J Environ Anal Toxicol
Volume 11:S7, 2021

Environmental & Analytical Toxicology

Research Article

Open Access

Evaluation of the Methodology for the Chemical Extraction of Phorbol Esters contained in *Jatropha Curcas* Oil

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Abstract

The use of resources that are of natural origin and that the practices are sustainable both in the harvest and manufacture of other products are increasingly poignant in the world society. In this sense, the use of the Pinhão Manso fruit (*Jatropha curcas*), an oilseed similar to mannone and soy, can be used as an input for various products in the Chemical Industry ranging from biodiesel to fine chemicals. The current work is a prologue to the work of evaluating the methodology for extracting phorbol esters by means of solvent extraction. The solvent initially used was ethanol and for comparison, regarding the presence of phorbol esters, the albumen was pressed to obtain the oil. The characterizations were performed by FTIR, NMR and HPLC. Through the analysis of FTIR, NMR and HPLC, the presence of common fatty acids was observed. There is also evidence that even in small concentrations there is a presence of phorbol esters, even when the extraction is carried out by polar solvent.

Keywords: Chemical extraction • Gasoline • Distillation • Fatty acids

Introduction

In Brazil, jatropha has been cultivated by producers in the Northeast, Midwest and Southeast, with the main purpose of producing biodiesel, which in theory biodiesel production can reach 1,100 to 1,700 liters of biodiesel/ha. According to Saturnino and collaborators [1] international development agencies and governments have researched and disseminated this culture in countries in Africa, Asia, South and Central America, as a producer of oil for the manufacture of biodiesel, which aroused the interest of businessmen Brazilians. Since 2004, some entrepreneurs have been planting Jatropha in Brazil and producing seeds in their crops. However, research on this species is still in its initial phase when compared to other oilseeds and much remains to be studied.

The cultivation of physic nut and the use of its fruit can have several other applications, other than the production of biodiesel. An alternative is the production of agroecological chemical pesticides of an organic and biodegradable nature of the seed makes them bet on other applications.

According to El-Gamassy the Jatropha fruit can be used in different fronts, such as: Biodiesel, its oil can also be used for lighting in small soap and candle production properties [2].

Mujumadar [3] and Abdel Shafy [4] showed that other parts of the plant also have varied applications, such as use in folk medicine, veterinary use, anti-inflammatory, anticoagulant, acaricidal and insecticidal properties. Jatropha seeds contain approximately 25%-30% protein. After removing the oil, proteins remain in the residue of the pinion seed. Proteins from Jatropha seeds have some similarity to well-known oilseed proteins, such as soy, canola, or sunflower protein. In contrast to soy and sunflower, Jatropha seed contains some toxic compounds, such as curcine and phorbol esters, which makes Jatropha proteins unsuitable for some applications in the food sector.

Therefore, the use of these substances in the manufacture of pesticides for some crops would be important since pest control is currently carried out by the uncontrolled use of pesticides in various crops.

Pest control associated with the cultivation of food for the Capixaba and Brazilian population is of great importance. Generally, in order to have an effective control, the use of pesticides in the crop is resorted to, which causes the wrong handling of these toxic and harmful to health compounds, as well as overdosing in order to eradicate the existing pests, resulting in the contamination of food that will pass to consumers and mainly the contamination of all individuals who mishandle these inputs.

Several methodologies for pest control have been used in the cultivation of food such as: Organic, barriers, repellent plant, plant with action insecticide, wood ash and insecticides and fungicides based on synthetic pesticides [5].

The objective of this work is to verify the presence of these toxic substances present in this species for the composition of a natural agroecological defensive. As well as achieving standardization in oil extraction from seeds; providing better conservation of them and the applicability of their products and by-products in the control of pests that compete with food crops, in an agroecological way, in the State of Espírito Santo.

Materials and Methods

Experimental part

The samples from *Jatropha curcas* were provided by the Experimental Farm of the Federal Institute of Espírito Santo (IFES)-Santa Teresa Campus. The seeds used come from *Jatropha curcas* L. fruits, obtained through collections. They were manually extracted from the fruits and dried in the shade on a screened platform.

After collecting the fruits, the peel was separated from the seed (albumen), which was carried out manually. The albumen was separated and preserved until the oil was obtained for future characterization.

The extraction of essential oil was carried out through two routes:

1. Pressing using the 15-ton hydraulic press: Model P15000 BOUVENAU.

2. Solvent extraction.

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Received: September 02, 2021; **Accepted:** September 16, 2021; **Published:** September 23, 2021

The chemical extraction route was performed using the previously pressed Jatropha seed. The extraction was performed in a Soxhlet apparatus, using ethanol as solvent, for a time interval of approximately 8 hours.

After extraction by Soxlhet, the alcoholic solution was evaporated until complete removal of the solvent in order to completely separate the oil phase from the solvent. The oil obtained was stored in an amber container and sealed for future analysis.

Quantification of the content of compounds present in the oil by pressing and by the solvent extraction method.

The characterizations of oils for the validation of the presence of phorbol esters were performed by: High Performance Liquid Chromatography (HPLC) with a Mass Spectrometry detector (HPLC-EM), provided by the company Quimplan; by Fourier Transform Infrared Spectoscopy (FTIR) and H1 Nuclear Magnetic Resonance, the latter performed at the Institute of Macromolecules, IMA/UFRJ.

Reagents

P.A. ethyl alcohol was used as a solvent for extraction, acquired by Vetec and used without previous treatment.

Infrared spectroscopy

The Fourier Transform Infrared Spectroscopy (FTIR) analyzes were performed at the Instrumental Support Laboratory of the Macromolecule Institute (IMA/UFRJ), in a Frontier PerkinElmer Spectrum Version 10.4.2 spectrophotometer, on potassium bromide (KBr) tablets in the range of 400-4000 cm^{-1}, with a resolution of 4 cm^{-1}.

Nuclear magnetic resonance

NMR spectra were recorded on Varian Mercury VX spectrometer (300 MHz/25°C) using deuterated chloroform (CDC13) as a solvent and reference.

High performance liquid chromatography

The chromatography analyzes were performed in an Ultra Performance Liquid Chromatography equipment coupled to the triple quadrupole mass selector UPLC-MS/MS.

Chromatographic analysis was performed on a C_{18} column (5 µm, 2.750 mm) Isocratic elution. Program was used with mobile phase, it consisted of Solvent (50 mm of ammonium acetate+acetonitrile, 9+1). The flow rate was 0.2 ml/min, the injection was 40 µl volume of MS/MS conditions. MS/MS was performed on a Micromass Quattro Ultima Spectrometer equipped with ESI source.

Results and Discussion

The extraction of jatropha oil was carried out in two different ways. The most practical and low cost of the process is the pressing process. The crude oil was characterized by FTIR, NMR H1 and by HPLC to verify the composition of the studied sample and to evaluate the presence of phenolic groups, such as phorbol esters. The second extraction to evaluate the purity of the studied sample was through extraction by solvent using ethanol as solvent. The characterization of the oil was carried out by FTIR where it was possible to verify in a better attribution to the functional groups present in the oil; however the yield is considerably lower for each extraction step compared to the pressing process, apart from the expense of solvent for the extraction process oil. Figure 1 shows the FTIR spectra for a sample of crude oil obtained by pressing and Figure 2 shows the oil extracted by means of solvent or ethanol.

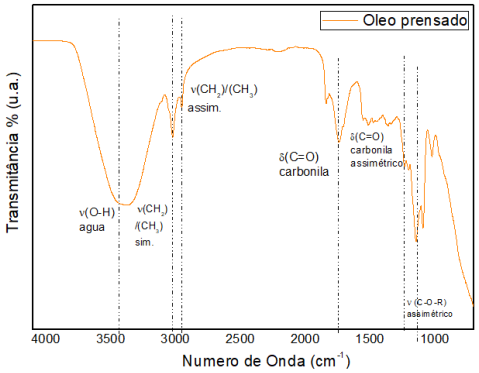

Figure 1. FTIR spectrum for the *Jatropha Curcas* oil sample obtained by pressing.

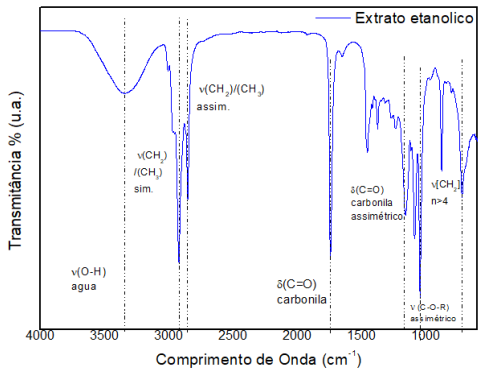

Figure 2. FTIR spectrum for the *Jatropha Curcas* oil sample obtained by extraction in soxlhet.

Infrared vibrational spectroscopy

In Figure 1, the peaks attributed to the functional groups obtained by pressing are less intense than the bands in Figure 2 that were obtained by ethanol extraction and then solvent evaporation, that is, by the ethanolic extraction method, the oil obtained is purer reducing the presence of natural solvent, which is water.

The assignments of the bands shown in Figure 1 are also present in the figure, but now with greater intensity of the bands showing in greater detail the composition of the functional groups present in jatropha oil Figure 2.

The values in Table 1 are represented by the peaks shown in Figure 1, where the displacements in the region from 2925 to 2856 cm^{-1} are observed, the existence of the absorption bands corresponding to the symmetrical and asymmetric stretching of the C-H connections of the CH_3 and CH_2 groups. The existence of such groups is also confirmed by the presence of absorption bands in 1456 and 1377 cm^{-1} corresponding to the angular deformations of the C-H bond. The presence of long carbon chains is verified by the vibration of fragment (CH_2) n, being n ≥ 4, which occurs in 721.3 cm^{-1}. The existence of double bonds (C=C) is confirmed by the absorption band in two regions, one of the double bond between carbons that occurs in 1642 cm^{-1} and the other in 879 cm^{-1}, which is related to the replacement of the ligands in the carbons of the double bond, in this case suggesting the predominance of vinyl substitution for this spectrum. The 1743.6 cm^{-1} band represents the stretching of the carbonyl group (C=O) for groups of carboxylic acids and the axial deformation of C–C (C=O)-O of saturated esters present in groups as carboxylic acids and in esters respectively Table 1.

Table 1. EAssignments of characteristic bands for Pinhão Manso oil.

Number of associated peaks	X (cm^{-1})	Y (%T)	Deformation stretches of relative groups	Relative intensity
1	3270,21	67,98	N (O-H) água	F
2	2922,49	80,51	N (CH_2) e n (CH_3) assimétricos	F
3	2853,07	85,87	N (CH_2) e n (CH_3) simétricos	F
4	1743,61	77.53	D (C=O) carboníla	F
5	1642,69	9,7	n (C=C)	F
6	1456,43	87,87	d (C-H) e d (C-H) assimétricos	M
7	1377,89	90,24	d (C-H) e d (C-H) simétricos	M
8	1160,11	81,54	d (C=O) carboníla ass.	F
9	1089,27	79,16	d (C–O) axial	M
10	1047,34	73,22	N (C-O-R) assimétrico	F
11	879,79	86,01	N (C=C) vinílico	M
12	721.38	83.31	(CH_2)n³ 4	M

The absorption bands that appear with low intensity around 1020-960 cm^{-1} are attributed to the vibrations of asymmetric stretching of the C-O bond, characteristic of esters.

Similar peaks are found in physic nut fatty acids and in the presence of phorbol esters there are two variations in the analyzed spectrum, which is the appearance of two peaks, the first in 1050 and the second at 940 cm^{-1}, deformation outside the plane of the CH bond for the cyclohexane group and axial deformation in the plane of the CO bond for the same group [6].

Nuclear Magnetic Resonance (H1 NMR)

The oil obtained by pressing was characterized by the nuclear magnetic resonance spectrum using the Hydrogen nucleus (NMR-H1). The solvent used was deuterated chloroform for the NMR-H1 analysis of the oil (Figure 3).

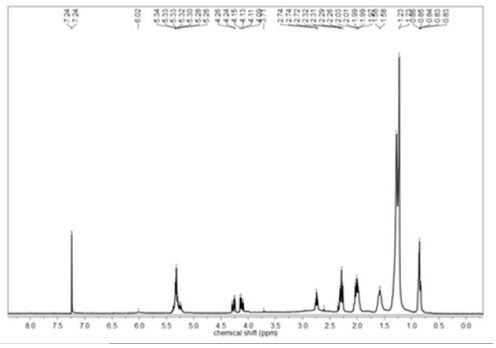

Figure 3. H1 Nuclear Magnetic Resonance Spectrum for the pressed oil sample.

The H1 NMR spectrum showed in Figure 3 shows shifts that indicate the presence of fatty acids, which are part of its composition. The composition of the oil is very heterogeneous, including the presence of methyl/ethyl esters, fatty acids, saturated and unsaturated, according to the HPLC analysis performed for the sample of pressed oil from physic nut and the possible presence of aromatic compounds such as phenols.

The H1 NMR spectrum for *Jatropha curcas* oil must present a signal at 3.7 ppm which is characteristic of hydrogen linked to the oxymethyl group referring to methyl esters, the presence of multiplets in 4.12-4.24 and 5.30-5.34 also confirm the presence of hydrogen attached to the oxymethyl group characteristic of triglycerides present in Jatropha oil [7].

The chemical shifts observed in the NMR spectrum functional groups of esters. The displacement at 5.4-5.5 ppm refers to the hydrogens linked in the transposition at the C=C bonds, for unsaturation of unsaturated fatty acids present in Jatropha oils.

Multiplets between 4.04-4.26 and 5.06-5.34 ppm are chemical shifts, referring to the hydrogens linked to the vicinal carbons of the carboxyl group of esters, linked to oxygen (H3C-O-(C=O) R), present in the composition of jatropha oil [8] and the displacements of Hydrogens in the vicinal carbon to the carbonyl group (C=O) of both esters and carboxyl acids appear at 2.75 to 2.02 ppm respectively. The presence of several signs in this region of the spectrum is due to the high heterogeneity of the oil composition.

For displacements less than 2 ppm, there are signs for the hydrogens that are located along the long carbon chain of fatty acids and possible esters that make up *Jatropha curcas* oil.

The presence of an intense peak at 7.24 ppm is correlated to the use of deuterated chloroform used as a solvent to perform the H1 NMR analysis.

High Performance Liquid Chromatography (HPLC)

Jatropha oil extracted by pressing was analyzed by HPLC and this chromatogram profile analysis showed, on average, a content of approximately 20.9% (v/v) of saturated fatty acids and 58.9% (v/v) unsaturated fatty acids. Oleic, linoleic and palmitic acids are the most abundant fatty acids present in the oil. The oil also presented 20% of two high molecular weight alcohols derived from flavonoids.

The chromatographic profile of *Jatropha curcas* oil extracted by pressing is shown in Table 2, which shows several contributions of fatty acids, these are shown in Table 2

Table 2. Composition of fatty acids present in the oil extracted by pressing Composition (fatty acids).

S.No	Nomencla-tura usual	Nomenclatura IUPAC	Massa molecular (g/mol)	Conc. % (v/v)
1	Palmítico (C_{16}: 0)	Hexadecanoico	256,424	12,7
2	Esteárico (C_{18}: 0)	Octadecanoico	284,477	8,2
3	Oleico (C_{18}:1)	CIS 9-Otadecenoico	282,461	15,7
4	Linoleico (C_{18}:2)	CIS (9Z,12Z)-9,12-Ácido Octadecadienoico.	280,445	22,3
5	1-Monooleolyglycerol	9- Octadecenyoinycacid (Z) -, 2, 3 – di hidropropil ester	884,213	8,6
6	Plastochromanol 8	2H-1Benzopyran-6-ol, 3,4 dihidro-2,7,8,trimetil	751,237	6,2
7	Sitosterol	(3b) – Stigmast-5-en-3-ol	414,707	6,1
	Total		450,566	79,8

The chromatography analyzes did not detect the presence of the molecular structures of the phorbol esters, for the oil extracted by pressing [7]. The presence of these structures of phorbol esters in liquid chromatography analyzes, under similar conditions, appear in a retention time between 8 to 12 minutes in low concentration, around 2% to 5% (v/v) in the oil.

The presence of unsaturated and saturated fatty acids, for the most part, are good indicators for the production of biodiesel, since these oils are essential for the production of biodiesel.

The chromatographic profile shows that the composition of fatty acids and other components that are present in Jatropha oil extracted by ethanol are very similar to the oil produced by pressing. The components present in this chromatogram are the same as shown in Figure 3, showing the same retention times, but with greater intensity.

However, Figure 3 shows a small contribution between the retention times 8 to 12 minutes, suggesting the presence of phorbol esters [8-10]. As the analysis was carried out in an exploratory way, a better analysis in this retention time range, we will be able to create a methodology for isolating phorbol esters, which can be used in future applications, such as natural agroecological defensive.

Conclusion

The methodology of extraction and initial characterization of the components present in the albumen of *Jatropha curcas* is in accordance with the literature showing a greater amount for unsaturated fatty acids in detriment to saturated ones, shown in the chromatographic profiles presented in this work.

The determination of phorbol esters proved to be promising, since in the first solvent extraction it suggests the presence of a small concentration of these molecules in the *Jatropha curcas* oil, but a more in-depth analysis from the point of view of HPLC using the fragment resource better mother in the mass spectrum, would favor the elucidation of these molecules in *Jatropha curcas* oil. And in this way create a methodology to isolate these molecules and apply them as a natural product.

The H1 NMR analyzes together with the FTIR analyzes for the two analyzed oils corroborate the presence of the functional groups of esters, triglycerides, unsaturated and saturated fatty acids. For the two oils obtained in this work, both come from the albumen of *Jatropha curcas*, the oil obtained by the extraction has a higher purity and less presence of water, evidenced by the characterization of FTIR, which can camouflage interfering in the analysis of the peaks related to the analyzed functional groups.

For the analysis of H1-NMR corroborates the HPLC/MS analyzes showing the presence of esters and fatty acids for the sample of the pressed *Jatropha curcas* oil.

For future work, a more in-depth study of solvent extraction methods, varying the composition and types of solvents, associated with a better analysis of the oil obtained, for quantitative validation of phorbol esters molecules will be fundamental for the isolation of these molecules and in the future the application as natural agroecological pesticides improving planting and harvesting in the state of Espírito Santo.

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How to cite this article: Loureiro, Felipe Augusto Moro and Alana Lemos Cavalcante de Oliveira. “Evaluation of the Methodology for the Chemical Extraction of Phorbol Esters contained in *Jatropha Curcas* Oil”. *J Environ Anal Toxicol* 11 (2021): 003.