembitsky, V. & Moussaieff, A. (2005). Myrrh-*Commiphora* chemistry. *Biomedical Papers*, *149*(1), 3-27. doi: 10.5507/bp.2005.001

Lima, T. A. A. C., Ribeiro, J. E. L. S., Marques, M. O. M., Facanali, R., & Lima, M. P. (2016). Estimulo para produção de resina em *Protium hebetatum* Daly e avaliação dos constituintes químicos voláteis. *Scientia Amazonia*, *5*(3), 21-24. Recovered from https://scientia-amazonia.org/wp-content/uploads/2016/10/v5-n3-21-24-2016.pdf

Lima, T. A. A. C., Cunha, L. P., Ribeiro, J. E. L. S., Marques, M. O. M., & Lima, M. P. (2021). Evaluation of volatile constituents, exudation of resin and occurrence of galls of *Protium aracouchini* (Aubl.) Marchand. *Acta Brasiliensis*, *5*(3), 88-91.doi: 10.22571/2526-4338559.

Lima, T. A. A. C., Queiroz, D. P., Melo, L. E. S., Oliveira, L. M., Ribeiro, J. E. L. S., Marques, M. O. M., & Lima, M. P. (2022). 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. *Research, Society and Development*, *11*(7), e46111730255. doi: 10.33448/rsd-v11i7.30255.

Maffei, M. E. (2020). Plant natural sources of the endocannabinoid (*E*)-β-caryophyllene: A Systematic quantitative analysis of published literature. *International Journal of Molecular Sciences*, *21*(8), 6540. doi: 10.3390/ijms 21186540

McLafferty, F. W. & Stauffer, D. (1989). The Wiley/NBS Registry of Mass Spectral Data. New York: Wiley –Interscience Pub.

Oliveira, L. M., Queiroz, D. P. K., Melo, L. E. S., Marques, M. O. M., Facanali, R. & Lima, M. P. (2018). Constituintes voláteis dos galhos de quatro espécies de *Protium* ocorrentes na flora da Reserva Ducke. *Scientia Amazonia*, *7*(1), 68-73. Recovered from http://scientia-amazonia.org/wp-content/uploads/2017/08/v7-n1-68-73-2018.pdf

Pinto, D. S., Carvalho, L. E., Lima, M. P., Marques, M. O. M., Facanali, R. & Ribeiro, J. E. L. S. (2010). Volatiles of foliar rachis, branches and resin elicited by insects from *Protium hebetatum* grows wild in Amazon. *Journal of Essential Oil Bearing Plants*, *13*(6), 699-703. doi: 10.1080/0972060X.2010.10643881

Ribeiro, J. E. S., Hopkins, M. J. G., Vicentini, A., Sothers, C. A., Costa, M. A. S., Brito, J. M., Souza, M. A. D., Martins, L. H. P., Lohmann, L. G., Assunção, P. C. L., Pereira, E. C., Silva, C. F., Mesquita, M, R. & Procópio, L. C. (1999). Flora da Reserva Ducke: Guia de Identificação das Plantas Vasculares de uma Floresta de Terra-firme na Amazônia Central. Manaus: INPA/DFID (Eds.), Manaus-Amazonas.

Santana, R. C., Santos Rosa, A. S., Mateus, M. H. S., Soares, D. C., Atella, G., Guimarães, A. C., Carlos Siani, A. C., Ramos, M. F. S., Saraiva, E. M., &Silva, L. H. P. (2020). *In vitro* leishmanicidal activity of monoterpenes presente in two species of *Protium* (Burseraceae) on *Leishmania amazonenses*. *Journal of Ethnopharmacology*, *15*, 112981. doi: 10.1016/j.jep.2020.112 981

Shen, T. & Lou, H. X. (2008). Bioactive constituents of myrrh and frankincense, two simultaneously prescribed gum resins in chinese traditional medicine. *Chemistry & Biodiversity*, *5*(4), 540-553. doi: 10.1002/cbdv.200890051

Siani, A. C., Ramos, M. F. S., Menezes-de-Lima Jr., Ribeiro-dos-Santos, R., Fernandez-Ferreira, E., Soares, R. O. A., Rosas, E. C., Susunaga, G. S., Guimarães, A. C., Zoghbi, M. G. B. & Henriques, M. G. M. (1999). Evaluation of anti-inflammatory-related activity of essential oils from the leaves and resin of species of *Protium*. *Journal of Ethnopharmacology*, *66*(1), 57-69. doi: 10.1016/s0378-8741(98)00148-2

Zuñiga, R. M., Sampaio, P. T. B. & Veiga-Junior, V. F. (2017). Use of ethephon in the extraction of resin in two species of *Protium* Burm f. and evaluation of its effect on hexane extract composition in *Protium strumosum* Daly. *Journal of Essential Oil Research*, *29*(5),1-6. doi: 10.1080/10412905.2017.130340