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Extraction, determination of the fatty profile and potential application of oil from the seeds of *Bombacopsis retusa* (Mart. & Zucc.) A. Robyns

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ABSTRACT. In Brazil there is a semi-arid region of the Bahia State where the transition between caatinga and cerrado prevails. The *Bombacopsis retusa* (Mart. & Zucc.) A. Robyns belongs to Bombacaceae family, and is frequent in this region. Fruit of *Bombacopsis retusa* have seeds edible by native fauna and appreciated as appetizer nut in small local villages. With the purpose of verifying the potential application of the oil of this species in different industrial segments, the crude oil of *Bombacopsis retusa* seeds was extracted with Soxhlet apparatus with 55.5% yield. The physical and chemical analyses to determine the oleochemical indices showed acid value of 0.09 mg KOH^{-1} g^{-1}, iodine value of 62.80 g $I2^{-1}$ 100 g^{-1}, saponification index of 192.80 mg KOH^{-1} g^{-1}, refractive index (40°C) of 1.468 and a melting point of 43.90°C. To determine the fatty profile of this oil was applied the CG-DIC that revealed the predominance of palmitic (59.98%), oleic (19.25%) and linoleic (8.47%) acids, besides the saturated/unsaturated fatty acids ratio equal to 2.70. These results suggested that the oil of *Bombacopsis retusa* is a functional, sustainable and competitive raw-material to be used in agro-energy, food, pharmaceutical and cosmetic industries.

Keywords: *Bombacaceae*, fixed oil, gas chromatography, fatty-acids.

Extração, determinação do perfil graxo e potencial de aplicação do óleo das sementes de *Bombacopsis retusa* (Mart. & Zucc.) A. Robyns

RESUMO. No Brasil existe uma região semi-árida no Estado da Bahia onde a transição entre o bioma caatinga e cerrado é predominante. A *Bombacopsis retusa* (Mart. & Zucc.) A. Robyns pertence a família Bombacaceae e é frequente nesta região. Os frutos da *Bombacopsis retusa* apresentam sementes comestíveis pela fauna nativa e apreciação como castanha aperitiva em pequenos povoados locais. No intuito de verificar o potencial de aplicação do óleo desta espécie em diferentes segmentos industriais, o óleo bruto das sementes da *Bombacopsis retusa* pôde ser obtido através da extração com dispositivo de Soxhlet em 55,50% de rendimento. As análises físico-químicas realizadas para determinação dos índices oleoquímicos mostraram índice de acidez de 0,09 mg KOH^{-1} g^{-1}, índice de iodo de 62,80 g I_2^{-1} 100 g^{-1}, índice de saponificação de 192,80 mg KOH^{-1} g^{-1}, índice de refração (40°C) de 1,468 e ponto de fusão de 43,90ºC. Para determinação do perfil graxo deste óleo foi aplicada a CG-DIC que revelou a predominância dos ácidos palmítico (59,98%), oleico (19,25%) e linoleico (8,47%), além da relação do total de ácidos graxos saturados/insaturados que foi de 2,70. Tais resultados sugerem que o óleo da *Bombacopsis retusa* se trata de uma matéria-prima funcional, sustentável e competitiva para o aproveitamento nas indústrias de agroenergia, alimentícia, farmacêutica e cosmética.

Palavras-chave: *Bombacaceae*, óleo fixo, cromatografia gasosa, ácidos graxos.

Introduction

Brazil has different biomes where the flora still retains richnesses to be exploited in a sustainable manner, contributing to the preservation of several species, and consequently the biodiversity. While Amazon is a world reference as a supplier of plant raw materials, the Brazilian Northeast has regions with unique flora promising to generate natural resources.

The semi-arid region of Bahia State still presents a transition between caatinga and cerrado. This region has been threatened by the lack of sustainable development, with increasing desertification process. Promising plant species have been lost, and could already be in cultivars aimed at adding value to their products by the adherence of local communities to the policies of regional sustainability

Bombacopsis retusa (Mart. & Zucc.) A. Robyns, [syn. *Pachira retusa* (Mart. & Zucc.) Fern. Alonso] belongs to the family Bombacaceae, and genus *Bombacopsis* occuring in transition regions between caatinga and cerrado. In Bahia State, this species is frequently found in the Chapada Diamantina, Morro do Chapéu and in Xique-Xique, in the serra de Santo Inácio.

As shown in the Figure 1, this species occurs in sandstone outcrops, being characterized by crowded leaves at the ends of branches with three to five leaflets of retuse apex. In flowering, flowers have linear to lanceolate petals, of white-greenish color, with white stamens and orange panthers, and in fruiting, fruit are dry and capsular (DU BOCAGE; SALES, 2002). Fruit contain seeds from which is extracted a light-yellow fixed oil. The seed is popularly known as the deer nut, once kernel are consumed by small rodents, brown brocket deer, and even by the local population as an appetizer.

In local communities, the economic exploitation of *Bombacopsis retusa* is made by using the fixed oil from seeds to produce soap. There are several methods to extract plant oils, which mix mechanical and chemical processes. The oldest method considered inefficient as for satisfactory yields, but used in the economy of the local community, is the mechanical pressing, also known as discontinuous pressure. In this case, kernels are cold pressed obtaining the crude oil, which is washed with water and separated in the form of a solid fatty material. Only this fraction of solidified waxy oil is used for soap production.

Other more efficient methods are described in literature (MANDARINO; ROESSING, 2001; MONTENEGRO et al., 2008) such as the continuous pressure or 'expeller'; solvent extraction and the combined method 'expeller'-solvent, where the oil is partially extracted by mechanical pressure in continuous presses known as 'expellers', followed by extraction with organic solvent. In this case, after seed pressing and removal of crude oil, the cake retained in the press is subjected to organic solvent that dissolves the residual oil of the cake, which becomes practically no oil. The solvent is recovered and the oil separated from the solvent is mixed to the crude oil removed from pressing. The method provides satisfactory yields, since in the cake or in the extracted meal there is less than 1% oil, which also has best quality due to the shorter exposure time to high temperature.

Considering that Brazil has a privileged position in ethanol production and aiming to eliminate the use of petroleum derivatives in oilseed processing, Freitas et al. (2007) performed a hot-ethanol extraction with the oil from Brazil nuts (*Bertholletia excelsa* H.B.K.) obtaining 75% yield after oil recovery process of micelles formed in the process. Nonetheless, for Brum et al. (2009) the most used process in laboratory, the Soxhlet extraction with apolar solvent, is still advantageous in the extraction of fixed oils, since the sample is always in contact with the solvent by continuous renewal. In this case, the system temperature is kept relatively high and constant, and the method is very simple and does not require specialized training and allows extracting a greater amount of oil in relation to other methods. Besides, there is also no need to filter micelles after the end of extraction, as in other methods, because the sample remains shrouded in a cartridge throughout the procedure.

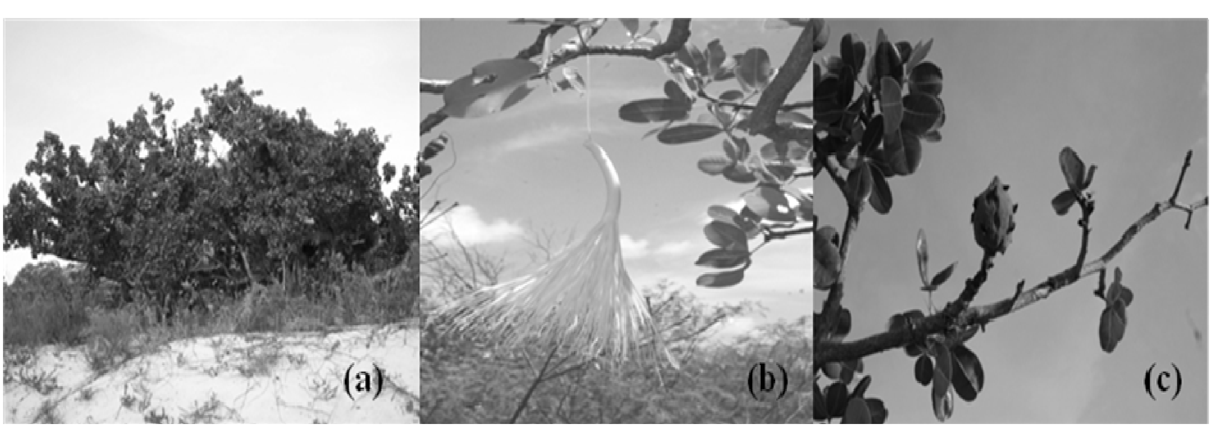

Figure 1. Photographic sequence of *Bombacopsis retusa* (Mart. & Zucc.) A. Robyns: tree in sandstone outcrop (a); flowering tree with leaves crowded at the ends (b) and fruiting tree with ripe fruit capsule (c).

From this popular use of the plain nut oil, without previous reports in literature, this study proposed to extract the oil of the seed of *Bombacopsis retusa* with apolar solvent, and determine the oleochemical indices and fatty profile to verify the potential use in products. The fixed oil of *Bombacopsis retusa* seeds had its fatty composition characterized by gas chromatography with flame ionization detection (GC-FID).

Material and methods

Seed collection and processing

Seeds of *Bombacopsis retusa* were collected in the municipality of Piemonte de Diamantina, Morro do Chapéu (Bahia State), in sandy soil, from the spontaneous opening of fruit, between September 2010 to February 2011, with extended fruiting period. According to Robyns (1963) it is close to the species *Bombacopsis paraense* (Ducke) A. Robyns should bloom from September to November, fruiting in November. The identification of the botanical species *Bombacopsis retusa* (Mart. & Zucc.) A. Robyns was performed by the botanist Maria Lenise Silva Guedes, at the herbarium Alexandre Leal Costa of the Biology Institute of the Federal University of Bahia State. The dried voucher specimen was deposited under the record ACCB 65850, whose taxonomical classification allows adopting the synonymy: *Pachira retusa* (Mart. & Zucc) Fern. Alonso.

Seeds were transported and stored at room temperature and protected from direct light. In laboratory, dry seeds were divided into monthly batches, and each was dated. In the present study, we used seeds from a single batch selected according to size. Seeds were taken to forced air oven at 40ºC to reduce the moisture in approximately 10%, which facilitated the manual removal of the shell. Exposed kernels presented white and sebaceous endocarp corresponding to about 65% weight of whole seed, in the evaluated batch. These kernels were ground in industrial blender and then pulverized into a knife mill and the resulting powder after sieved (180 microns mesh size) was classified as fine, as specified in the 5th edition of the Brazilian Pharmacopoeia (FB, 2010).

Obtaining the oil

The fine waxy powder was placed into a Soxhlet extraction cartridge, the process of continuous extraction proceeded in hexane for 12 hours on a thermostated electric blanket. After this, a yellow oil was achieved and submitted to rotary evaporation with heating in thermo-controlled bath not surpassing 40ºC, in order to remove the residual hexane and prevent decomposition of heat-sensitive substances. The solvent-free crude oil was viscous, pale yellow, and placed in amber vials under refrigeration until chemical analyses, in order to avoid photo- and thermal-oxidation that can compromise a genuine determination of the fat profile.

Analysis of oil extracted from seeds

Peroxide value – milliequivalents of active oxygen in one kilogram of oil, calculated from the iodine released from potassium iodide, performed according to American Oil Chemists'Society, AOCS Cd 8-53 (AOCS, 1993).

Acid value – amount in mg of base required to neutralize the free fatty acids present in 1 g of oil, determined according to AOCS Cd 3d-63 (AOCS, 1993).

Saponification value - amount in mg of potassium hydroxide required to saponify 1 g of fat or oil. The material is saponified under reflux of alcoholic solution of potassium hydroxide in excess. The amount of alkali consumed is determined by titration with hydrochloric acid and phenolphthalein. For this, it was adopted the method of AOCS Cd 3c - 91 (AOCS, 1993).

Iodine value – measure of unsaturation of oil and fats, calculated as the amount of iodine in grans absorbed by 100 grams of sample, performed as recommended by AOCS Cd 1 - 25 (AOCS, 1993).

Refractive index – characteristic for each oil type, i.e., is closely related to the degree of saturation, but is affected by other factors, such as levels of free fatty acids, oxidation and thermal treatment. It was employed the method described in Analytical Standards of Instituto Adolfo Lutz (IAL, 2008) that uses the Abbe refractometer.

Melting point – expressed in ºC, was determined by the sample in capillary tube method inserted in the device to determine the melting point, equipped with thermometer and microscope, as proposed by the Institute Adolfo Lutz (IAL, 2008).

Fatty acid profile – oil from *Bombacopsis retusa* seeds was derivatized using boron trifluoride and methanol (BF_3 $MeOH^{-1}$), as proposed by Ackman (1998). The formation of methyl esters of fatty acids of oil composition was verified by thin layer chromatography and infra-red, as described by Jork et al. (1990). For thin layer chromatography, it was used petroleum ether: ethyl ether: acetic acid as eluent mixture in the proportion of

90:10:1 with sulfochromic disclosure. The sample containing the methyl esters was taken to infrared spectroscopic analysis by Fourier transform (IV-TF) performing scanning between 4000-600 cm^{-1}. The esterified sample was analyzed by gas chromatography with flame ionization detection - CG-DIC (VARIAN – CP 3800), with detector temperature at 250ºC, capillary column CP-WAX, and nitrogen carrier gas flow of 30 Ml $min.^{-1}$, oven temperature at 180ºC and of injector at 240ºC. The identification of fatty acids (FA) was undertaken by comparing the retention time (rt) of sample components with the standard of FAME (fatty acids methyl esters) 189-19 SIGMA and quantified as percentage.

Results and discussion

Seeds of *Bombacopsis retusa* were angular shaped with average diameter of 2.05 ± 0.01cm for the whole seed, as described in literature (DU BOCAGE; SALES, 2002). Also, on the surface of most seeds there were several striations starting from a single point, which were described as clear and longitudinal, matching the botanical description in literature on some species of the family Bombacaceae (SILVA et al., 2012).

Of the whole seeds, were separated the shell from the kernel, observing that 25.0% weight of nut was relative to shell and 70.0% to kernel. The Figure 2 illustrates the shape and longitudinal striation of whole seeds of a same batch, as well as the width (cm) of the whole seed (with shell) and of the kernel (without shell).

After extraction, the yield of crude oil of seeds (kernels) of *Bombacopsis retusa* was 55.60%, and values of saponification, acid, peroxide, iodine, refraction and the melting point were calculated by mean values obtained in triplicates followed by their standard deviations. In Table 1 are listed the oleochemical indices determined for the oil extracted from seeds of *Bombacopsis retusa*.

The peroxide value is an oleochemical parameter widely used to measure the state of oxidation of fats and oils. The extraction process used and storage conditions, both of oil and seeds, showed that the value of 5.65 ± 0.04 (meq kg^{-1}) relative to peroxide value calculated for the oil from *Bombacopsis retusa* seeds is in accordance with the RDC # 482 of the National Health Surveillance Agency (ANVISA, 1999), in which the value of oils widely used for consumption should not exceed 10.0 meq kg^{-1}.

The content of free fatty acids of crude oils depends on their quality. In general, increasing acidity declines the oil quality, justifying the importance of calculating this index to evaluate the hydrolytic rancidity of the oil or fat (OSAWA et al., 2006). According to the Ordinance # 255 of the National Petroleum Agency (ANP, 2003), the biodiesel should not have acid value above 0.80 mg KOH g^{-1}. Thus the fresh crude oil of *Bombacopsis retusa* seeds showed potential for obtaining biodiesel.

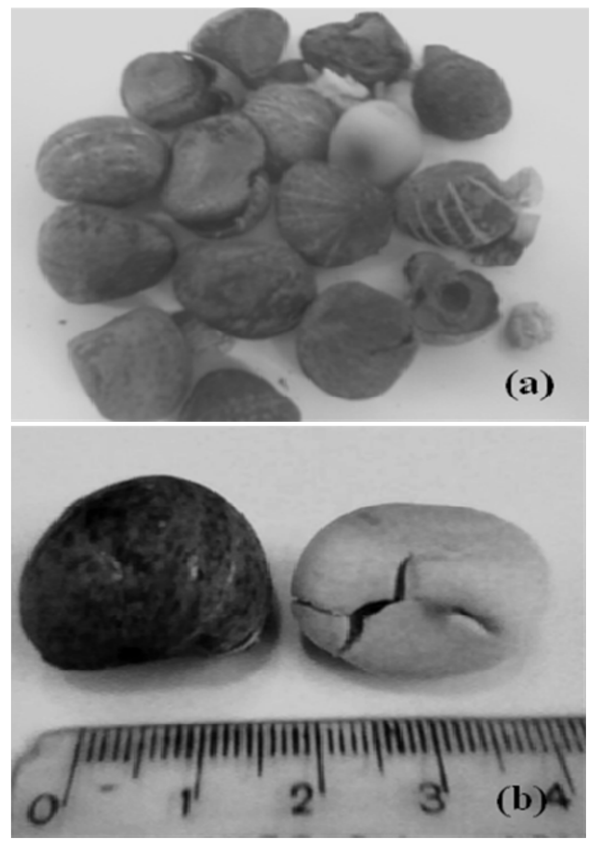

Figure 2. Whole seeds of *Bombacopsis retusa* (Mart. & Zucc.) A. Robyns (a) and a whole seed with its respective kernel (b).

Table 1. Results of values of peroxide, acid, saponification, iodine, refraction and melting point of oil of seeds of *Bombacopsis retusa*.

Oleochemical parameters	(X ± SD*)
Peroxide value (meq kg^{-1})	5.65 ± 0.04
Acid value (mg KOH^{-1} g^{-1})	0.09 ± 0.00
Saponification value (mg KOH^{-1} g^{-1})	192.80 ± 0.10
Iodine value (g I_2 100 g^{-1})	62.80 ± 0.30
Refractive index (40ºC)	1.468 ± 0.001
Melting point (ºC)	43.90 ± 0.10

X mean of values found; SD standard deviation.

Considering the saponification value, it was verified 192.80 mg KOH^{-1} g^{-1} for the oil extracted from *Bombacopsis retusa* seeds. This value lies within the range reported by the Codex Alimentarius Commission (1999), including for conventional plant oils, such as palm oil (190-209 mg KOH^{-1} g^{-1}).

The iodine value was 62.80 ± 0.30 g I2 100 g^{-1}. This value indicates the degree of unsaturation of an oil, thus it is an empirical value that should be compared to others obtained for plant oils with potential industrial application. Chaves et al. (2004) observed an iodine value of 66.30 g I_2 100 g^{-1} for the oil from seeds of chichá (*Sterculia striata*). But lower iodine values are mentioned by Silva (2011), such as 42 g de I_2 100 g^{-1} for cocoa seed oil and 45.90 g I_2 100 g^{-1} for oil from cupuassu seeds (*Theobroma grandiflorum*) and 45.17 g I_2 100 g^{-1} for oil from water chestnut seeds (*Pachira aquatica* Aubl.) also belonging to the family Bombacaceae. All these oils are widely used in cosmetic and food industries.

The refractive index is mainly related to saturation degree and the ratio of cis/trans double bonds of fatty acids, besides being influenced by oxidation processes. In the analyzed oil, the refractive index at 40°C was 1.468 ± 0.001 in the range of those determined for the soybean and sunflower oils (1.466-1.470 and 1.461-1.468, respectively), according to the Codex Alimentarium Commission (1999).

The melting point of a fixed oil is associated with the content of unsaturated fatty acids in its composition, the higher the saturated/unsaturated fatty acids ratio in a fixed oil, the great the tendency to increase of the melting point. The oil of *Bombacopsis retusa* seeds showed a fat composition of 63.47% saturated fatty acids, and the melting point found was 43.90°C ± 0.10. This result has a consistency when compared to oil from water chestnut seeds (*Pachira aquatica* Aubl.) which presented a composition with about 49.00% saturated fatty acids, and melting point at 41.90 ± 0.10°C (JORGE; LUZIA, 2012).

To determine the fat profile of oil from *Bombacopsis retusa* seeds, the derivatization of fatty acids of the sample with BF_3 $MeOH^{-1}$ was verified by the formation of a solid product which submitted to thin layer chromatography has revealed the spots relative to fatty acid methyl esters. The FT-IR spectroscopic analysis showed the bands typical of methyl esters (POULENAT et al., 2003), such as in 3010 cm^{-1}, assigned to the axial stretching of C-H methyl, and between 1710 cm^{-1} and 1743 cm^{-1} relative to stretchings C=O and C-O typical of the ester function.

Under the conditions described for gas chromatography with flame ionization detection (CG-DIC) the chromathogram (Figure 3) allowed verifying the major fatty acids comprising the oil of kernels of *Bombacopsis retusa*.

As discriminated in the Table 2, the predominance of methyl esters of saturated fatty acids in 63.47 ± 1.16% with the palmitic acid as the major component (59.98 ± 0.61%), and a total content of 29.28 ± 1.12% of methyl esters of unsaturated fatty acids.

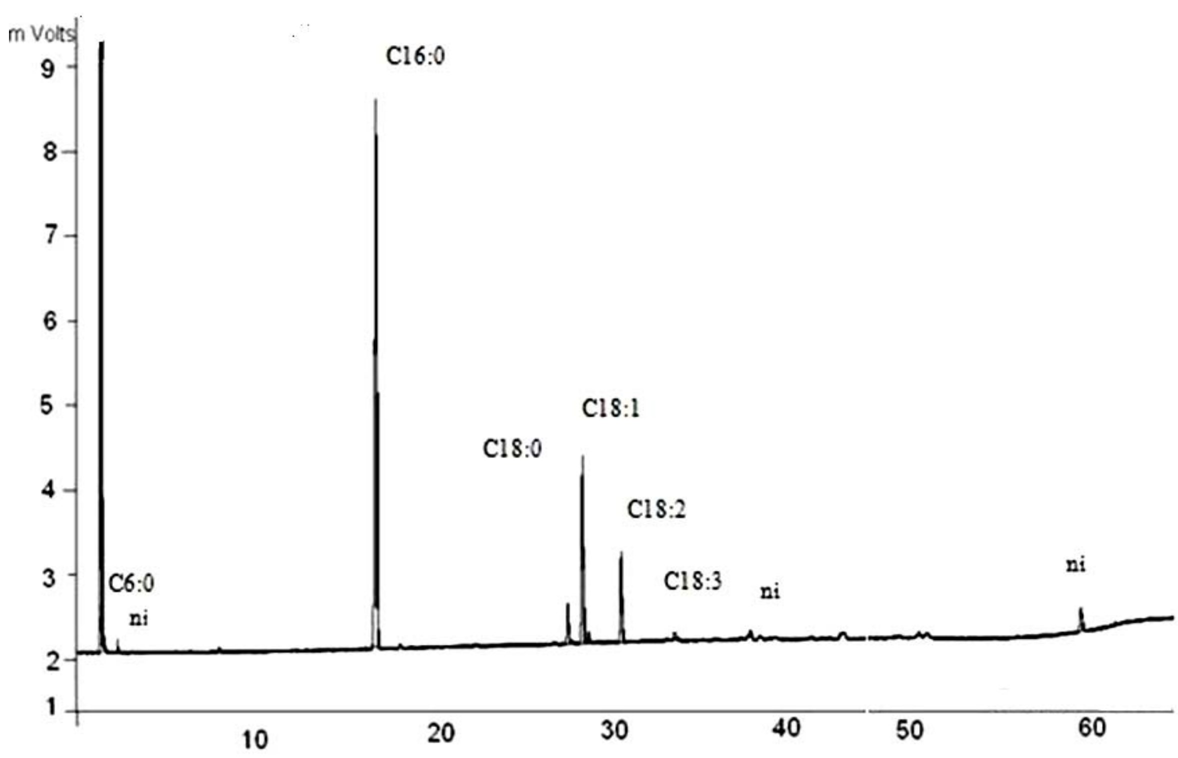

Figure 3. Chromathogram of the fat composition identified and unidentified (ni) of the oil extracted from seeds of *Bombacopsis retusa* (Mart. & Zucc.) A. Robyns / standard of FAME 189-19 SIGMA.

Table 2. Composition of fatty acids (% area) of crude oil of seeds of *Bombacopsis retusa* (Mart. & Zucc.) A. Robyns obtained by CG-DIC.

Tr	Fatty acid	Mean (%) ± SD**	
1.852	Caproic C 6:0	0.18	± 0.08
16.854	Palmitic C16:0	59.98	± 0.61
27.462	Stearic C 18:0	3.31	± 0.40
28.260	Oleic C 18: 1ω9c	19.25	± 0.72
30.398	Linoleic C18:2ω6c	8.47	± 0.21
33.352	Linolenic C18:3ω3	1.56	± 0.38
	ni*	7.26	± 0.68
	Total of sat.	63.47	
	Total of unsat.	29.28	
	sat./unsat. ratio	2.17	

*Percentage of fatty acids non-identified (*ni) by the standard FAME 189-19 SIGMA. **Percentage of fatty acids identified with standard deviation (SD) of the mean obtained in triplicate. Tr- retention time; sat.- saturated; unsat.- unsaturated.

The ratio obtained between the content of saturated/unsaturated fatty acid was compared with the other oils extracted from oilseeds found in Northeast Brazil, such as licuri (*Syagrus coronata (Martius) Beccari*) and macaúba (*Acrocomia aculeata*) that have been studied as for their potential application for production of biodiesel and foodstuffs (BELTRÃO; OLIVEIRA, 2007). The profile of fatty acids of these species present a saturated/unsaturated fatty acid ratio higher than 1.0. The oil extracted from *Bombacopsis retusa* seeds had 55.60% extraction yield, and a saturated/unsaturated fatty acid ratio of 2.17 and when inverse, i.e. unsaturated/saturated of 0.45.

Similar results were registered by Cargnin (2007) regarding the yield for other fixed oils and with potential for biodiesel production. The babassu nut oil (*Orbignya martiniana*) is extracted at 66.0%, but shows an unsaturated/saturated fatty acid ratio of 0.20. The palm oil (*Elaeis guianensis*), in turn presents only 22.0% yield, exhibiting an unsaturated/saturated fatty acid ratio of 0.35. Both oils have been made feasible in the agro-energy sector, once the content of unsaturated in this product is important for the suitable density of the oil, but if high can make it vulnerable to oxidation (DABDOUB et al., 2009). Therefore, the high content of saturated fatty acids compared with the lower content of unsaturated fatty acids in the oil of *Bombacopsis retusa* can become a further advantage in the potential use of this oil to produce biodiesel, because fatty acids are susceptible to oxidation reactions accelerated by the exposure to oxygen at high temperatures, conditions that are relevant during the use of the product as fuel.

Recent studies on oleochemical characterization aiming the specific use in food industry were developed with seeds of *Pachira aquatica* Aublet, known as water chestnut or nut of Maranhão, also belonging to the family Bombacaceae (JORGE; LUZIA, 2012). The lipid fraction of the seeds of this species has a saturated/unsaturated fatty acid ratio of 1.04, given the high content of total unsaturated, also had the palmitic acid as the major component, followed by the oleic and linoleic acids, as observed for *Bombacopsis retusa*. Kim et al. (2008) evaluated several fatty acids in seed oils, as promoters of cutaneous absorption of drugs and have stated the essential role of the palmitic acid, suggesting a potential use of *Bombacopsis retusa* in the pharmaceutical industry.

Fixed plant oils from Brazilian species relative to saturated/unsaturated fatty acids ratio higher than 1.0 have been frequently used in the cosmetic industry as agents of consistency in the production of creams and solid soaps, and when this ratio is lower than 1.0, plant oils tend to impart softness to the products (PEREIRA, 2009). In this way, the oil of *Bombacopsis retusa* (Mart. & Zucc.) A. Robyns also indicates functionality to make up cosmetic formulations.

Conclusion

The oil of *Bombacopsis retusa* seeds had a fat profile with important emphasis on palmitic, oleic and linoleic acids. The total fat identified of saturated was 63.47% and of unsaturated was 29.28%. The predominance of palmitic acid in the oil of *Bombacopsis retusa* has consistency with the fat profile found for *Paquira aquatic* which is also a Bombacaceae species. In summary, the oil of *Bombacopsis retusa* has potential for the same segments of application of oils widespread in northeastern Brazil with added value, such as the babassu nut oil (*Orbignya martiniana*) and palm oil (*Elaeis guianensis*). Furthermore, the species *Bombacopsis retusa*, through its seeds, can also play a sustainable economic role for the existing vegetation between cerrado and caatinga, mainly contributing to slow the process of desertification of the semi-arid of Bahia State.

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