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Chemical study of the seeds of *Ximenia americana*: analysis of methyl esters by gas chromatography coupled to mass spectrometry

Abstract

Fresh seeds of *Ximenia americana* presented high levels of protein (19.77%) and oil (27.71%). The oil components of the seeds, obtained by extraction with hexane, were subjected to the saponification/methylation reactions to produce methyl esters. The esters were then analyzed by gas chromatography-mass spectrometry allowing the identification of ten fatty acids. The main ones were oleic (55%), ximeninic (17%) and cis-19-octacosenóico (10%). The analysis process involved the fragmentation pattern exhibited by the methyl derivayives in their respective mass spectra.

Keywords: *Ximenia Americana,* Olacaceae, fatty acids; ximeninic acid; GC/MS

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Introduction

Beans are esterified natural fats have a prominent role in human nutrition, playing important biological functions for metabolism. Bones of the earth and the walls of the walls of the walls of the walls of the walls of the walls of the walls and the walls of the walls of the walls of the building blocks of the floor and the walls of the floor, resultando em proteção cardiovascular.[1] You may know that you are getting enough of these products to be used in the manufacture of sabers and detergents, or acidic products, for example, which are food-based. Although unsaturated fatty acids are the healthiest, studies are still needed to improve the conservation of edible oils, especially in oils with a high concentration of unsaturated as a result of auto-oxidation (rancidity).[2] It is worth adding the use of natural fatty acids for the production of biofuels by esterification with light alcohols (methanol, ethanol).Considering the main objective of the study, that is, the identification of *X. americana* seed oil components by gas chromatography coupled to mass spectrometry (CG / MS) and also for the purpose of comparison with the analysis made from derived by silylation,[3] components of the oil were converted to esters of fatty acids by means of methylation reaction. Basically, two reactional pathways have been most frequently used to convert fatty acids to methyl esters: mild alkaline methanolysis and saponification followed by fatty acid methylation (saponification/methylation). The mild alkaline methanolysis is a single-stage transesterification reaction catalyzed by an alkali in the presence of methanol, which is mainly effective for the formation of fatty acid methyl esters from ester-linked lipids.[4] The pathway via saponification/methylation is efficient to produce fatty acid methyl esters (and other methylated compounds) from fatty acids and free aldehydes, as well as from ester, ether and amine lipids. The process is carried out in two steps, both at elevated temperature, which includes base catalyzed saponification (usually NaOH or KOH) and acid catalyzed methylation (HCl or H_2SO_4) in the presence of methanol. In the first step alkali metal (saponification) long chain salts are formed, which, in a second step, are converted into volatile forms (methyl esters) for analysis by gas chromatography coupled to mass spectrometry (GC/MS).

Considering the already known biological activities of the bark, stem and roots of *X. americana*[5] and considering its abundance in the Brazilian Northeast, the present study aimed to contribute with the chemical knowledge of the species regarding the seeds of its edible fruits. Seeds with a high lipid mass, high levels of total protein (19.77%) and oil (27.71%) could eventually take advantage of nutraceutical (human or animal), medicinal, cosmetic,[6–8] or as raw material for the production of biodiesel.

Experimental

General methods

Gas chromatography coupled to mass spectrometry (GC/MS) analyzes were performed on Shimadzu GC-2010 apparatus coupled to a GCMS-QP2010SE mass spectrometer equipped with Rtx®-5MS (95% dimethylpolysiloxane and 5% diphenyl) column of 30 m, 0.25 mm internal diameter and 0.25μm film thickness of the fixed phase. Conditions: 80°C (3 min) to 280°C (5 min) at 5°C/min, then 20°C/min to 300°C (5 min) using He as drag gas at a flow rate of 1.7mL / min; injector temperature of 250°C and detector of 300°C. The analysis with the mass detector was in the scan mode with analysis time in 40 min; the recording of the mass spectra was in the range of 35 to 500 Daltons by impact of electrons with ionization energy of 70 eV (voltage of 1,5 KV), quadrupole type analyzer and source of ions at 240°C; cc were performed using Merck H60 gel, and tlc with Si gel Merck 60 F_{254}. Total protein content (%) in the grains of *X. americana.* The initial experiment consisted in determining the total protein content in the enzymatic system used, that is, the internal part of the grains of *X. americana*. Using the methodology of Nogueira and Souza,[9] a considerable content of 19.77% was found.

Moisture content (%) in grains of *X. americana*

The grains (30.974g) were oven-dried at a temperature of 105°C for 24h, time required to obtain a constant mass (21.394g). The moisture content was obtained according to the equation below, with a value of 31.32%.

$$Moisture(\%) = \frac{mi - mf}{mi} X100$$

At where:

mi = initial seed mass.

mf = final seed mass.

Oil content in (%) in grains of *X. americana*

The oil content was determined from the internal part of the grains (dehydrated matter = 21.394g) using a Soxhlet type extractor and hexane as solvent in a continuous and uninterrupted extraction process for 6:00 h. After this time, the solvent was evaporated under reduced pressure to give a mass of fixed oil (5.90 g), which represented 27.71% oil in the beans.

Obtaining extracts

Fresh seeds after drying at room temperature for one week were reduced in small granules. A sample (424.0g) was extracted with hexane (1: 3m/v) at room temperature four consecutive times, each extraction lasting for three days. The solutions were filtered, pooled and the hexane removed in vacuo yielding the hexane extract as yellow dense oil (EHXA, 148.0g). Part (20.3g) of this oil was subjected to a silica gel filter column eluted successively with hexane, dichloromethane and ethyl acetate. The solvents were evaporated under reduced pressure giving the FHEHXA (11.45g), FDEHXA (2.17g) and FAEHXA (1.56 g) fractions respectively.

Obtaining the methyl esters

Saponification: To one part (10.0 g) of the hexane fraction (FHEHXA) in MeOH (80 mL) was added KOH (10.0 g) and the mixture was kept under reflux for 1h. After cooling, distilled H 2 O (240mL) was added and the alkaline hydroalcohol solution was extracted with hexane (3 x 50mL) in decantation funnel. The organic phases were combined, dried over Na_2SO_4 and concentrated under reduced pressure, the unsaponifiables giving the pale yellow solid (1.87g). The hydroalcoholic phase was acidified with 20% HCl to pH 3-4 and then subjected to extraction with AcOEt (3 x 50mL). The organic phases were combined, dried over Na_2SO_4 and concentrated under reduced pressure to give the saponifiables as a bleached solid (7.15g).

Methylation: To one part (2.0 g) of the saponifiable material in MeOH (20.0mL) was added concentrated HCl (1.0 mL) and the mixture maintained under reflux for 1 hour. After cooling to room temperature, H_2O (10mL) was added, the reaction mixture was extracted with CH_2Cl_2 (3x10mL) and the organic phases were combined and dried with Na_2SO_4. Concentration under reduced pressure gave the methylated crude product (1.39g) which was then purified on a silica gel chromatographic column using hexane, hexane/CH_2Cl_2/ hexane/AcOEt mixtures as eluents. The AGME fractions 24-28 (0.69g), AGME 29-30 (0.36g) and AGME 31-39 (0.19g) (AGME = methylated fatty acids), eluted with 8: 2 hexane- CH_2Cl_2, 1: 1 hexane-CH_2Cl_2 and 6: 4 hexane-ACOEt, respectively, in higher amounts and higher tlc purity were then subjected to GC / MS analysis.

Results and discussion

Analysis of methylated derivatives x identification of fatty acids

The hexane fraction (FHEHXA) was subjected to saponification reaction (KOH / MeOH) to obtain the fatty acid salts (Saponification). These, after acidification, gave the free fatty acids which were then esterified, (MeOH/HCl) to yield the corresponding methyl esters (AGME). Silica gel column chromatography of the crude reaction product gave the fractions AGME 24-28, AGME 29-30 and AGME 31-39 (Methylation).

Analysis of AGME 24-28 in CG/MS showed the presence of ten fatty acids (Table 1), identified by the molecular ions.^{M} + (consistent with the respective molecular formulas) corresponding to the respective methyl esters represented by the peaks with retention (RT) and percentages (%) in the total ion chromatogram (Figure 1).

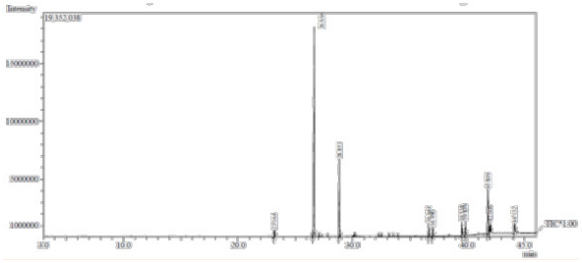

Figure 1 Chromatogram of AGME total ions 24-28

Mass spectra (MS) of the methylated components were also compared to mass spectra of fatty acid methyl esters reported in the literature and were consistent with the proposed structures, exhibiting characteristic m/z ratio fragments, as described below. The main components were Z-octadec-9-enoic acid (55.05%), octadeca-9-in-11-trans-enoic acid (17.38%) and Z-docos-13-enoic acids known as oleic acids, ximeninic and cis-19-octacosenoic acids, respectively (Table 1).

Table 1 Fatty acids identified as methyl esters (AGME 24-28)

Substance	Common name	T_R (min)	Content (%)	M.M	F.M
Haxadecanoic acid	Palmitic acid	23,16	1,46	256	$C_{16}H_{32}O_2$
Cis-octadec-9-enoic acid	Oleic acid	26,63	55,05	282	$C_{18}H_{34}O_2$
Octadeca-9-yn-11-trans-enoic acid	ximeninic acid	28,85	17,38	278	$C_{18}H_{30}O_2$
Cis-tetracos-15-enoic acid	Nervonic acid	36,64	3,00	366	$C_{24}H_{46}O_2$
Tetracosanoic acid	Lignoceric acid	36,99	2,05	368	$C_{24}H_{48}O_2$
Cis-hexacos-17-enoic acid	Ximenic acid	39,54	3,06	394	$C_{26}H_{50}O_2$
Hexacosanoic acid	Ceric acid	39,84	3,34	396	$C_{26}H_{52}O_2$
Cis-octacos-19-enoic acid	Acid Ximenixico[a]	41,81	9,85	422	$C_{28}H_{54}O_2$
Octacosanoic acid	Montanic acid	42,01	2,28	424	$C_{28}H_{52}O_2$
Trans-triacont-21-enoic acid	Lumequeic acid	44,13	2,22	450	$C_{30}H_{58}O_2$

T_R, Retention time; M.M, Molar mass (g mol^{-1}); F.M, Molecular formula.

A, common name used in this work.

The four saturated fatty acids (1, 5, 7 and 9) exhibited practically the same fragmentation pattern, with peaks due to breaks in the alkane chain [M-15 (CH_3) to M-183 ($C_{13}H_{27}$, 1), M -295 ($C_{21}H_{43}$, 5), M-323 ($C_{23}H_{47}$, 7) and M-351 ($C_{25}H_{51}$, 9), major peaks in *m/z* 41, 43, 55, 57, 74, 87 and 143 and base peak in m/z 74, the latter, resulting from Diels-Alder retro fragmentation.

1.T_R 23,164: M^+ *m/z* 270 (fórmula molecular $C_{17}H_{34}O_2$), 239 [(M-31)$^+$, perda de OCH_3], 227 (M-43)$^+$, 213 (M-57)$^+$, 199 (M-71)$^+$, 185 (M-85)$^+$, 171 (M-99)$^+$, 143 ($C_6H_{13}CO_2CH_3$)$^+$, 101 ($CH_2CH_2CH_2CO_2CH_3$)$^+$, 87 ($CH_2CH_2CO_2CH_3$)$^+$, 74 [H_2C=C(OH)OCH_3]$^{+\bullet}$, 55 (H_2CCH=C=O)$^+$, 57 (C_4H_9)$^+$, 43 (C_3H_7)$^+$ e 41 (C_3H_5)$^+$: Palmitic acid.

5. T_R 36,990: M^+ 382 (fórmula molecular $C_{25}H_{50}O_2$), 367 (M-15), 351 (M-31), 353 (M-29), 339 (M-43), 325 (M-57), 311 (M-71), 297 (M-85), 283 (M-99). Os picos principais foram em *m/z* 41 (C_3H_5)$^+$, 43 (C_3H_7)$^+$, 55 (H_2CCH=C=O)$^+$, 57 (C_4H_9)$^+$, 74 (H_2C=C(OH)OCH_3)$^{+\bullet}$, 87 ($CH_2CH_2CO_2CH_3$)$^+$ e 143 ($C_6H_{12}CO_2CH_3$)$^+$: Lignoceric Acid

.7. T_R 39,538: M^+ 410 (fórmula molecular $C_{27}H_{54}O_2$), 395 (M-15), 381 (M-29), 367 (M-43), 353 (M-57), 339 (M-71), 325 (M-85) e 311 (M-99). Os picos principais foram em *m/z* 41 (C_3H_5)$^+$, 43 (C_3H_7)$^+$, 55 ((H_2CCH=C=O)$^+$, 57 (C_4H_9)$^+$, 74 (H_2C=C(OH)OCH_3)$^{+\bullet}$, 87 ($CH_2CH_2CO_2CH_3$)$^+$ e 143 ($C_6H_{12}CO_2CH_3$)$^+$: Cerylic acid.

9. T_R 42,006: M^+ 438 (fórmula molecular $C_{29}H_{58}O_2$), 409 (M-29), 395 (M-43), 381 (M-57), 367 (M-71), 353 (M-85) e 339 (M-99). Os picos principais em *m/z* 41 (C_3H_5)$^+$, 43 (C_3H_7)$^+$, 57 (C_4H_7)$^+$, 55 (H_2CCH=C=O)$^+$, 74 (H_2C=C(OH)OCH_3)$^{+\bullet}$, 87 ($CH_2CH_2CO_2CH_3$)$^+$ e 143 ($C_6H_{12}CO_2CH_3$)$^+$; Mushroom Acid.

The five monounsaturated acids (2, 4, 6, 8 and 10) exhibited the same pattern of fragmentation, with the principal peaks in *m/z* 41, 43, 55, 69, 74, 83, 97, 98 and 111. It is worth highlighting in all these cases the peak due to the fragment in M-32 (M-CH3OH), as well as the base peak in *m/z* 55.

2. T_R 26,634, M^+ 296 (fórmula molecular $C_{19}H_{36}O_2$), *m/z*: 265 (M-31), 264 (M-32), 222 (M-74), 111 (CH_3CH_2CH=$CHCH_2CH_2CH_2CH_2$)$^+$, 98 (CH_3CH_2CH=$CHCH_2CH_2CH_3$)$^+$, 97 (CH_3CH_2CH=$CHCH_2CH_2CH_2$)$^+$, 87 ($CH_2CH_2CO_2CH_3$)$^+$, 83 ($CH_2CH_2CH_2CH_2CH$=CH_2)$^+$, 74 ([H_2C=C(OH)OCH_3]$^+$, 69 ($CH_2CH_2CH_2CH$=CH_2)$^+$, 55 [(CH_2CH_2CH=CH_2)$^+$ e/ou H_2C=CH-CO)$^+$], 43 (C_3H_7)$^+$ e 41 (CH_2CH=CH_2)$^+$; Oleic acid.

4. T_R 36.644, M 380 (fórmula molecular $C_{25}H_{48}O_2$), *m/z*: 349 (M-31), 348 (M-32), 306 (M-74), 111 (CH_3CH_2CH=$CHCH_2CH_2CH_2CH_2$)$^+$, 97 (CH_3CH_2CH=$CHCH_2CH_2CH_2$)$^+$, 83 ($CH_2CH_2CH_2CH_2CH$=CH_2)$^+$, 74 ([H_2C=C(OH)OCH_3]$^+$, 69 ($CH_2CH_2CH_2CH$=CH_2)$^+$, 57 (C_4H_9)$^+$, 55 [(CH_2CH_2CH=CH_2)$^+$ e/ou H_2C=CH-CO)$^+$], 43 (C_3H_7)$^+$, 41 (C_3H_5)$^+$; Nervous Acid.

6. T_R 39,538, M^+ 408 (fórmula molecular $C_{27}H_{52}O_2$), *m/z*: 377 (M-31), 376 (M-32), 334 (M-74), 111 (CH_3CH_2CH=$CHCH_2CH_2CH_2CH_2$)$^+$, 97 (CH_3CH_2CH=$CHCH_2CH_2CH_2$)$^+$, 83 ($CH_2CH_2CH_2CH_2CH$=CH_2)$^+$, 74 ([H_2C=C(OH)OCH_3]$^+$, 69 ($CH_2CH_2CH_2CH$=CH_2)$^+$, 57 (C_4H_9)$^+$, 55 [(CH_2CH_2CH=CH_2)$^+$ e/ou H_2C=CH-CO)$^+$], 43 (C_3H_7)$^+$, 41 (C_3H_5)$^+$; Ximenic Acid.

8. T_R 41,809, M^+ 436 (fórmula molecular $C_{29}H_{56}O_2$), *m/z*: 405 (M-31), 404 (M-32), 362 (M-74), 111 (CH_3CH_2CH=$CHCH_2CH_2CH_2CH_2$)$^+$, 97 (CH_3CH_2CH=$CHCH_2CH_2CH_2$)$^+$, 83 ($CH_2CH_2CH_2CH_2CH$=CH_2)$^+$, 74 ([H_2C=C(OH)OCH_3]$^+$, 69 ($CH_2CH_2CH_2CH$=CH_2)$^+$, 57 (C_4H_9)$^+$, 55 [(CH_2CH_2CH=CH_2)$^+$ e/ou H_2C=CH-CO)$^+$], 43 (C_3H_7)$^+$, 41 (C_3H_5)$^+$; Ximenoic acid (cis-19-octacosenoic acid).

10. T_R 44,132, M^+ 464 (fórmula molecular $C_{31}H_{60}O_2$), *m/z*: 433 (M-31), 432 (M-32), 390 (M-74), 111 (CH_3CH_2CH=$CHCH_2CH_2CH_2CH_2$)$^+$, 97 (CH_3CH_2CH=$CHCH_2CH_2CH_2$)$^+$, 83 ($CH_2CH_2CH_2CH_2CH$=CH_2)$^+$, 74 ([H_2C=C(OH)OCH_3]$^+$, 69 ($CH_2CH_2CH_2CH$=CH_2)$^+$, 57 (C_4H_9)$^+$, 55 [(CH_2CH_2CH=CH_2)$^+$ e/ou H_2C=CH-CO)$^+$], 43 (C_3H_7)$^+$, 41 (C_3H_5)$^+$;Lumequeic Acid.

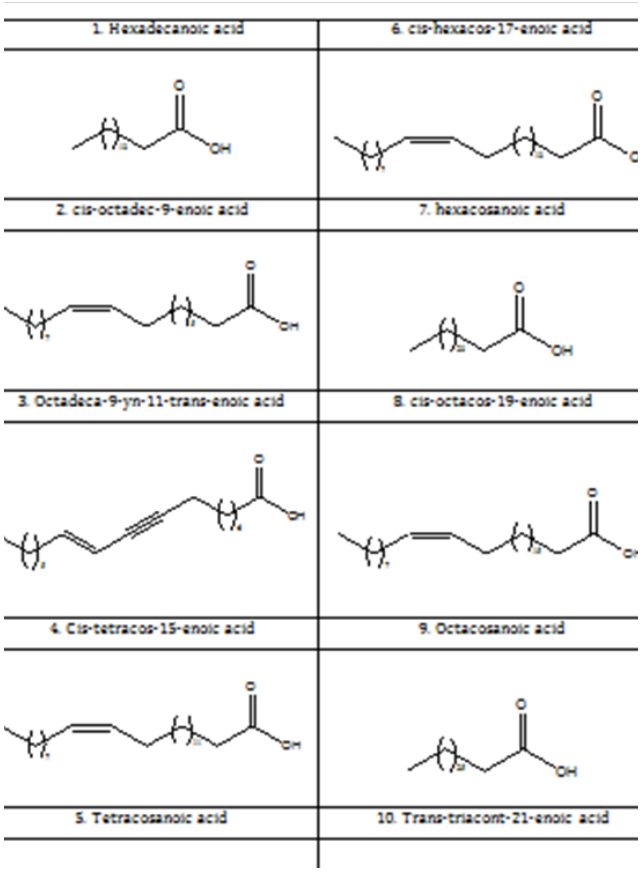

Figure 2 Structures of fatty acids identified in grains of *X. americana*.

In the case of the component with retention time 28,853 (3) a distinct fragmentation pattern was observed. Except for the peaks in *m/z* 41 and 43, fragments common to all and the peak in *m/z* 55, observed in the unsaturated acid spectra 2, 4, 6, 8 and 10, the mass spectrum of 3 showed the peaks *m/z* 67, 79, 80, 93 and 150, with the base peak in *m/z* 79. The highlights were the peaks due to fragments in *m/z* 261 (M-31 loss of OCH_3), 219 -CH_2CH_2CH = CH (CH_2) 5 CH_3] + en / z 150 [H_2 C = CHCH = CH (CH_2) 5 CH_3]., both indicative of a C_{18}-enine structure with the triple bond between C_9 and C_{10} carbons.[10,11] The base peak in *m/z* 79 (C_6H_7) + indicated the presence of more than one double or triple bond in the fatty acid chain.[12]

3. 28,853, M^+ 292 (fórmula molecular $C_{19}H_{32}O_2$), *m/z*: 261 (M-31), 219 (M-73), 164, 150, 135 ([H_2C=C=CHCH=$CH(CH_2)_5$]$^+$, 121 [[H_2C=C=CHCH=$CH(CH_2)_4$]$^+$, 107 [H_2C=C=CHCH=$CH(CH_2)_3$]$^+$, 93 [H_2C=C=CHCH=$CH(CH_2)_2$]$^+$, 79 [H_2C=C=CHCH=$CHCH_2$]$^+$, 67 [$HCC(CH_2)_3$]$^+$, 55 (C_3H_3O/C_4H_7)$^+$, 43 (C_3H_7)$^+$ e 41 (C_3H_5)$^+$; Ximeminic Acid.

The structures of the fatty acids identified in the grains of *X. americana* are shown in Figure 2. The fractions AGME 29-30 and AGME 32-39 (Methylation), By GC/MS analysis, practically led to the same constituents of the AGME fraction 24-28.

Conclusion

Analysis of the oil of *X. americana* seeds by gas chromatography coupled to mass spectrometry using the saponification/methylation route allowed the identification of ten fatty acids. Four of these acids (palmitic, oleic, ximeninic and lignoceric) had already been identified

by analyzing the oil of these seeds via derivatization by silylation, also by gas chromatography coupled to mass spectrometry. It was possible to identify other six fatty acids (ceric, nerve, montane, ximenic, oxymic and lumeochemical), as well as to confirm the presence of the first four, contributing significantly to the knowledge of the composition of the oil of the species under study. It is worth mentioning the high content (19.77%) of total proteins and the high percentage (about 27.71%) of oil in the seeds of the edible fruits of this plant species, which, together with its spread in Northeast Brazil, possible use as a nutraceutical, in cosmetology or even for the production of alkyl fatty acid monoesters (biodiesel). In addition, the present study contributed to increase knowledge about the medicinal potential of *X. americana*. Cataplasms of plants of the genus Ximênia are used as masks for treatment of the skin. The ximeninic acid and its ethyl ester (ximenoil) have topical action, being used in the form of emulsions with anti-cellulite action.

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Conflicts of interest

The authors declare no conflicts of interest.

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