



## Pharmacognosy

# Development of a gas chromatography method for quantification of triterpenes in the commercial oleoresins from *Protium* species

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### Abstract

Crude non-volatile oleoresin from Burseraceae species (*breu*), a non-timber forest product of the Amazon, still lacks methods for its quality control even though its pharmacological activities have been associated to the major triterpenes  $\alpha$ -amyrin ( $\alpha$ AA),  $\beta$ -amyrin,  $\alpha$ -amyrenone,  $\beta$ -amyrenone and lupeol (LU). Its chemical characterization has usually been determined by gas chromatography (GC) using columns with phases of low polarity, which are ineffective in the separation of  $\alpha$ AA and LU. The present study aimed to develop a GC method to characterize and quantify triterpenes in commercial *breu* with good selectivity and resolution. Columns with phases of different polarity (DB-35, DB-1701, DB-17HT and DB-Innowax) were tested, and DB-17HT separated  $\alpha$ AA and LU without signal suppression or high retention of other constituents. This column allowed the quantification of *breu* triterpenes using flame ionization detection and cholesterol as a reference standard. Good similarity between the calibration curves of cholesterol and  $\alpha$ AA was verified. Internal and external standardizations led to different ( $p < 0.05$ ) quantifications, but the latter showed less deviation ( $RSD \leq 3\%$ ). This work provides a reproducible GC method for the quality control of commercial *breu*, clearly evidencing the presence of lupeol and allowing the accurate quantification of major triterpenes.

**Key words:** gas chromatography, oleoresin, *Protium*, quantification, triterpenes.

### Resumo

A oleoresina bruta não volátil das espécies de Burseraceae (*breu*), um produto florestal não madeireiro da Amazônia, ainda carece de métodos para seu controle de qualidade, embora suas atividades farmacológicas tenham sido associadas aos principais triterpenos  $\alpha$ -amirina ( $\alpha$ AA),  $\beta$ -amirina,  $\alpha$ -amirenona,  $\beta$ -amirenona e lupeol (LU). Sua caracterização química tem sido geralmente determinada por cromatografia gasosa (CG), utilizando colunas com fases de baixa polaridade, que são ineficazes na separação de  $\alpha$ AA e LU. O presente estudo teve como objetivo desenvolver um método de CG para caracterizar e quantificar triterpenos em breu comercial com boa seletividade e resolução. Colunas com fases de diferentes polaridades (DB-35, DB-1701, DB-17HT e DB-Innowax) foram testadas, e DB-17HT separou  $\alpha$ AA e LU sem supressão de sinal ou alta retenção de outros constituintes. Esta coluna permitiu a quantificação de triterpenos do *breu* utilizando detecção de ionização de chama e colesterol como padrão de referência. A boa similaridade entre as curvas de calibração do colesterol e  $\alpha$ AA foi verificada. Padronizações interna e externa levaram a quantificações diferentes ( $p < 0,05$ ), mas a última apresentou menor desvio ( $RSD \leq 3\%$ ). Este trabalho fornece um método de CG reprodutível para o controle de qualidade do *breu* comercial, evidenciando claramente a presença do lupeol e permitindo a quantificação precisa dos principais triterpenos.

**Palavras-chave:** cromatografia a gás, oleoresina, *Protium*, quantificação, triterpenos.

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## Introduction

Oleoresins produced by species of Burseraceae Kunth, popularly known as *breu* or *almécega* (Hernández-Vázquez *et al.* 2010), are among the Amazonian non-timber forest products (NTFP) with the highest commercial potential. These materials are mainly sourced from species of *Protium* Burm.f. and closely related genera (Rüdiger & Veiga-Junior 2013).

The Amazonian *breu* is primarily constituted by a mixture of triterpenes and volatile monoterpenes (Langenheim 2003). The latter are usually separated by submitting the raw material (as fresh as possible) to steam distillation (Siani *et al.* 1999). The resulting essential oil is demanded by perfumery and diverse pharmaceutical products (Costa 1975), besides presenting a wide range of biological properties (Murthy *et al.* 2016). Conversely, the solid residue product of distillation is constituted by the crude triterpene fraction, resembling the hard *breu* collected from the soil beneath the oleoresin-producing tree, where it ages after spontaneous detachment from the tree branches (Siani *et al.* 2012). It has been reported that the commercial oleoresins of *breu* have usually presented exclusively a mixture of triterpenes (Siani *et al.* 1999). This material is a solid that eventually is softened by heating into an amorphous material able to caulk boats, repair wooden objects and act as an excellent film-forming constituent in varnish and waterproofing agent (Costa 1975; Ramos *et al.* 2000).

Although imbued with high potential for commercialization, this dry raw material is sold at low prices in local markets scattered throughout the Amazonian region. Adding value to the Amazonian *breu* would require organization of its production niche and amelioration of the already existing initiatives aiming at exploiting it from this source (UNDP 2012; Silva *et al.* 2016). In this context, the developing methods is an important step to qualify the raw material chemically and to propose some level of standardization for such a raw material (Siani *et al.* 2017).

Efforts have been exerted for establishing the chemical profile of the non-volatile fraction of *breu*, which is mostly constituted by a mixture of the pentacyclic triterpene alcohols  $\alpha$ -amyrin and  $\beta$ -amyrin (Hernández-Vázquez *et al.* 2010), complemented with the corresponding ketones as well as minor amounts of diols and acidic tetracyclic species (Susunaga *et al.* 2001; Vieira

Júnior *et al.* 2005; Siani *et al.* 2012; Rüdiger & Veiga-Junior 2013). These major amyryns have been reported as responsible for the anti-inflammatory, antinociceptive, hepatoprotective and gastroprotective activities of *breu* (Rüdiger *et al.* 2007; Aragão *et al.* 2008; Melo *et al.* 2011).

Gas chromatography (GC) with columns using low polarity stationary phases, composed of polydimethylsiloxanes with 1 to 5% phenyl groups, has been usually applied for the chemical analysis of *breu*. This probably occurs because these columns are efficient in separating the monoterpenes and sesquiterpenes present in the volatile fraction of this oleoresin (Ramos *et al.* 2000; Siani *et al.* 2012; Satyal *et al.* 2017), which has been extensively studied in view of its versatility of applications for the cosmetic industries. Thus, there is a clear tendency to reproduce these analytical conditions in the characterization of the non-volatile fraction of *breu*, which is rich in triterpenes (Maia *et al.* 2000; Guimarães & Siani 2007; Silva *et al.* 2009; Siani *et al.* 2012; Lima *et al.* 2014). However, such stationary phases imply coelution of the  $\alpha$ -amyrin and lupeol, which become indistinguishable when using flame ionization detection (FID) and thus require a mass spectrometry detector for the quantification of these compounds, as seen for other matrices (Moggia *et al.* 2016). In fact, the GC-FID method has not been reported for assay of triterpenes so far. In this way, the present study aims to develop a feasible GC-FID method to quantify the triterpenoid components in this important NTFP.

## Material and Methods

Oleoresin, chemicals and GC columns

A commercial sample (3 kg) of raw *breu* was acquired in a popular market in the municipality of Manaus, Amazonas state, Brazil, in 2001. The material was stored in proper cardboard containers that were maintained in dry places until its utilization.

HPLC/UV grade chloroform (99.8% purity) and dichloromethane were purchased from Tedia (Fairfield, USA). Analytical grade diethyl ether was acquired from Vetec (Rio de Janeiro, Brazil). Cholesterol (95%) and  $\alpha$ -amyrin ( $\geq 98\%$ ) were furnished by Sigma Aldrich (Missouri, USA).

Columns with stationary phases (Agilent, California) of different polarity were applied: three based on polydimethylsiloxane, DB-35 (35% phenyl, 30 m  $\times$  250  $\mu$ m  $\times$  0.25  $\mu$ m film thickness),

DB-1701 (14% cyanopropyl-phenyl, 30 m × 320 μm × 0.25 μm), and DB-17HT (50% phenyl, 30 m × 250 μm × 0.15 μm), and one based on polyethylene glycol, DB-Innowax (30 m × 250 μm × 0.25 μm).

#### Pretreatment of the oleoresin

The commercial hard oleoresin was fragmented into small pieces with the aid of a hammer and then manually pulverized with a pestle in a porcelain mortar. The powdered *breu* was mechanically sieved through a set of stainless sieves (Bertel, Brazil), and particles with sizes below 32 mesh (500 μm) were selected and conditioned at room temperature in an amber glass vessel. This was labeled as Processed Breu Oleoresin (PBO). The percent moisture level was determined in an automatic digital drying scale (Marte ID series 1.8, model ID50), subjecting a 1 g-sample to 105 °C in a quartz-coated infrared heat source until reaching constant weight. The melting point was determined through digital equipment (MQAPF-302 model, Microquímica, Palhoça, Brazil) at the heating rate of 5 °C/min.

#### Gas chromatography with flame ionization detection (GC-FID)

GC-FID was performed in an Agilent 6890N chromatograph equipped with an autoinjection system (Agilent 7683) (Palo Alto, CA, USA). Hydrogen as carrier gas at constant flow rate of 1.8 mL/min; injector set at 290 °C and split ratio 1:25; and injection volume of 1.0 μL. Oven temperature ranged from 50 °C (held for 5 minutes) to 230 °C at 20 °C/min, followed by 5 °C/min until 350 °C (held for 9 minutes). The detector was set at 400 °C and was operated with air at 320 mL/min, hydrogen at 31 mL/min and nitrogen at 10 mL/min.

The calibration curve of cholesterol was built by triplicate injections at eight concentration levels: 2.5, 5.0, 10.0, 50.0, 100, 250, 400, and 500 μg/mL. FID did not respond linearly to a 1000 μg/mL solution that was also injected. The individual concentrations of the triterpenes in sample dilutions were calculated by external standardization, considering the parameters of the linear equation, which were estimated by the least-squares method of the calibration curve of cholesterol (external standard, ES). Concentrations in PBO were determined considering the standard purity and the weight and moisture of the sample. The sensibility of the method was verified by comparing the calibration curve of ES with that

obtained from α-amyrin in the same working range (2.5–500 μg/mL).

The limit of detection (0.4 μg/mL) and the limit of quantitation (1.3 μg/mL) were determined from three other calibration curves (ICH 1997) which were built with cholesterol dilutions at 1.0, 2.5, 5, 10, and 50 μg/mL.

The PBO (100 mg) was weighed at 0.1 precision in a 100 mL volumetric flask and completely dissolved with CHCl<sub>3</sub>, giving a clear solution and so filtration was not performed to avoid concentration errors due to solvent evaporation. Sample dilutions were prepared in triplicate. For studying the application of internal standardization, a 0.1 mg/mL cholesterol solution in CHCl<sub>3</sub> was applied as solvent for other three weightings.

#### Gas chromatography with mass spectrometry (GC-MS)

The samples were completely dissolved at 1 mg/mL in CHCl<sub>3</sub> at room temperature and placed in sealed 1.5 mL vials. The individual constituents of PBO were identified by GC using a chromatograph of the same model as that in GC-FID, but equipped with an Agilent 5973N mass spectrometer (Palo Alto, CA, USA). Runs were performed in the DB-17HT column with helium as carrier gas at flow rate of 1.0 mL/min. The injector was set at 250 °C, and the split ratio was 1:10. Oven temperature was programmed as for the GC-FID procedure. Ion source, transfer line and quadrupole were set at 250, 290 and 150 °C, respectively, with electron impact ionization at 70 eV. Acquisition mass ranged from 70 to 800 Da.

The constituents were identified by comparing their fragmentation patterns to those of the Wiley 275 library (Agilent MSD Productivity ChemStation) and to those found in the literature (including fragmentation pathways) and other mass spectral reference libraries (Vilegas *et al.* 1997; Noronha 2001; Carvalho *et al.* 2010; HMDB 2017; NIST 2017; SDBS 2017).

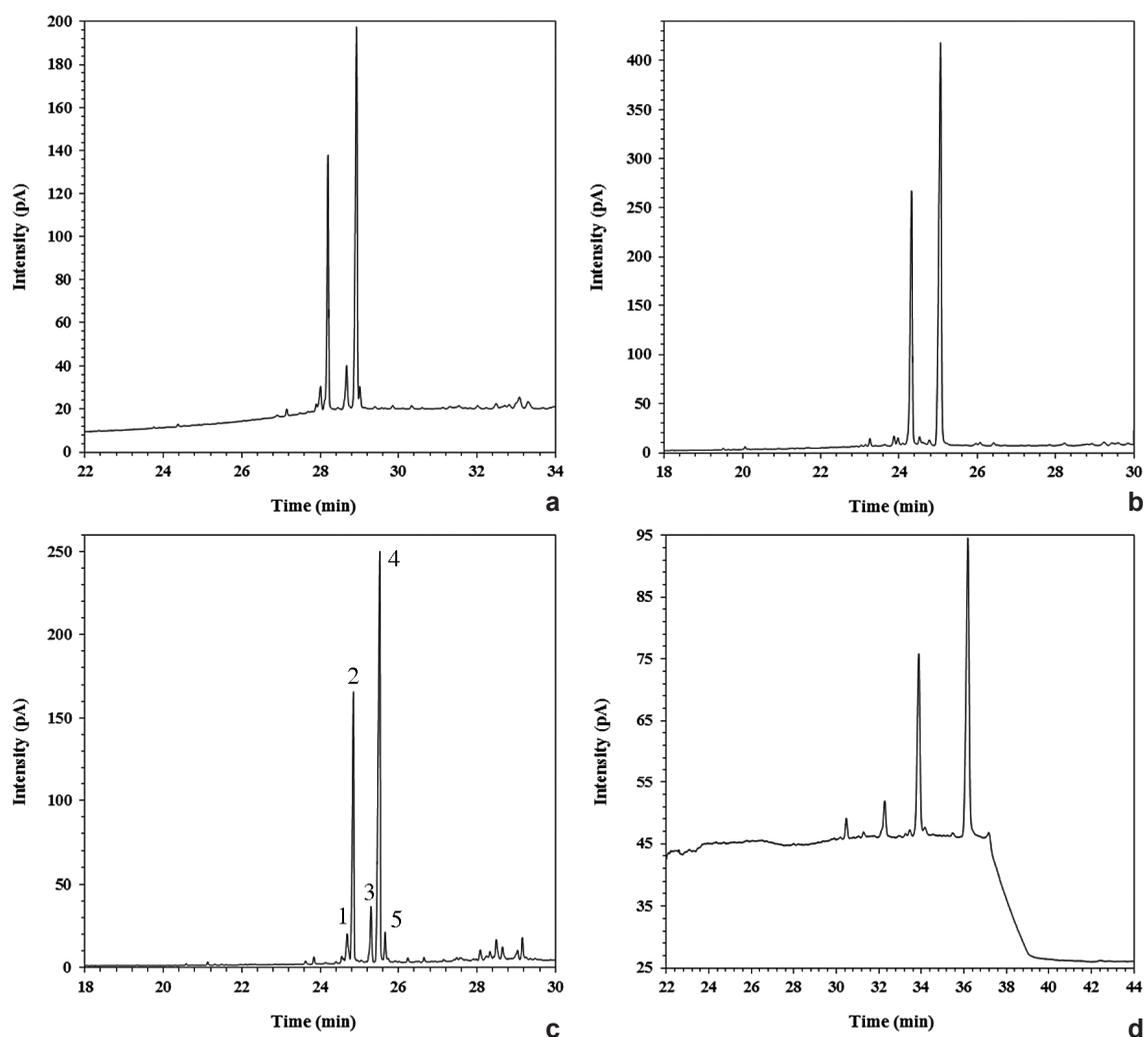
#### Results and Discussion

The raw commercial oleoresin from a popular market was characterized as a mechanically resistant dark solid. After cracking, the remaining wood debris and other inorganic impurities from the tree trunks commonly present in the collected oleoresin (Siani *et al.* 2012) were removed off. The resulting material (3 kg) was divided in fine

particles by sieving (PBO, 2.5 kg) that presented 1.75% (w/w) average humidity and melted at 100–120 °C.

GC-FID was applied for chemically characterizing and checking the separation performance of the PBO components using different columns. Initially, the programming of the column oven was based on a previous study (Siani *et al.* 2012), in which the authors used a DB-5 column, but poor separation efficiency was found for two of the interesting triterpenes,  $\alpha$ -amyrin and lupeol, and several other expected minor peaks. Any pre-treatment to remove monoterpenes and sesquiterpenes was spared since these compounds elute below 200 °C,

as also shown by Satyal *et al.* (2017), and so do not interfere with the signals of triterpenes (elution above 290 °C). Thus, GC-FID analyses of PBO in columns with other stationary phases (DB-35, DB-1701, DB-17HT and DB-Innowax) were performed, and typical chromatograms are presented in Figure 1a-d. Among the columns based on phenyl methylpolysiloxane phases - DB-5, DB-35 and DB-17HT - the latter proved to present the best separation efficiency, evidencing minority compounds around main peaks and the elution of more retained compounds (retention time of 28–30 min). The DB-35 column also shows improved resolution for the interesting triterpenes, comparatively to DB-5, but with worst resolution



**Figure 1** – a-d. Typical GG-FID chromatograms of *breu* in different stationary phases – a. DB-35; b. DB-1701; c. DB-17HT, with peaks of major triterpenes assigned as identified by GC-MS (Tab. 1); d. DB-Innowax.

than the DB-17HT column. There was evidence of intensity suppression of minorities at times around the main signals on the DB-1701 column. Finally, the most polar DB-Innowax column resulted in only four relevant signals in the PBO chromatogram with prolonged run time, probably due to their strong interaction with the triterpenes that would lead to interference in subsequent runs. Thus, in view of the resolution between  $\alpha$ -amyrin and lupeol without signal suppression and lack of resolution among the other main triterpenes, the DB-17HT column was considered as the most suitable to analyze *breu* by GC, being thus selected for further development.

It was possible to assign the five well-resolved triterpenes of PBO in Figure 1 through GC-MS analysis using the DB-17HT column (Tab. 1):  $\beta$ -amyrenone,  $\beta$ -amyrin,  $\alpha$ -amyrenone,  $\alpha$ -amyrin, and lupeol. The presence of these substances corroborates the literature reporting isolated lupeol in *breu* oleresin (Maia *et al.* 2000; Guimarães & Siani 2007), but it has not been detected directly by any analytical method so far.

It is important to note that, as a general rule, oleanane-type triterpenes elute before their ursane isomers, as observed by retention times on low and medium polarity columns (Siani *et al.* 1999; Maia *et al.* 2000). In the present study, this feature was considered to punctuate the identities of the triterpenes, besides the respective mass fragmentations. This assertion is also corroborated by the usual predominance of  $\alpha$ -amyrin on  $\beta$ -amyrin, and reproduced for the corresponding ketones ( $\alpha$ -amyrenone on  $\beta$ -amyrenone) in *breu* (Maia *et al.* 2000; Guimarães & Siani 2007; Silva *et al.* 2009; Siani *et al.* 2012; Lima *et al.* 2014; Melo *et al.* 2019). In fact, the retention time of  $\alpha$ -amyrin

in PBO (25.5 min) was verified with that obtained through injection of standard.

The quantification of PBO components was performed through GC-FID, using cholesterol as ES. The equation of the calibration curve was determined as  $y = 0.961x - 12.772$ , where  $y$  is equal to the integration area of the cholesterol signal and  $x$  corresponds to the concentration of the ES in  $\mu\text{g/mL}$ . The linear Pearson's correlation ( $r$ ) equal to 0.996 and the relative standard deviation (RSD)  $\leq 1.0\%$  were in compliance with ICH criteria of  $r > 0.990$  and  $\text{RSD} < 5\%$  (ICH 1997).

Cholesterol was selected as ES in view of (i) commercial availability of the certified material, (ii) lower market value (approximately 200-fold) than the amyrin isomer standards, and (iii) structural similarity (Lanças 2009) to the majority of pentacyclic triterpenes in *breu*, which FID response can be very similar to the analyte (Dietz 1967). In fact, this chemical has already been applied previously as internal standard for the quantification by GC of oleanolic acid, ursolic acid,  $\alpha$ -amyrin, and lupeol in blueberry cutine. However, mass spectrometry (MS) detection was necessary for quantification of these compounds in view of the analysis having been performed in a low polarity column (Elite-5MS), similar to DB-5 (Moggia *et al.* 2016). Moreover, a verification of similarities between the response factors by the detection of cholesterol and triterpenes should be necessary to demonstrate the accuracy of the use of ES for purposes of quantification of triterpenes in *breu*.

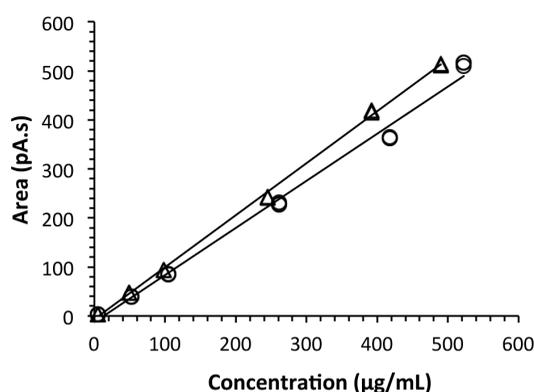
In our study, the standard was applied as ES because this procedure consumes less chemical material and extractive steps were not required, which implies enhanced recovery. Moreover, our

**Table 1** – Fragmentation by GC-MS and quantification of the main triterpenes found in PBO by the proposed GC-FID method using DB-17HT column and standardization with cholesterol.

Peak*	$t_R$ (min)	m/z	Compound	By standardization	
				external	internal
1	24.68	281(100), 133, 147, 95, 424[M <sup>+</sup> ], 409	$\beta$ -amyrenone	2.38±0.07	1.91±0.23
2	24.85	218(100), 203, 189, 175, 95, 135, 161, 426[M <sup>+</sup> ], 411	$\beta$ -amyrin	9.28±0.26	8.14±0.98
3	25.30	281(100), 133, 147, 95, 424[M <sup>+</sup> ], 409	$\alpha$ -amyrenone	3.27±0.08	2.71±0.32
4	25.52	218(100), 203, 189, 175, 95, 135, 161, 426[M <sup>+</sup> ], 411	$\alpha$ -amyrin	18.93±0.57	16.87±2.06
5	25.66	218(100), 95, 189, 107, 109, 121, 135, 203, 426[M <sup>+</sup> ], 411	lupeol	4.52±0.11	3.85±0.46

\* Peaks assigned in the Fig. 1c.  $t_R$  = Retention time. Determination by weighing in triplicate. Compounds identified by the fragmentation patterns in the literature (Vilegas *et al.* 1997; Noronha 2001; Carvalho *et al.* 2010) and in the mass spectral reference libraries Wiley 275 (Agilent), HMDB (2017), NIST (2017) and SDBS (2017).

method involves a less costly and more readily available detector (FID in place of MS), which is also more suitable in terms of similarity of response between the ES and the intended analytes. Nonetheless, the similarities between response factors were checked by FID in the present study. The analytical curves of  $\alpha$ -amyrin ( $y = 1.061x - 6.679$ ,  $r = 0.999$ ,  $RSD \leq 0.52\%$ ) and cholesterol indicated very similar sensitivities by FID (Fig. 2). The ratio between both sensitivities is equal to 1.027, closer than one unit ( $< 3\%$  error), indicating excellent accuracy in using cholesterol as a reference for quantification of triterpenes.



**Figure 2** – Analytical curves of cholesterol (circles) and  $\alpha$ -amyrin (triangles).

Table 1 shows the quantification by external standardization and by internal standardization of the main triterpenes found in the PBO. The quantitation methods indicate different ( $p < 0.05$ ) individual content, through a paired  $t$ -test (two-tailed), however, the external standardization is preferred in view of its lower uncertainty (low %RSD). The standardization using cholesterol is quite advantageous in comparison with the technique of area normalization, which was determined by the ratio between individual and total integration areas (Grinberg & Rodriguez 2019) and improperly overestimated (2.5-times higher) the content of triterpenes (data not shown).

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### References

- Aragão GF, Pinheiro MCC, Bandeira PN, Lemos TLG & Viana GSB (2008) Analgesic and anti-inflammatory activities of the isomeric mixture of alpha-and beta-amyrin from *Protium heptaphyllum* (Aubl.) March. *Journal of Herbal Pharmacotherapy* 7: 31-47.
- Carvalho TC, Polizeli AM, Turatti ICC, Severiano ME, Carvalho CE, Ambrósio SR, Crotti AEM, Figueiredo US, Vieira PC & Furtado NAJC (2010) Screening of filamentous fungi to identify biocatalysts for lupeol biotransformation. *Molecules* 2010: 6140-6151.
- Costa AF (1975) *Farmacognosia*. Vol. 1. Fundação Calouste Gulbenkian, Lisboa. Pp. 841-842.
- Grinberg N & Rodriguez S (2019) *Ewing's analytical instrumentation handbook*. 4<sup>th</sup> ed. CRC Press, Boca Raton. 646p.
- Dietz WA (1967) Response factors for gas chromatographic analyses. *Journal of Chromatographic Science* 5: 68-71.
- Guimarães AC & Siani AC (2007) Triterpenes from the leaves of *Protium strumosum*. *Revista Fitos* 3: 67-76.
- Hernández-Vázquez L, Mangas S, Palazón J & Navarro-Ocaña A (2010) Valuable medicinal plants and resins: commercial phytochemicals with bioactive properties. *Industrial Crops and Products* 31: 476-480.
- HMDB - The Human Metabolome Database (2016) The metabolomics innovation centre. Available at <[http://www.hmdb.ca/spectra/c\\_ms/search](http://www.hmdb.ca/spectra/c_ms/search)>. Access on 10 November 2017.
- ICH - Internacional Conference on Harmonization (1997) *Guide for industry: Q2B validation of analytical procedures: methodology*. Current Step 4 version dated 19 may 1997. Available at <<https://www.fda.gov/regulatory-information/search-fda-guidance-documents/q2b-validation-analytical-procedures-methodology>>. Access on 10 October 2017.
- Lanças FM (2009) A cromatografia líquida moderna e a espectrometria de massas: finalmente "compatíveis"? *Scientia Chromatographica* 1: 35-61.
- Langenheim JH (2003) *Plant resins: chemistry, evolution and ethnobotany*. Timber Press, Portland. 612p.
- Lima EM, Nascimento AM, Lenz D, Scherer R, Meyrelles SS, Boëchat GAP, Andrade TU & Endringer DC (2014) Triterpenes from the *Protium heptaphyllum* resin - chemical composition and cytotoxicity. *Brazilian Journal of Pharmacognosy* 24: 399-407.

- Maia RM, Barbosa RP, Cruz FG, Roque NF & Fascio M (2000) Triterpenos da resina de *Protium heptaphyllum* March (Burseraceae): caracterização em misturas binárias. *Química Nova* 23: 623-626.
- Melo CM, Morais TC, Tomé AR, Brito GAC, Chaves MH, Rao VS & Santos FA (2011) Anti-inflammatory effect of  $\alpha$ , $\beta$ -amyrin, a triterpene from *Protium heptaphyllum*, on cerulein-induced acute pancreatitis in mice. *Inflammation Research* 60: 673-681.
- Melo KM, Oliveira FTB, Silva RAC, Quinderé ALG, Marino Filho JDB, Araújo AJ, Pereira EDB, Carvalho AA, Chaves MH, Rao VS & Santos FA (2019)  $\alpha$ ,  $\beta$ -Amyrin, a pentacyclic triterpenoid from *Protium heptaphyllum* suppresses adipocyte differentiation accompanied by down regulation of PPAR $\gamma$  and C/EBP $\alpha$  in 3T3-L1 cells. *Biomedicine & Pharmacotherapy* 109: 1860-1866.
- Moggia C, Graell J, Lara I, Schmeda-Hirschmann G, Thomas-Valdés S & Lobos GA (2016) Fruit characteristics and cuticle triterpenes as related to postharvest quality of highbush blueberries. *Scientia Horticulturae* 211: 449-457.
- Murthy KSR, Reddy MC, Rani SS & Pullaiah T (2016) Bioactive principles and biological properties of essential oils of Burseraceae: a review. *Journal of Pharmacognosy and Phytochemistry* 5: 247-258.
- NIST - National Institute of Standards and Technology (2017) NIST standard reference data. Standard Reference Data Act. Available at <<https://webbook.nist.gov/chemistry/name-ser/>>. Access on 15 October 2017.
- Noronha JPC (2001) Metabolitos secundários do fruto de *Arbutus unedo* L. (Medronho). Dissertação de Doutorado. Universidade Nova de Lisboa, Lisboa. 274p.
- Ramos MFS, Siani AC, Tappin MRR, Guimarães AC & Ribeiro JELS (2000) Essential oils from oleoresins of *Protium* spp. of the Amazon region. *Flavour and Fragrance Journal* 15: 383-387.
- Rüdiger AL & Veiga-Junior VF (2013) Chemodiversity of ursane- and oleanane-type triterpenes in Amazonian Burseraceae oleoresins. *Chemistry & Biodiversity* 10: 1142-1153.
- Rüdiger AL, Siani AC & Veiga-Junior VF (2007) The chemistry and pharmacology of the South America genus *Protium* Burm. f. (Burseraceae). *Pharmacognosy Reviews* 1: 93-104.
- Satyal P, Powers CN, Parducci VR, McFeeters RL & Setzer WN (2017) Chemical composition, enantiomeric distribution, and antifungal activity of the oleoresin essential oil of *Protium amazonicum* from Ecuador. *Medicines* 4: 70.
- SDBS - Spectral Database for Organic Compounds (2017) SDBS-Mass. National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba. Available at <[https://sdb.sdb.aist.go.jp/sdb/cgi-bin/direct\\_frame\\_top.cgi](https://sdb.sdb.aist.go.jp/sdb/cgi-bin/direct_frame_top.cgi)>. Access on 13 December 2017.
- Siani AC, Moraes R & Veiga Junior VF (2017) Toward establishing the productive chain for triterpene-based Amazonian oleoresins as valuable non-timber forest products. *Open Journal of Forestry* 7: 188-208.
- Siani AC, Nakamura MJ, Tappin MRR, Monteiro SS, Guimarães AC & Ramos MFS (2012) Chemical composition of South American Burseraceae non-volatile oleoresins and preliminary solubility assessment of their commercial blend. *Phytochemical Analysis* 23: 529-539.
- Siani AC, Ramos MFS, Guimarães AC, Susunaga GS & Zoghbi MGB (1999) Volatile constituents from oleoresin of *Protium heptaphyllum* (Aubl.) March. *Journal of Essential Oil Research* 11: 72-74.
- Silva ER, Oliveira DR, Melo MFF, Bizzo HR & Leitão SG (2016) Report on the Malungo expedition to the Erepecuru river, Oriximiná, Brazil. Part I: is there a difference between black and white breu? *Revista Brasileira de Farmacognosia* 26: 647-656.
- Silva JRA, Zoghbi MGB, Pinto AC, Godoy RLO & Amaral ACF (2009) Analysis of the hexane extracts from seven oleoresins of *Protium* species. *Journal of Essential Oil Research* 21: 305-308.
- Susunaga GS, Siani AC, Pizzolatti MG, Yunes RA & Monache FD (2001) Triterpenes from the resin of *Protium heptaphyllum*. *Fitoterapia* 72: 709-711.
- UNDP - United Nations Development Programme (2012) Equator initiative case studies. Green Life Association of Amazonia (AVIVE), Brazil. Available at <[https://www.equatorinitiative.org/wp-content/uploads/2017/05/case\\_1348160963\\_EN.pdf](https://www.equatorinitiative.org/wp-content/uploads/2017/05/case_1348160963_EN.pdf)>. Access on 12 December 2018.
- Vieira Júnior GM, Souza CML & Chaves MH (2005) The *Protium heptaphyllum* resin: isolation, structural characterization and evaluation of thermal properties. *Química Nova* 28: 183-187.
- Vilegas JHY, Lanças FM, Vilegas W & Pozetti GL (1997) Further triterpenes, steroids and furocoumarins from Brazilian medicinal plants of *Dorstenia* genus (Moraceae). *Journal of the Brazilian Chemical Society* 8: 529-535.

