



 Cite this: *RSC Adv.*, 2021, 11, 33160

Ultrasound-assisted lipid extractions, enriched with sterols and tetranortriterpenoids, from *Carapa guianensis* seeds and the application of lipidomics using GC/MS

 André Silva dos Reis,^a Alberdan Silva Santos ^{*a} and José Francisco de Carvalho Gonçalves^b

This study describes the optimized stages of lipid extraction assisted by ultrasound to increase the concentrations of limonoids and steroids from andiroba seeds, identified as *Carapa guianensis* Aublet, and the lipidome analyzed by TLC and GC/MS. After boiling and peeling, crushed seeds were subjected to extractions with *n*-hexane (G1), acetone (G2), and methanol (G3) in an ultrasonic bath for 30 minutes at 50 °C. These extracts were analyzed by analytical TLC; aliquots of each extract and a marketable oil, used as a reference, were transesterified followed by silylation with BSTFA + 1% TMCS, and analyzed by GC/MS. The reference oil and the biomass of the seeds did not present significant differences in the profiles of free fatty acids (FFA). However, differences were observed in the profiles of tetranortriterpenoids (TnTT) (limonoids) in the seeds. Afterward, the analysis of the extracts G1, G2, and G3 detected free steroids: campesterol, stigmaterol, and β -sitosterol; and limonoids: 7-deacetyl-7-oxogedunin, 6 α -acetoxo-gedunin, deacetylgedunin, and epoxyacetylgedunin. The use of lipidomic techniques associated with ultrasound-assisted extraction was applied for the first time to enhance the triterpenoids and steroids, mainly in G3, describing a faster and more economical process, and allowing a one-step lipidome analysis of the andiroba seeds.

 Received 20th June 2021
 Accepted 30th August 2021

DOI: 10.1039/d1ra04776k

rsc.li/rsc-advances

Introduction

The Andirobeira (*Carapa guianensis* Aublet.) is a large plant that belongs to the Meliaceae family, first described by a French botanist known as Jean-Baptiste Christopher Fusée Aublet in 1775 in French Guiana.¹ Even before its classification, Brazil's native population used the seeds mainly for medicinal purposes and created a general denomination of andiroba. In the north of Brazil, andiroba has many utilities: its wood is considered noble and much appreciated for the construction of houses and manufacturing of furniture,² the seed's oil is used by the industry for manufacturing of soap,³ cosmetics,^{4,5} and insect repellent candles,⁶ and also presents anti-inflammatory properties.^{7,8}

Over the last few decades, studies have intensified mainly to confirm new biological activities with andiroba oil, which demonstrates the importance of this species for biodiesel production as an alternative fuel for isolated communities from the Amazon Forest for the generation of electric energy by

generators.⁹ The oil extracted by mechanical pressing supplies the insecticidal,^{10–12} pharmaceutical,¹ and cosmetics¹³ industries in the production of soap, shampoo, and body oils on an industrial scale.¹⁴ In the medicinal uses, the fractions oil enriched with gedunin, a limonoid present in andiroba oil, was reported to combat the inflammatory disease caused by the action of T lymphocytes during an allergic reaction.¹⁵

The process of industrial extraction of the oil guarantees a 30% yield by the double pressing of the crushed seeds with 8% moisture.¹⁶ Usually, andiroba oil is extracted in two ways: by cooking followed by maceration and heating in the sun to release the oil; this method is widely used by traditional communities. Another method is to press the seeds *in nature* employing hydraulic presses.¹⁷

In general, several studies relate the profiles of fatty acids present in andiroba oil and others describe the presence of triterpenoids, classified as limonoids.^{18–21} However, until now, no study has shown systematic lipidic research on andiroba seeds targeting the lipid profile of the metabolites present in those seeds, mainly tetranortriterpenoids and steroids, who are referenced for presenting relevant biological activities, such as anti-inflammatory, and other factors that may be associated with the development of the disease.^{7,8,22,23} In this aspect, this study applied lipidomic techniques to analyze steroids and

^aLaboratory of Systematic Investigation in Biotechnology and Molecular Biodiversity, Federal University of Pará (UFPA), Belém, PA, 66075-110, Brazil. E-mail: alberdan@ufpa.br

^bLaboratory of Plant Physiology and Biochemistry, National Institute of Amazonian Research (MCTI-INPA), Manaus, AM, 69060-020, Brazil



tetranortriterpenoids as well as other lipids, adopting ultrasonic-assisted extraction associated with apolar, polar, and polar medium to obtain all the lipids present in the seeds of *C. guianensis*. Since lipid-assisted extraction of steroids and tetranortriterpenoids has been used in other vegetable species to obtain lipid classes very efficiently,^{24,25} it has not yet been applied for the analysis of the seeds of *Carapa guianensis* Aubl.

In this study, the application of the techniques of ultrasound-assisted extraction with concentrated limonoids and steroids and other lipids, and the lipidomic applied to the oil and seeds of *C. guianensis* were shown to be highly efficient. It describes faster extraction, time and solvent savings, as well as the use of a small amount of the samples, which are promising methodologies for future lipids extractions and analysis.

Experimental

Andiroba's seeds collecting

The seeds of Andirobeira (*Carapa guianensis* Aubl.) were collected at Manaus-AM, Brazil with the latitude south: 02° 35' 987" and longitude west: 60° 02' 238" by collaborators from INPA (National Research Institute of the Amazon). The seeds were kept frozen at -20 °C until processed. The SisGen authorization was numbered: AF60078.

Obtention of extracts by solvent-assisted ultrasound

The seeds were baked for 20 minutes at 100 °C water temperature in a closed 5 L stainless steel vessel under a constant pressure system. After cooking, at 30 °C, the seeds were peeled and the homogenized, 60 g crushed seeds were transferred to a 500 mL conical glass jar. The extraction was performed in a UNIQUE ultrasonic bath, model USC - 1800 of 40 kHz frequency, and power 155 Watts RMS, maintained at a constant temperature of 50 °C for 30 minutes. The solvents used were *n*-hexane, acetone, and methanol (analytical grade), it was used 200 mL volume for each solvent, and the extraction procedure was repeated three times for each solvent. The hexane extract (G1) was filtered off, the biomass extracted again to the obtention of the acetone extract (G2), and the methanolic extract (G3) under the same conditions previously mentioned. After evaporation of the solvents each extract had its measured mass, the analysis of the metabolic classes were performed by analytical-TLC and the separation of the bands was performed by preparative TLC.

Method of detection of steroids and triterpenoids by TLC

The triterpenoid detection method, adapted from Kovac-Bessvic *et al.*²⁶ and Hossain & Ismail,²⁷ was performed in TLC-analytical, using a 5 × 10 cm aluminum plate coated with 60 Å silica gel of pore diameter (SILICYCLE) and 250 μm of layer thickness. Volumes of 5 μL of each sample (G1), (G2) and (G3) were applied with a capillary tube over a distance of 0.8 cm from the base of the plate with a spacing of 1 cm between the samples. The solvent system used for sample elution on the plate was *n*-hexane/ether/ethyl acetate in the proportions of 6 : 2 : 2 (v/v) and two drops of acetic acid. The method to exposed TLC plate followed the Magrini's method²⁸ with adaptation, which

a solution of sulfuric vanillin as the developer was used: solution A [(10% vanillin in ethanol (m/v)) and solution B [(10% sulfuric acid in ethanol (v/v))] at the ratio of 1 : 1 (v/v) for detections of the metabolic classes.

Reaction of lipid transesterification in alkaline medium and sample preparation

Transesterification in an alkaline environment adapted from Souza²⁹ and Geris *et al.*³⁰ was applied to the samples. Aliquots of 10 mg of each sample were transferred to well-identified polypropylene tapered tubes (TBx-1), followed by heating for 2 minutes in a water bath at 40 °C. For each tube was added 100 μL of 2 N potassium methoxide and kept in an ultrasonic bath for 2 minutes at 30 °C (±2). Then 200 μL of *n*-hexane, stirred manually for 10 seconds, centrifuged for 1 minute at 10 000 RPM; the organic phase of each sample was transferred to new tubes (TBx-2). A volume of 110 μL of 2 N HCl was added to each tube (TBx-1) of each sample, shaking manually for 10 seconds. Thereafter, 200 μL of the CH₂Cl₂ : *n*-hexane mixture was added in a ratio of 1 : 1 (v/v), stirred again for 10 seconds, 1 mL of deionized water was added to remove the excess acid from the organic phase, centrifuged under the same conditions as before. The supernatant from each solution was transferred to its respective tube (TBx-2) and the samples were concentrated with the inert gas flow at (35 °C/5 psi/20 min). The concentrated samples were sent for derivatization with BSTFA + 1% TMCS and subsequent GC/MS analysis.

Method for the analysis of carbohydrates and phenolic compounds

The methanolic extract (G3 extract) was analyzed a second time under the same conditions as in the previous item, observing that in this procedure the addition of 20 μL of dry pyridine was carried out for the complete solubilization of the higher polarity compounds.

Derivatization of samples

The derivatization of the samples was performed with *N,O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) + 1% trimethylchlorosilane (TMCS) to sieve the free molecules that were not transesterified and the others with free hydroxyls. In the tube samples (TBx-2) 50 μL of the derivatizing agent was added and the tubes were kept in an ultrasonic bath for 20 min at 30 °C. The solvents were then evaporated at 35 °C/5 psi/20 min and 500 μL of *n*-hexane : CH₂Cl₂ in the proportion of 1 : 1 (v/v) were added to the tubes (TBx-2) respectively, 10 seconds, and the contents transferred into 2 mL glass flasks with lid and septum and analyzed by GC/MS.

Sample analysis by GC-MS

Mass spectrometric analysis were performed using a Thermo Scientific Trace 1300 Gas Chromatograph (GC) coupled to a Thermo Scientific MS-ISQ Single Quadrupole mass spectrometer with AI 1310 autosampler, equipped with a ZB capillary column -5HT (30 m × 0.25 mm × 0.1 μm) or DB-5 (15 m × 0.25 mm × 0.1 μm), helium gas was used as the carrier at a flow rate of 1 mL min⁻¹. Sample injection of 1.0 μL in splitless mode. The injector operated at 220 °C



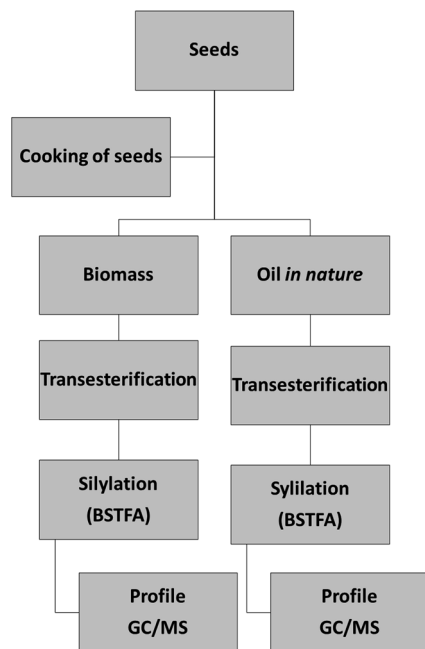


Fig. 1 The flowchart representing the process steps to obtain the chemical profiles of the oil and the andiroba seeds.

and the oven temperature setting started at 50 °C to 200 °C (8 °C min⁻¹), remaining for 1 min, rising to 300 °C (15 °C min⁻¹), being maintained for 5 min, rising again to 350 °C (15 °C min⁻¹) and remaining for another 9 minutes. MS-ISQ operated with the interface at 280 °C, ionization source at 280 °C, the mass range band (40–1000 Da) with 1 scan per s, and electronic ionization operated at 70 eV. The identifications of the substances were carried out by comparing the mass spectra with those of the commercial libraries NIST2011, WILEY2009, FAMES2011 × retention time. Lipid concentration was acquired by calculating peak area normalization and validation of retention rates by calculating retention indices from homologous hydrocarbons.

Results and discussion

Analysis of lipid profile of crude oil and seeds of *Carapa guianensis*

One of the great difficulties of analyzing molecular profiles of fixed substances, such as the micrometabolites produced by plants (lipids, phenolics, alkaloids, steroids, and several other classes) is to analyze the complex matrix in a single analysis. In this sense, we try to detect or identify all metabolites in a single run analysis or single technique analysis (*e.g.* GC/MS). However, when trying to analyze lipids, the difficulties present themselves

Table 1 Content of lipids compounds found in the analysis of the *in natura* oil and the biomass of the andiroba seed (*Carapa guianensis* Aubl.)^a

| N | Compounds | RT | KI | INO (%) | Sd± | ABM(%) | Sd± |
|----|-----------------------------|-------|------|---------|------|--------|------|
| 1 | Glycerol 3TMS | 9.71 | 1108 | 1.50 | 0.07 | 0.10 | 0.00 |
| 2 | 2,3-Ditert-butylphenol 1TMS | 13.17 | 1241 | — | — | 0.20 | 0.01 |
| 3 | Miristic acid 1TMS | 17.74 | 1456 | — | — | 0.35 | 0.02 |
| 4 | Methyl palmitoleate | 18.47 | 1494 | 2.13 | 0.09 | 0.70 | 0.03 |
| 5 | Methyl palmitate | 18.81 | 1512 | 17.26 | 0.85 | 16.03 | 0.69 |
| 6 | Palmitoleic acid 1TMS | 19.80 | 1566 | — | — | 0.71 | 0.04 |
| 7 | Palmitic acid 1TMS | 20.17 | 1588 | 11.70 | 0.59 | 11.50 | 0.67 |
| 8 | Methyl oleate | 20.83 | 1626 | 30.80 | 1.61 | 25.80 | 1.28 |
| 9 | Methyl estearate | 21.00 | 1635 | 10.46 | 0.44 | 7.80 | 0.39 |
| 10 | Oleic acid1TMS | 21.87 | 1687 | 15.01 | 0.76 | 20.42 | 1.01 |
| 11 | Stearic acid 1TMS | 22.06 | 1699 | 3.98 | 0.17 | 6.20 | 0.31 |
| 12 | Methyl 11-eicosenoate | 22.47 | 1724 | — | — | 0.31 | 0.02 |
| 13 | Methyl araquidoate | 22.52 | 1727 | 0.42 | 0.02 | 2.40 | 0.12 |
| 14 | Methyl araquidonate | 22.77 | 1742 | 2.60 | 0.15 | — | — |
| 15 | Adipic acid 2TMS | 23.29 | 1775 | — | — | 0.66 | 0.03 |
| 16 | Araquidic acid 1TMS | 23.77 | 1806 | — | — | 0.80 | 0.04 |
| 17 | Methyl behenoate | 24.38 | 1846 | 0.75 | 0.03 | 0.64 | 0.03 |
| 18 | Bdca – 2TMS | 24.48 | 1852 | — | — | 0.95 | 0.05 |
| 19 | Malic acid 2TMS | 24.75 | 1870 | — | — | 0.39 | 0.02 |
| 20 | Behenic acid 1TMS | 25.16 | 1897 | — | — | 0.11 | 0.01 |
| 21 | Methyl lignocerate | 25.85 | 1945 | 0.43 | 0.02 | 0.45 | 0.02 |
| 22 | Squalene | 26.48 | 1989 | — | — | 0.22 | 0.03 |
| 23 | Stigmasterol 1TMS | 29.70 | 2226 | — | — | 0.11 | 0.01 |
| 24 | β-Sitosterol 1TMS | 30.28 | 2293 | 0.20 | 0.01 | 0.14 | 0.01 |
| 25 | N.I. | 30.84 | 2395 | — | — | 1.80 | 0.09 |
| 26 | N.I. | 32.38 | 2664 | — | — | 0.14 | 0.01 |
| 27 | N.I. | 32.81 | 2738 | — | — | 0.31 | 0.02 |
| 28 | 7-Deacetoxy-7-oxogedunin | 34.08 | 2953 | — | — | 0.35 | 0.02 |

^a TMS: trimethylsilyl, RT: retention time, KI: Kovat's retention index calculated on DB-5 MS capillary column using homologous series of *n*-alkanes, (%) compound abundance in the sample, INO: *in natura* oil, ABM: andiroba biomass, Bdca = 1,2-benzenedicarboxylic acid 2TMS, Sd = standard deviation, NI = not identified.



with greater intensity due to the different polarities, sizes, and affinities that these molecules present. A strategy used in this study to analyze the lipids from the oil and seeds *in natura* of *C. guianensis* was to apply the transesterification directly in the samples and silylation reactions to have all substances derivatized and analyzed by GC/MS. The flowchart in Fig. 1 describes the sequences used to obtain and analyze the chemical profiles of the oil and fresh seeds.

The transesterification reaction in the experimental condition allowed the release of the fatty acids that constituted the triglyceride molecules (TAG), making possible the detection of these compounds in the form of methyl esters, not interfering in the composition of the triterpenoids and steroids. It was observed that in the retention time of 9.7 minutes, in both analyses, the compound propanetriol-3TMS also known as glycerol trimethylsilylated, a byproduct generated in the transesterification of TAG, DAG, and/or MAG, was detected, confirming that the transesterification reactions in the oil and seed were efficient, also denoting efficient derivatization.

The chemical profiles of oil and seeds samples have three main classes of metabolites: fatty acids, steroids, and triterpenoids; in parallel, glycerol, squalene (a polyunsaturated hydrocarbon of the triterpene type) and unidentified substances can also be observed. It is noteworthy that the relevance of this analysis is based on identifying all free substances containing hydroxyl group, consistent with the presence of free fatty acids that characterize the acidity of the oil, being palmitic (11.70%) and oleic (15.01%) acids the responsible for this characteristic; and with the other steroidal or triterpenoid molecules favoring their volatilizations and, that can be observed by their derivatizations, presenting the TMS group linked to their structures (Table 1).

The methyl esters characterize the fatty acids that were in the form of acylglycerols, that is, that were linked to the glycerol, showing the level of oil integrity in the matrix. In this study, it is observed that in seeds, a higher level of substances that were bound to the cell wall was detected and that was released by hydrolysis, which can be derivatized and/or detected. In this case, the applied methodologies are efficient in the extraction and detection of substances that, generally, are bound in the walls or the cell membranes. It is observed that the levels of detection of

substances were higher in seeds than in oil. This is because during the extractions, the seeds are pressed or a liquid–solid extraction is done using mechanical, magnetic, sound, or percolation systems. In this case, the results show greater visibility of the chemical profile of these seeds; this is due to the presence of limonoids, which are substances of great importance in this species, due to their biological activities. Besides that, it can be seen that steroids are detected on a wider spectrum than in oil. Some of the substances present in these chemical profiles could not be identified, but their mass fragments are described (Table 2).

In a resumed way the analysis of the lipid profile of the cooked seed andiroba biomass (SAB) by GC/MS appears as an efficient application of the lipidomic technique to detect compounds that are not concentrated in the oil by press extraction. These results describe the possibility of concentrating the limonoids and steroids in the *in natura* oil.

Previous analysis of the crude oil and that hydrolyzed by enzyme describe identity fatty acids profile Salgado *et al.*¹⁹ very similar to the profile found in this study, however, only fatty acids were detected by means of GC/MS, which may be related to the process of extraction of lipids, which compared to the study in question, it was possible to detect steroids and tetranortriterpenoids obtained by ultrasound-assisted solvent extraction.

The values of the oleic acid concentration present high concentration value confirm that andiroba seed is an important source of this compound.^{31–33} Experimental evidence demonstrates *in vitro* and *ex vivo*, the ability of oleic acid to accelerate

Table 2 Mass fragments of the unidentified substances in the *in natura* oil samples and seed biomass resulting from the GC/MS analysis^a

| Samples | RT | <i>m/z</i> |
|---------|-------|--|
| ABM | 30.84 | 57(100), 91(12), 131(8), 147(45), 191(10), 237(10), 308(11), 329(2), 385(2), 441(98), 442(38), 443(5), 631(1), 646(5), 662(1) |
| | 32.38 | 43(39), 55(25), 59(10), 67(22), 70(47), 91(72), 95(80), 105(100), 121(62), 137(30), 148(42), 164(25), 210(15), 243(10), 257(62), 331(10), 359(15), 470(40), 497(1) |
| | 32.81 | 57(100), 91(11), 147(15), 175(5), 191(20), 253(5), 291(5), 391(30), 367(2), 443(1), 535(2), 591(3), 647(35), 648(20), 662(18), 664(3) |

^a INO: *in natura* oil, ABM: andiroba biomass, RT: retention time, *m/z*: mass fragments.

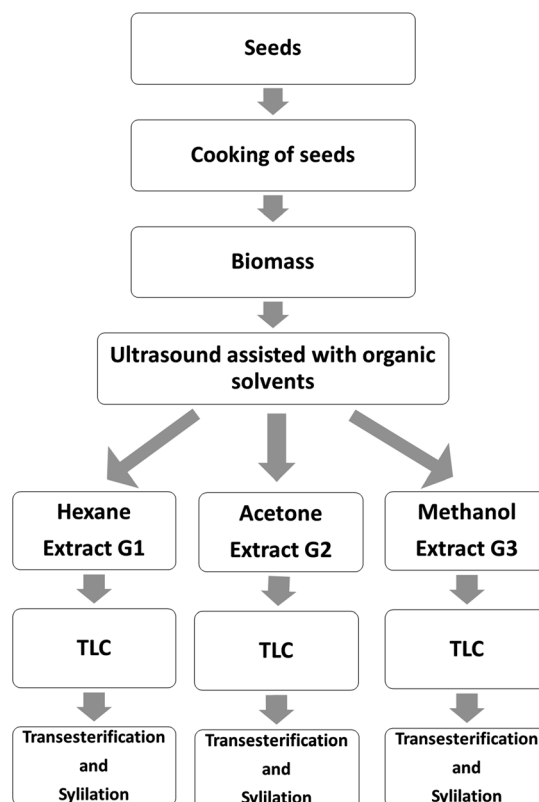


Fig. 2 Flowchart of the process of obtaining the different extracts and the analysis of the lipidic profile of the *Carapa guianensis*' seeds.



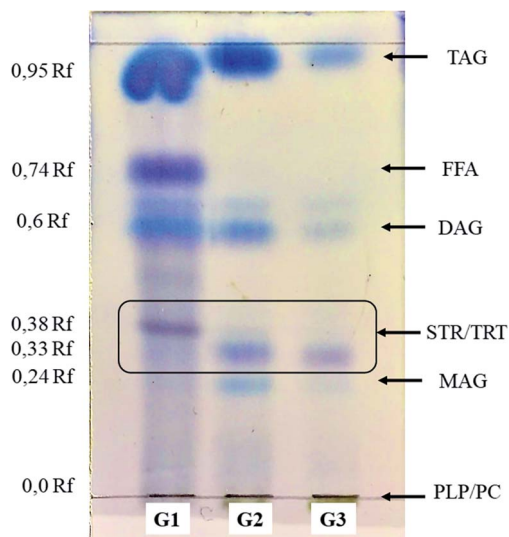


Fig. 3 Analytical thin-layer chromatography showing the lipid class separations in the extracts of the andiroba seeds. G1 = hexane extract; G2 = acetone extract; G3 = methanol extract. PLP = phospholipids; PC = phenolic compounds; MAG = monoacylglycerol; STR/TRT = steroids and tetranortriterpenoids; DAG = diacylglycerol; FFA = free fatty acids; TAG = triglycerides. Eluent: hexane/ether/ethyl acetate (6 : 2 : 2) 2 drops of acetic acid. Developer: sulfuric vanillin.

the healing process through supplementation, and this fact associated with the presence of this substance in high concentrations in andiroba oil may be related to the healing effect.³⁴ Although the biological potential of this oil is as an anti-inflammatory, which is associated with the limonoids compounds^{7,14,35} in this case, the synergic action could act, once minimizing the inflammation acting in favor of the healing.

Another important detection verified in the analysis of the biomass of the seeds was the non-major metabolic classes known as steroid and limonoids, which for the first time techniques of lipidomics was applied directly in the seeds, innovating a different type of study had not yet developed in the seeds of *C. guianensis* allowing the detection of all tetranortriterpenoids. It is important to stand out that most of the studies carried out to obtain this class of compound directly from the oil and use a large quantity of oil to obtain the minimum amount.^{11,22,33,36–38} The technological aim is to obtain concentrated fraction containing the steroids stigmaterol and β -sitosterol and other concentrated fraction containing the tetranortriterpenoids. In this case, the 7-deacetoxy-7-oxogedunin and the steroids found in the biomass analysis, as well as the other limonoids, are substances of pharmacological interest, mainly because they act synergistically demonstrating anti-allergic effects and the ability to inhibit

Table 3 Substances found in the analysis of the extracts of *C. guianensis* seeds with their respective retention times (TR) and percentage area (%)^a

| N | Compounds | RT | KI | G1% | Sd1 ± | G2% | Sd2 ± | G3% | Sd3 ± |
|----|---|-------|------|------|-------|------|-------|------|-------|
| 1 | Glycerol 3TMS | 9.77 | 1110 | 1.15 | 0.05 | 0.24 | 0.01 | — | — |
| 2 | N.I. | 15.39 | 1340 | 0.14 | 0.01 | — | — | 0.45 | 0.02 |
| 3 | Methyl miristate | 15.75 | 1356 | 0.11 | 0.00 | — | — | — | — |
| 4 | Miristic acid 1TMS | 17.59 | 1448 | 0.20 | 0.01 | 0.22 | 0.01 | 0.30 | 0.01 |
| 5 | Methyl palmitoleate | 18.33 | 1487 | 0.60 | 0.03 | 0.44 | 0.02 | 0.30 | 0.01 |
| 6 | Methyl palmitate | 18.79 | 1511 | 12.7 | 0.63 | 11.9 | 0.67 | 12.8 | 0.54 |
| 7 | Palmitoleic acid 1TMS | 20.06 | 1581 | 0.80 | 0.04 | 1.01 | 0.06 | 0.61 | 0.03 |
| 8 | Palmitic acid 1TMS | 20.52 | 1608 | 16.8 | 0.75 | 15.8 | 0.89 | 15.2 | 0.65 |
| 9 | Methyl oleate | 20.78 | 1623 | 26.4 | 1.26 | 24.9 | 1.41 | 29.8 | 1.26 |
| 10 | Methyl stearate | 21.27 | 1651 | 4.90 | 0.21 | 4.80 | 0.27 | 3.80 | 0.16 |
| 11 | Oleic acid 1TMS | 22.16 | 1705 | 25.8 | 1.09 | 28.9 | 1.63 | 26.7 | 1.13 |
| 12 | Stearic acid 1TMS | 22.40 | 1720 | 4.80 | 0.20 | 5.50 | 0.31 | 5.00 | 0.21 |
| 13 | Methyl hexadecanoate, 9,10-(Z)-methylene- | 22.70 | 1738 | 0.21 | 0.01 | 0.15 | 0.01 | — | — |
| 14 | Methyl arachidonate | 22.88 | 1749 | 1.55 | 0.08 | 1.14 | 0.06 | 0.71 | 0.03 |
| 15 | Suberic acid 2TMS | 23.35 | 1779 | 0.53 | 0.02 | — | — | — | — |
| 16 | Arachidic acid 1TMS | 23.79 | 1807 | 1.41 | 0.06 | 0.40 | 0.02 | — | — |
| 17 | Methyl behenoate | 24.18 | 1832 | 0.48 | 0.01 | 0.36 | 0.02 | 0.23 | 0.01 |
| 18 | Albuterol | 24.54 | 1856 | 0.24 | 0.01 | 0.20 | 0.01 | 0.94 | 0.04 |
| 19 | Methyl lignocerate | 25.33 | 1909 | 0.27 | 0.02 | 0.22 | 0.01 | 0.14 | 0.01 |
| 20 | Campesterol 1TMS | 28.72 | 2151 | — | — | — | — | 0.12 | 0.01 |
| 21 | β -Sitosterol 1TMS | 30.15 | 2282 | 0.17 | 0.01 | 0.14 | 0.01 | 0.32 | 0.01 |
| 22 | N.I. | 30.19 | 2291 | 0.27 | 0.02 | 0.32 | 0.02 | 0.92 | 0.04 |
| 23 | 2,6-Di- <i>tert</i> -butylphenol | 32.41 | 2669 | 0.14 | 0.01 | 0.12 | 0.01 | 0.33 | 0.01 |
| 24 | Gedunin | 33.70 | 2889 | 0.09 | 0.00 | — | 5.71 | 0.58 | 0.02 |
| 25 | 7-Deacetoxy-7-oxogedunin | 34.01 | 2941 | — | — | 1.25 | 0.07 | — | — |
| 26 | Calystegine B2 4TMS | 34.27 | 2984 | 0.07 | 0.00 | — | — | — | — |
| 27 | Deacetylgedunin | 34.52 | 3025 | 0.81 | 0.03 | 0.25 | 0.01 | 0.18 | 0.01 |
| 28 | 6 α -Acetoxygedunin | 34.73 | 3059 | — | — | 0.30 | 0.02 | 0.29 | 0.01 |
| 29 | N.I. | 34.91 | 3090 | 0.14 | 0.01 | 0.90 | 0.05 | 0.20 | 0.01 |
| 30 | N.I. | 35.47 | 3180 | — | 0.05 | 0.32 | 0.02 | — | — |
| 31 | Epoxideacetylgedunin | 36.15 | 3289 | — | 0.01 | 0.30 | 0.02 | 0.12 | 0.01 |

^a TMS: trimethylsilyl, RT: retention time, (%) compound abundance in the sample, KI: Kovat's retention index calculated on DB-5 MS capillary column using homologous series of *n*-alkanes, Sd = standard deviation, G1: hexane extract, G2: acetone extract, G3: methanol extract, NI: compound not identified.



Table 4 Fragments of the substances not identified in the analyzes of the extracts of the seeds of *Carapa guianensis*^a

| Extracts | RT | <i>m/z</i> |
|---------------|-------|--|
| G2 | 35.47 | 43(22), 73(100), 95(45), 105(12), 161(12), 201(5), 225(5), 402(1), 403(17), 404(5), 495(1) |
| G1 and G3 | 15.39 | 57(48), 82(10), 97(100), 99(20), 137(10), 180(8), 193(2), 235(2), 292(1) |
| G1, G2 and G3 | 30.19 | 57(100), 91(10), 147(30), 191(5), 237(5), 308(6), 385(1), 441(20), 444(2), 646(1) |
| | 34.91 | 43(15), 73(100), 95(30), 121(20), 149(12), 157(72), 237(5), 279(3), 297(10), 315(6), 387(18), 388(5) |

^a G1: hexane extract, G2: acetone extract, G3: methanol extract, RT: retention time, *m/z*: mass fragments.

eosinophil migration, as well as the activation of T lymphocytes⁸ and their predominant properties action against the inflammatory process.^{9,39–41}

Selection of the solvent to enhancing limonoids in the lipid extracts from *Carapa guianensis*' seeds assisted by ultrasound

The process of obtaining lipid extracts from cooked andiroba seeds assisted by ultrasound showed a difference based on the hydrolysis of substances bound in the cell wall. This hydrolysis is

not catalyzed, only the temperature and the presence of water act, which can present low efficiency, however, it is known that small amounts of limonoids and steroids attached to the walls, but the amounts present can make differences when released. In this case, the solvents used acted by solubilizing the related substances, increasing efficiency, and reducing the volume of solvent and the extraction time. The lipid extracts obtained showed different yields: 10.1 g for G1 (hexane extract), 6.1 g for G2 (acetone extract), and 3.4 g for G3 (methanolic extract). The flowchart presents the sequencing of the extractions processes, Fig. 2.

The G1, G2, and G3 extracts were analyzed by TLC to investigate the profiles of the lipid metabolic classes present, using a chemical developer sulfuric vanillin (SV), allowing the detection and confirmation of the presence of triglycerides (monoacyl, diacyl, and triacylglycerols), phospholipids, free fatty acids, steroids and tetranortriterpenoids in seeds. The separations followed the methodology described by Ganstone, Harwood, and Dijkstra⁴² for lipids using TLC analytical techniques, where phospholipids, due to their polarity, are at the base. Detections of the steroid and triterpenoid bands were based on the appearance of violet color after staining the bands with (SV), according to Kavoc-Besovic *et al.*,²⁶ Fig. 3.

These metabolic profiles of the extracts (G1), (G2) and (G3) show notably differences, especially related to the limonoids and steroids compounds, being evident in the extracts G2 and G3, which showed an increment concentration of steroids campesterol (G3), stigmasterol, β -sitosterol (G1/G3) and tetranortriterpenoids: 7-desacetoxy-7-oxogedunin (G2), deacetylgedunin (G1/G2), gedunin (G1/G3), 6 α -acetoxygedunin (G2/G3), and epoxydeacetylgedunin (G2), Table 3.

Initially, it seems that the solvents do not present relevant extractions influence, although when pyridine was used to improve the methanol extraction its influences in the derivatization of all polar molecules present in the G3-P (methanolic extract with pyridine) extract, including sugars, and micro phenolic molecules, Table 4. This addition takes out all limonoids and steroids present but also takes out all molecules not desired like sugars and phenolic acids hydrolyzed from cell walls or that are present in the growth cell as described by Lima *et al.*⁴³ and Moraes *et al.*⁴⁴ The detection and analysis of these polar molecules may be related to the solubility of these substances in solvents of medium and high polarity and associated with the exposure of the ultrasonic bath that turns polar molecules more easily extracted. Some compounds could not be identified due to the lack of mass spectrum pattern. Table 5 is composed of the mass fragments of the unidentified substances

Table 5 GC/MS analysis of the substance profile found in the methanolic extract with pyridine (G3-P) and their respective retention times (RT)^a

| N | Compounds | RT | KI | G3-P (%) | Sd \pm |
|----|----------------------------|-------|------|----------|----------|
| 1 | Tiglic acid 1TMS | 5.6 | 925 | 1.20 | 0.06 |
| 2 | 3-Heptanol 1TMS | 5.95 | 939 | 1.02 | 0.05 |
| 3 | Benzoic acid 1TMS | 9.12 | 1069 | 0.95 | 0.02 |
| 4 | Glycerol 3TMS | 9.74 | 1109 | 0.66 | 0.03 |
| 5 | Butanedioic acid 2TMS | 10.34 | 1130 | 0.41 | 0.02 |
| 6 | N.I. | 15.86 | 1362 | 0.86 | 0.04 |
| 7 | N.I. | 16.21 | 1379 | 1.10 | 0.05 |
| 8 | Xylitol 5TMS | 16.6 | 1398 | 0.33 | 0.02 |
| 9 | D-fructose 5TMS | 17.69 | 1453 | 1.76 | 0.09 |
| 10 | Palmitato de metila | 18.74 | 1508 | 4.45 | 0.18 |
| 11 | D-Glucitol 6TMS | 19.28 | 1538 | 0.43 | 0.02 |
| 12 | D-glicose 5TMS | 19.89 | 1572 | 1.85 | 0.09 |
| 13 | Palmiticacid 1TMS | 20.21 | 1590 | 16.8 | 0.63 |
| 14 | Methylolate | 20.71 | 1618 | 8.13 | 0.40 |
| 15 | Methyl stearate | 20.97 | 1634 | 0.72 | 0.04 |
| 16 | Oleic acid 1TMS | 21.95 | 1692 | 28.4 | 1.21 |
| 17 | Stearic acid 1TMS | 22.15 | 1704 | 5.90 | 0.23 |
| 18 | Arachidic acid 1TMS | 23.49 | 1811 | 0.33 | 0.02 |
| 19 | Suberic acid 2TMS | 24.66 | 1864 | 1.02 | 0.05 |
| 20 | Malic acid 3TMS | 24.96 | 1884 | 1.14 | 0.06 |
| 21 | Campesterol 1TMS | 29.62 | 2220 | 0.22 | 0.01 |
| 22 | Stigmasterol 1TMS | 29.83 | 2236 | 0.17 | 0.01 |
| 23 | β -Sitosterol 1TMS | 30.25 | 2288 | 0.61 | 0.03 |
| 24 | Gedunin | 33.65 | 2881 | 0.79 | 0.04 |
| 25 | 7-Deacetoxy-7-oxogedunin | 34.09 | 2954 | 2.53 | 0.13 |
| 26 | N.I. | 34.77 | 3066 | 1.40 | 0.07 |
| 27 | Desacetylgedunin | 35.14 | 3127 | 1.60 | 0.08 |
| 28 | 6 α -Acetoxygedunin | 35.32 | 3156 | 0.24 | 0.01 |
| 29 | N.I. | 35.64 | 3207 | 6.27 | 0.25 |
| 31 | Epoxydeacetylgedunin | 36.23 | 3302 | 0.70 | 0.03 |

^a TMS: trimethylsilyl, RT: retention time, KI: Kovat's retention index calculated on the DB-5 MS capillary column using homologous series of *n*-alkanes, Sd = standard deviation, (%) compound abundance in the sample.



Table 6 Fragments of the substances not identified in the analyzes of the methanolic extract of the seeds of *Carapa guianensis* with the addition of pyridine (G3-P)^a

| RT | <i>m/z</i> |
|-------|---|
| 15.86 | 43(20), 57(55), 69(26), 83(18), 97(100), 111(8), 123(18), 137(10), 179(10), 193(2), 235(1) |
| 16.21 | 70(68), 73(35), 84(12), 130(10), 157(30), 158(12), 159(5), 242(100), 243(20), 244(5), 275(1), 330(5), 332(1) |
| 34.77 | 73(52), 75(8), 147(20), 157(75), 158(15), 159(5), 217(100), 231(70), 246(20), 275(10), 325(1), 414(1) |
| 35.64 | 73(10), 96(10), 135(10), 177(5), 191(15), 207(100), 208(25), 221(5), 253(15), 281(25), 282(5), 325(1), 341(2), 355(2), 405(1), 429(1) |
| 36.23 | 73(100), 95(50), 131(25), 149(15), 189(12), 213(10), 267(5), 313(8), 331(2), 403(40), 404(10), 419(5), 495(1) |

^a RT: retention time, *m/z*: mass fragments.

and presents their respective retention times, which may help in future identification.

The tetranortriterpenoid detected in the analysis of the three extracts of andiroba seeds and identified by comparison of the CG/MS mass spectra library was desacetylgedunin (C₂₆H₃₂O₆) 440.53 MW, 513 [M+]³ and (*m/z* 299.28). This was also found in another study,⁴⁵ and in the analysis of the hexane extract of the seeds by GC/MS resulting in values of 440 MW and the base peak of 299 *m/z* confirm the presence of limonoids in fresh andiroba oil employing gas chromatography.¹⁸ Another study carried out¹⁹ when analyzing the andiroba oil profile by GC/MS, detected the presence of deacetylgeduninumine and epoxides acetylenedunine in the analyzed samples.¹⁹ The results obtained by Ferraris *et al.*¹⁵ using a fraction rich in gedunin in animals with induced allergic reactions, proves the efficiency of this substance in the non-activation of T lymphocytes during the inflammatory process caused by the allergic reaction, thus functioning as an excellent antiallergic (Table 6).

The steroid compounds detected and identified in the extracts are relevant for Amazonian vegetable oils Bataglion *et al.*,³¹ and that GC/MS analysis is not always successful in this type of identification, especially steroids and triterpenoids (limonoids). In this case, it is observed that the presence of the campesterol identified in the extract (G3), shows that the method used was efficient in the identification of this class of metabolite and that the highest or lowest quality of the analysis may be related, mainly to the treatment of the samples.

The ultrasonic-assisted extraction method demonstrates to be efficient for all classes of lipids present in the andiroba seed matrix, concentrating steroids, mostly tetranortriterpenoids, by increasing the concentration of these substances in the extracts of the seeds.

Conclusions

The application of the ultrasound-assisted lipid extraction technique proved to be an efficient method to increase the concentration of triterpenoids (steroids and

tetranortriterpenoids) in the extracts, from a small amount of andiroba seed biomass, which describes a simple and fast method that does not require large quantities of samples. The reduction in the extraction time and the volume of solvents used in obtaining the extracts were other important observed factors. The use of the lipidomics technique was presented as an alternative that replaced the classic techniques with greater success in obtaining the results in this study. It is noteworthy that this study obtained the largest number of identifications possible involving different lipid classes, including steroids and tetranortriterpenoids known as limonoids in a single analysis. The application of the technique in fresh oil and a solid matrix, as is the case of the biomass of the andiroba seeds, denotes versatility, proved as evidenced by the data of the lipid profiles obtained in this study. The lipidomics applied to the oil and seeds of *C. guianensis* shown to be highly efficient for the analysis and identification of the lipid substances, especially limonoids and steroids, with faster extraction, time, and solvent savings, as well as the use of a small amount of the samples, when compared to other methodologies described in the literature.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

We want to thank the Laboratory of Plant Physiology and Biochemistry, National Institute of Amazonian Research (MCTI-INPA) for sending the plant material, also, CAPES and PRO-PESP/UFPA for granting a scholarship and financial support.

Notes and references

- 1 A. J. E. A. Menezes, O histórico do sistema extrativo e a extração de óleo de andiroba cultivado no município de Tomé-Açu, Estado do Pará, in *XLIII CONGRESSO DA SOBER*, Sociedade Brasileira de Economia e Sociologia Rural, Ribeirão Preto, 2005, vol. 24, pp. 1–11.
- 2 A. P. Mendonça and I. D. K. Ferraz, Procedimento para Exportação Óleo de Andiroba no Estado do Amazonas, *Revista Fitos*, 2006, **2**, 42–45.
- 3 L. F. G. Amaral and I. M. Fierro, Profile of medicinal plants utilization through patente documents: the andiroba example, *Brazilian Journal of Pharmacognosy*, 2013, **23**, 716–722.
- 4 A. P. Mendonça and I. D. K. Ferraz, Óleo de Andiroba: processo tradicional de extração, uso e aspectos sociais no estado do Amazonas, *Acta Amazonica*, 2007, **37**, 353–364.
- 5 L. K. S. Pires, M. G. Grisotto and R. F. Grisotto, O uso de plantas da Amazônia na produção de bioprodutos para tratamento de pele, *Revista de Investigação Biomédica.*, 2017, **9**, 78–88.
- 6 J. N. P. Lourenço, L. M. M. Ferreira, G. C. Martins and D. G. Nascimento, *Produção, biometria de frutos e sementes e extração do óleo de andiroba (Carapa guianensis Aublet)*



- sob manejo comunitário em Parintins*, AM. Embrapa Amazônia Ocidental, Manaus -AM, Brasil, Documentos, 2017, vol. 138, p. 36p.
- 7 C. C. Penido, K. A. Costa, M. F. S. Pereira, J. F. G. Siani, A. C. Henriques and M. G. M. Oliveira, Inhibition of allergen-induced eosinophil recruitment by natural tetranortriterpenoids is mediated by the suppression of IL-5, CCL11/eotaxin and NFκB activation, *Int. Immunopharmacol.*, 2006, **6**, 109–121.
 - 8 F. K. Ferraris, R. Rodrigues, V. P. Silva, R. Figueiredo, C. Penido and M. G. M. Henriques, Modulation of T lymphocyte and eosinophil functions in vitro by natural tetranortriterpenoids isolated from *Carapa guianensis* Aublet, *Int. Immunopharmacol.*, 2011, **11**, 1–11.
 - 9 A. K. F. Carvalho, Síntese de biodiesel por transesterificação pela rota etílica: comparação do desempenho de catalisadores heterogêneos, 2011, *Dissertação (Mestrado em Ciências) – Escola de Engenharia de Lorena*, Universidade de São Paulo, Lorena, 2011, p. 105.
 - 10 R. N. C. Miranda Júnior, M. F. Dolabela, N. M. Silva, M. M. Póvoa and J. G. Maia, Antiplasmodial activity of the andiroba (*Carapa guianensis* Aubl., Meliaceae) oil and its limonoid-rich fraction, *J. Ethnopharmacol.*, 2012, **142**, 679–683.
 - 11 A. R. P. Ambrozin, A. C. Leite, F. C. Bueno, P. C. Vieira, J. B. Fernandes, O. C. Bueno, M. F. G. F. Silva, F. C. Pagnocca, M. J. A. Hebling and M. Bacci Júnior, Limonoids from Andiroba Oil and Cedrela fissilis and their Insecticidal Activity, *J. Braz. Chem. Soc.*, 2006, **17**, 542–547.
 - 12 J. Machado da Rosa, M. I. C. Boff, P. A. Gonçalves, P. Boff and M. Z. Nunes, Andiroba oil (*Carapa guianensis* Aubl) in the capture of the fruit fly (*Anastrepha fraterculus* Wiedemann) in Feijoa (Accasellowiana (Berg) Burret), *Idesia*, 2013, **31**, 97–101.
 - 13 Brasil, *Sub-rede de Dermocosméticos na Amazônia a partir do uso sustentável de sua biodiversidade com enfoques para as cadeias produtivas da: castanha-do-pará e dos óleos de andiroba e copaíba*, Centro de Gestão e Estudos Estratégicos, Brasília – DF, 2007, p. 209.
 - 14 A. P. Galdino, *Estudo de mercado de andiroba, buriti e murumuru*, Relatório de consultoria para Imazon, 2007.
 - 15 F. K. Ferraris, K. H. Moret, A. B. C. Figueiredo, C. Penido and M. G. M. O. Henriques, Gedunin, a natural tetranortriterpenoid, modulates T lymphocyte responses and ameliorates allergic inflammation, *Int. Immunopharmacol.*, 2012, **14**, 82–93.
 - 16 I. D. K. Ferraz, J. L. C. Camargo and P. T. B. Sampaio, *Carapa guianensis* Aubl. *ECarapaproceras D. C. Manual de sementes da Amazônia*, Instituto Nacional de Pesquisas da Amazônia – INPA, Manaus – AM, 2003.
 - 17 C. R. Souza, R. M. B. Lima, C. P. Azavedo and L. M. B. Rossi, *Andiroba (Carapa guianensis Abul.)*, Embrapa Amazônia Ocidental, Manaus, 1st edn, 2006.
 - 18 E. C. Cabral, G. F. Cruz, R. C. Simas, G. B. Sanvido, L. V. Gonçalves, R. V. P. Leal, R. C. F. Silva, J. C. T. Silva, L. E. S. Barata, V. S. Cunha, L. F. França, R. J. Daroda, G. F. Sá and M. N. Eberlin, Typification and quality control of the Andiroba (*Carapa guianensis*) oil via mass spectrometry fingerprinting, *Anal. Methods*, 2013, **5**, 1357–1630.
 - 19 S. S. R. Milhomem-Paixão, M. L. Fascineli, M. M. Roll, J. P. F. Longo, R. B. Azevedo, J. C. Pieczarka, H. L. C. Salgado, A. S. Santos and C. K. Grisolia, The lipidome, genotoxicity, hematotoxicity and antioxidant properties of andiroba oil from the Brazilian Amazon, *Genet. Mol. Biol.*, 2016, **39**, 248–256.
 - 20 H. L. C. Salgado, R. Barbosa-Júnior, L. K. M. Conceição, A. S. C. Carvalho and A. S. Santos, Enzymatic hydrolysis of crude oil and isolated acyglycerides from Andiroba's seed, *Int. J. Sci. Res.*, 2015, **4**(7), 132–134.
 - 21 M. R. R. Tappin, M. J. Nakamura, A. C. Siani and L. Lucchetti, Development of an HPLC method for the determination of tetranortriterpenoids in *Carapa guianensis* seed oil by experimental design, *J. Pharm. Biomed. Anal.*, 2008, **48**, 1090–1095.
 - 22 T. Inoue, Y. Negai, A. Mitooka, R. Ujike, O. Muraoka, T. Yamada and R. Tanaka, Carapanolides A and B: Unusual 9,10-seco-mexicanolides having a 2R,9S-oxygen bridge from the seeds of *Carapa guianensis*, *Tetrahedron Lett.*, 2012, **53**, 6685–6688.
 - 23 A. Sakamoto, Y. Tanaka, T. Yamada, T. Kikuchi, O. Muraoka, K. Ninomiyac, T. Morikawa and R. Tanaka, Andiololides W-Y from the flower oil of andiroba (*Carapa guianensis*, Meliaceae), *Fitoterapia*, 2015, **100**, 81–87.
 - 24 S. V. Sancheti and P. R. Gogate, Ultrasound assisted selective catalytic transfer hydrogenation of soybean oil using 5% Pd/C as catalytic under ambient conditions in water, *Ultrason. Sonochem.*, 2017, **38**, 161–167.
 - 25 E. C. Schinor, M. J. Salvador, I. C. C. Turatti, O. L. A. D. Zucchi and D. A. Dias, Comparison of classical and ultrasound-assisted extractions of steroids and triterpenoids from *Chresta* spp., *Ultrason. Sonochem.*, 2004, **11**, 415–421.
 - 26 E. E. Kovac-Besovic, K. Duric, Z. Kalodera and E. Sofic, Identification and isolation of pharmacologically active triterpenes in Betula cortex, *Betula pendula* Roth. Betulaceae, *Bosnian J. Basic Med. Sci.*, 2009, **9**, 31–38.
 - 27 M. A. Hossain and Z. Ismail, Isolated and characterization of triterpenes from the leaves of *Orthosiphon stamineus*, *Arabian J. Chem.*, 2013, **6**, 295–298.
 - 28 F. E. Magrini, A. Spech, J. Gaio, C. P. Girelli, I. H. Miguez Heinzen, V. C. Sartori and V. Cesio, Viability of *Cabrlea canjerana* extracts to control the South American fruit fly, *Anastrepha fraterculus*, *J. Insect Sci.*, 2014, **14**, 1–13.
 - 29 C. A. Souza, *Sistemas Catalíticos na Produção de Biodiesel por meio de Óleo Residual*, Unifei – Engenharia Hídrica, 2006.
 - 30 R. Geris, N. A. C. Santos, B. A. Amaral, I. S. Maia, V. D. Castro and J. R. M. Carvalho, Biodiesel de soja - Reação de transesterificação para aulas práticas de química orgânica, *Quim. Nova*, 2007, **30**, 1369–1373.
 - 31 G. A. Bataglion, F. M. A. Silva, J. M. Santos, F. N. Santos, M. T. Barcia, C. C. Lourenço, M. J. Salvador, H. T. Godoy, M. N. Marcos Eberlin and H. H. F. Koolen, Comprehensive characterization of lipids from Amazonian vegetable oils



- by mass spectrometry techniques, *Food Res. Int.*, 2014, **64**, 472–481.
- 32 K. Yamamoto, A. Yahada, K. Sasaki, K. Sakamoto, K. Ogawa and H. Ohta, Multivariate Analyses and Characterization of Volatile Components in Citrus Species, *Food Sci. Technol. Res.*, 2013, **19**, 39–49.
- 33 E. F. Silva, I. F. Montero, A. A. Melo Filho, R. C. Santos, P. R. E. Ribeiro, A. K. P. Costa and A. C. G. Melo, Propriedades físico-químicas por RMN de ^1H y constituyentes en el Aceite de *Carapa guianensis* por ESI-MS, *Orbital: Electron. J. Chem.*, 2014, **6**, 246–254.
- 34 H. G. Rodrigues, *Modulação do processo de cicatrização pelos ácidos oleico e linoleico*, Tese (Doutorado em Fisiologia Humana) – Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, 2011, p. 91.
- 35 P. V. Borges, K. H. Moret, R. N. Manjunathaiah, T. E. M. Costa, A. P. Monteiro, A. B. Carneiro, P. Pacheco, J. R. Temerozo, D. C. Bou-Habib, M. G. Henriques and C. Penido, Protective effect of gedunin on TLR-mediated inflammation by modulation of inflammasome activation and cytokine production: evidence of a multitarget compound, *Pharmacol. Res.*, 2017, **115**, 65–77.
- 36 T. Inoue, Y. Matsui, T. Kikuchi, Y. In, T. Yamada, O. Muraoka, S. Matsunaga and R. Tanaka, Guianolides A and B, new carbon skeletal limonoids from the seeds of *Carapa guianensis*, *Org. Lett.*, 2013, **15**, 3018–3021.
- 37 T. Inoue, Y. Matsui, T. Kikuchi, T. Yamada, Y. In, O. Muraoka, C. Sakai, K. Ninomiya, T. Morikawa and R. Tanaka, Carapanolides M-S from seeds of andiroba (*Carapa guianensis*, Meliaceae) and triglyceride metabolism-promoting activity in high glucose-pretreated HepG2 cells, *Tetrahedron*, 2015, **71**, 2753–2760.
- 38 Y. Matsui, T. Kikuchi, T. Inoue, O. Muraoka, T. Yamada and R. Tanaka, Carapanolides J-L from the seeds of *Carapa guianensis* (andiroba) and their effects on LPS-Activated NO production, *Molecules*, 2014, **19**, 17137–17140.
- 39 O. K. Iha, F. C. S. C. Alves, P. A. Z. Suarez, C. R. P. Silva, M. R. Meneghetti and S. M. P. Meneghetti, Potential application of *Terminalia catappa* L. and *Carapa guianensis* oils for biofuel production: Physical-chemical properties of neat vegetable oils, their methyl-esters and bio-oils (hydrocarbons), *Ind. Crops Prod.*, 2014, **52**, 95–98.
- 40 B. S. Nayak, J. Kanhai, D. M. Milene, L. P. Pereira and W. H. Swanston, Experimental evaluation of ethanolic extract of *carapa guianensis* L. leaf for its wound healing activity using three wound models, *J. Evidence-Based Complementary Altern. Med.*, 2011, **2011**, 1–6; K. Ninomiya, S. Miyazawa, K. Ozeki, N. Matsuo, O. Muraoka, T. Kikuchi, T. Yamada, R. Tanaka and T. Morikawa, Hepatoprotective limonoids from andiroba (*Carapa guianensis*), *Int. J. Mol. Sci.*, 2016, **17**.
- 41 F. D. Ganstone, J. L. Harwood and A. J. Dijkstra, *The Lipid handbook*, Marcel Dekker, Inc., New York, USA, 3rd. edn, 2007, p. 438.
- 42 E. G. S. Lima, T. B. Oliveria, S. S. Conceição, W. L. S. Ataíde, B. M. Maltarolo, G. A. S. Nogueira, C. F. Oliveira Neto, R. C. L. Costa and R. S. Okumura, Biochemical and physiological responses of Andiroba (*Carapa guianensis* Aubl.) seedling subjected to water deficit, *Aust. J. Crop Sci.*, 2015, **9**, 517–522.
- 43 F. K. C. Moraes, G. L. S. Castro, D. D. Silva Júnior, H. A. Pinheiro and R. A. Festucci-Buselli, Chloroplast pigments, gas exchange, and carbohydrates changes during *Carapa guianensis* leaflet expansion, *Photosynthetica*, 2011, **49**, 619–626.
- 44 V. P. Silva, R. R. Oliveira and M. R. Figueiredo, Isolation of Limonoids from Seeds of *Carapa guianensis* Aublet (Meliaceae) by High-Speed Countercurrent Chromatography, *Phytochem. Anal.*, 2009, **20**, 77–81.
- 45 V. P. Silva, R. R. Oliveira and M. R. Figueiredo, Isolation of Limonoids from Seeds of *Carapaguianensis* Aublet (Meliaceae) by High-Speed Countercurrent Chromatography, *Phytochem. Anal.*, 2009, **20**, 77–81.

