

Avaliação da composição química e da toxicidade do óleo essencial de folhas e frutos da *Jatropha gossypifolia* L.

Assessment of chemical composition and toxicity of the essential oil of leaves and fruits of *Jatropha gossypifolia* L.

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Resumo

As folhas e frutos da *Jatropha gossypifolia* foram avaliados quanto a sua composição química. O óleo essencial das folhas obtido por hidrodestilação e o material graxo do albúmen das sementes e o óleo das sementes foram avaliados quanto a composição química e toxicidade frente a *Artemia salina*. O rendimento na extração dos óleos foi de 26,6 %, 38,4 % e 0,0365 % para as sementes, albumen e folhas, respectivamente. As toxicidades dos óleos obtidos do albúmen e das sementes, frente a *Artemia salina*, foram de $74,25 \pm 0,21 \mu\text{g mL}^{-1}$ e $78,66 \pm 0,25 \mu\text{g mL}^{-1}$, respectivamente. O índice de saponificação e o índice de acidez do óleo do albúmen foram de $165,4 \text{ mg KOH g}^{-1}$ e de 10%, respectivamente. Cromatografia gasosa com detecção por ionização em chama (CG-DIC) foi utilizada para análise do perfil de ácidos graxos do albúmen e sementes. Alto percentual para o ácido heptadecanoico (C17:0) foi determinado para o óleo das sementes com 35,81 %. Treze compostos foram identificados no óleo essencial obtido de folha por cromatografia gasosa acoplada a espectrometria de massas (CG-EM). Os índices de Kovats para os compostos foram calculados e comparados aos da literatura.

Palavras-chave: Óleos voláteis. Ácidos graxos. CG-EM. Euphorbiaceae. *Jatropha gossypifolia* L.

Abstract

The leaves and fruits of *Jatropha gossypifolia* L. were evaluated for their chemical composition. The essential oil of the leaves, were obtained by hydrodistillation and the fatty material of albumen and seeds were evaluated for chemical composition and toxicity in the *Artemia salina* assay. The extraction yield of oil was 26.6 % 38.4 % and 0.0365 % for the seeds, leaves and albumen, respectively. The toxicity of the oil obtained from the albumen and seeds against in the *Artemia salina* were $74.25 \pm 0.21 \mu\text{g mL}^{-1}$ and $78.66 \pm 0.25 \mu\text{g mL}^{-1}$. The saponification and acidity index for the oil albumen was $165.4 \text{ mg KOH g}^{-1}$ and 10%, respectively. Gas chromatography with flame ionization detection (GC-FID) was used to analyze the fatty acid profile of albumen and seeds. High percentage (35.81 %) for heptadecanoic acid (C17:0) was determined for the oil form of the seeds. Twelve compounds were identified in the essential oil obtained from the leaves by gas chromatography-mass spectrometry (GC-MS). The Kovats Index for compounds were calculated and compared to those of literature.

Key words: Volatile oils. Fatty acids. GC-MS. Euphorbiaceae. *Jatropha gossypifolia* L.

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Introduction

In Brazil, many plants are used for domestic use as medicament. Several of these plants still unexplored and needs to be investigated through research for use as new drugs. In vegetation of the savannah it can be found the family Euphorbiaceae, represented by approximately 222 genera and 7.000 species. The genus *Jatropha* belongs to this family and consists of 175 tropical and subtropical species. The species *Jatropha gossypifolia* L. is found in the savannah where it can be known as purple pinion or angry pinion (OLIVEIRA et al., 2008; SERVIN et al., 2006).

It is known for its medicinal properties and as a pesticide, containing mainly lignins and diterpenes. *J. gossypifolia* is also found in other countries like India, where studies show the discovery of new diterpenes, flavonoids and lignans (OLIVEIRA et al., 2008). From the ethyl acetate/hexane (EtOAc/He) leaves extract of *J. gossypifolia* L., Biswanath et al. (2003) has isolated cleomiscosin A and a coumarin-lignin. Studies of Ravindranath et al. (2003) on the leaf extract obtained with dichloromethane/methanol (DCI/MeOH) led to isolation of a new diterpenoid with antibacterial bioactivity against *Staphylococcus aureus*. Oliveria (2008) shows the intoxication caused by fruits and leaves of *J. gossypifolia* L. in the feeding of sheep, but do not indicate the toxic principles responsible for the poisoning. In Brazil, few studies are related to the structural elucidation and discovery of new compounds.

This work shows the evaluation of the composition of leaves extracts and essential oil obtained from *J. gossypifolia*. The extract was investigated for biological activity against *Artemia salina*, acidity and saponification index, and the chemical composition of the essential oil and fatty acids.

Material and Methods

Collect of plant

The vegetable material (leaves and fruits of *Jatropha gossypifolia* L.) was collected on February 2012, in Naviraí-MS, in region of pastures near to the “Córrego do Touro”. (VASCONCELOS et al., 2011). The vegetable material was taken to laboratory to preparation of extraction of essential oils from the leaves, and of fixed oils from the fruits. The fruits were skinned for separation of albumen and seeds for extraction of fixed oils. The moisture content for fruits was determined according methodology described by Farmacopéia Brasileira (1988). The samples were added in beckers and placed in oven at 100 °C for 60 min.

Extraction of fixed oils

The oils were extracted by using a Soxhlet apparatus (INGERSOLL; FUSON; ROSS, 2009). Approximately 70.25 g of albumen samples and 49.25 g of seeds were involved in a paper filter and placed in a Soxhlet apparatus and extracted with 250 mL of hexane for 6 hours, at reflux. After extraction, the solvent was evaporated in rotary evaporator and the oil collected for calculating the yield. The oil were placed in a glass vial with screw cap and protected from light.

Extraction of essential oil

The extraction of essential oil was performed in apparatus type Clevenger by hydrodistillation (SANTOS et al., 2004). The leaves (875.9 g) were crushed, placed in 5 L flask and submitted to extraction for 6 hours. After this time, the oil was removed by use of the ethyl acetate (5 mL). The solvent was evaporated in ambient temperature, and the oil stored in glass vial with screw cap and protected from light.

Oil degumming

For the degumming process, the crude oil was heated at 80°C and added 1% (by weight of oil) of H₃PO₄ (85% commercial) and 3% distilled water. The mixture was stirred for 30 min and centrifuged to separation of the gum (VIEIRA et al., 2009). The supernatant from this centrifugation was taken to a separation funnel in which remained at rest for 30 min. After the formation of the upper phases were collected.

Saponification

To determination of the soaps content (1.0 g of oil) was dissolved in 50 mL of a KOH solution (0.5 mol L⁻¹) and heated at 60 °C, in a reflux system, for 30 min. The solution was titrated with H₂SO₄ (0.5 mol L⁻¹) with phenolphthalein as indicator. The same procedure was performed with KOH free in medium. The volume of acid was determined for calculus of the saponification index (SI) by equation 1 (ARAÚJO, 2009):

$$SI = \frac{(V_B - V_A) \times M_1 \times MM}{m_{sample}} \quad \text{Equation 1}$$

where V_B is volume (mL) of spent H₂SO₄ solution in the titration, V_A is volume (mL) of spent H₂SO₄ in the titration of the sample, M concentration KOH solution, MM molar mass of KOH, and m the mass sample.

Acidity Indict

To determination of the content of acidity indict in the oil extract from the albumen, 1.0 g of degummed oil was obtained and dissolved in 15 mL of ethyl ether/ethanol (2:1). The solution was titrated with KOH (0.5 mol L⁻¹), with phenolphthalein as indicator. The volume of base was determined and the acidity indict was determined by equation 2 (TIRITAN; BEUX, 2006):

$$Acidity = \frac{V \times M \times 56.1}{m_{sample}} \quad \text{Equation 2}$$

where V is the volume (mL) of KOH solution spent in the titration of the sample, M concentration KOH solution, f is the correction factor of KOH solution and m is the weight (g) of sample.

Cytotoxic Assay on *Artemia salina* sp.

The cytotoxic assay with *Artemia salina* was performed according the methodology described by MEYER et al., (1982) with modification. Marine saturated solution (38 g L⁻¹) was prepared by dissolution of sea salt in distilled water. For 500 mL the water the pH was adjusted between 8-9 and 1.5 g the *A. salina* eggs placed for hatching and subjected to shock light with 100 W lamp for 48 hours, protected from dust and insects. The oils obtained from the albumen, seed and leaves (10 mg) were solubilized in methanol and volume completed to 5 mL with distillate water. Aliquots of these solutions were taken and transferred to 5 mL tubes and the volume completed with marine water to a concentration of 500, 250, 100, 50 and 10 µg.L⁻¹ to each sample. Under the same condition the laccachol, a natural organic compound was perpetrated with positive control. For each dilution of the samples and controls, 10 larvae of *A. salina* was placed with a Pasteur pipette. After 24 hours under controlled lighting and temperature was performed counting of the living and dead larvae. The lethal dose-level of 50% (LD₅₀) was calculated by Probit analysis, using software Biostat 2008 v. 5.2.5 with 95% significance.

Identification of fatty acids

The trans-esterification was carried out according to ISO method 5509 (ISO, 1978) using KOH 2 mol L⁻¹ in methanol. Fatty acid methyl esters were analyzed using gas chromatography (GC) HP 5890

equipped with flame ionization detector (FID) and fused silica capillary column (60 m x 0.25 mm and 0.25 μm of 100% polyethyleneglycol). The N_2 flow (carrier gas) was 1.2 mL min^{-1} , with 30 mL min^{-1} and 300 mL min^{-1} , for H_2 and synthetic air, for the flame detector. The volume injected was 2.0 μL , using a sample splitter 1:80, with temperatures of the injector and detector at 250 $^\circ\text{C}$, respectively. The column temperature was 160 $^\circ\text{C} / 5 \text{ min} \rightarrow 5 \text{ }^\circ\text{C} / \text{min} \rightarrow 200 \text{ }^\circ\text{C} / 10 \text{ min} \rightarrow 3 \text{ }^\circ\text{C} / \text{min} \rightarrow 235 \text{ }^\circ\text{C} / 60 \text{ min}$. The peak areas were determined by the software N2000 Chromatostation. The identification of the fatty acid methyl esters (FAME) was performed by comparing the retention time of the sample constituents with a mixture of 37 standards of fatty acid methyl ester from Sigma-Aldrich. The percentage the each acid was performed by total area normalized through the equation $\%N = (Ac_r / \sum Ac_{total}) \times 100\%$, where $\%N$ is a percentage obtained, Ac_r is fatty acid obtained and Ac_{total} is total area this chromatographic peak.

Identification of compounds essential oil

The essential oil obtained from the leaves was analyzed by gas chromatography coupled to mass spectrometry (GC-MS Thermo Finigan). The analyses were performed in COMCAP in State University of Maringá (UEM). The condition of temperature of injector and detector were 250 $^\circ\text{C}$, respectively. The column temperature was 50 $^\circ\text{C}$ with elevation of 4 $^\circ\text{C} / \text{min}$ at 250 $^\circ\text{C} / 5 \text{ min}$, transference line of 240 $^\circ\text{C}$. The flow of carrier gas was helio in 1.2 mL.min^{-1} and ionization by electron impact (EI) of 70 eV. One micro liters of oil was injected in *split* mode with ratio 1:100. The confirmation and identification of the chromatographic peaks was performed by comparing the mass spectrum of the library NIST 32, Kovats indices (KI) calculation and comparing with Adams (2007) library.

Results

In Table 1 are showed the results for the fruits of *J. ribifolia*. The fruits obtained were separated in seeds (50.03 g) and albumens (72.15 g) for determination of moisture content according the methodology described by Farmacopéia Brasileira (1988). This method is based on the loss of water by drying in an oven with aim to determine the amount of volatile substances of any type disposed under the specified conditions. Table 1 show that the moisture content is low for seed and albumen.

Table 1 – Moisture content for seeds and albumen of the *J. gossypifolia* L. fruits (n=3).

Samples	Weight (g)	Moisture (%)*
Seeds	50.03	2.65 \pm 0.54
Albumen	72.15	1.57 \pm 0.54

Fonte: Dados do autor.

The content of oil extracted showed a yield of 26.6 % and 38.4 % for seeds and albumen, respectively. The oils had a faint smell of nuts with pale yellow coloration. The essential oil obtained from the leaves showed a green color and odorless. The hydrodistillation yield was the 0.0365 %. The degumming process of oil was important to remove the proteins, phospholipids, ash and impurities in analysis of the acidity and saponification.

Saponification and acidity indict

The saponification is a reaction between oils and alkali, in press and heating moderated. The reaction affords glycerol and free fatty acid in the soap form (OLIVEIRA et al., 2006). The value found for the SI of albumen oil was 165.4 mg KOH g^{-1} of sample. According to ANVISA (1999), the IS for vegetable oils should be between 189 and 195 mg KOH g^{-1} . The acidity indicts showing the value of 10

% (5.61 mg g⁻¹). This value is high to the acidity index, which cannot reach values above 1 % to the vegetable oils. (ALVES et al., 2009; DORSA et al., 2004). These free fatty acids are undesirable element that can cause undesirable odor and flavor (MORETTO; FETT, 1998).

Cytotoxicity assay on *Artemia salina*

The *Artemia salina* L brine shrimp assay is a simple and efficient method for the evaluation of acute toxicity of extracts and oils obtained from plants. According David et al. (2001) plant extracts

presenting DL50 >1000µg mL⁻¹, in the cytotoxic assay, are considered inactive. To DL50 < 100 µg mL⁻¹ is highly active and may present cytotoxic activity. Extracts with DL50 ≥ 100 and ≤ 1000 µg mL⁻¹ have moderate activity. Table 2 shows the values of DL50 obtained for oils extracted. Leaves and albumen essential oils showed cytotoxic activity, with DL50 lower than 100 µg mL⁻¹, while the seed oil presents moderated activity, with DL50 ≥ 100 and ≤ 1000 µg mL⁻¹. This result is satisfactory since are reported in literature that *jatropha* has been used in fighting diseases such as cancer, hemorrhoids, burn, venereal disease etc. thus proving the cytotoxic activity presented (GBEASSOR et al., 1989).

Table 3 – Percentagem of fatty acids obtained for the extract oil the seeds and albumen the *J. gossypifolia* L.

Fatty acids	*Seeds (%)	Albúmen (%)
C13:0 (<i>tridecanoic acid</i>)	0.08	-
C14:0 (<i>mirystic acid</i>)	0.19	0.05
C15:0 (<i>pentadecanoic acid</i>)	0.03	0.04
C16:0 (<i>palmitic acid</i>)	3.98	12.72
C16:1 (<i>palmitoleic acid</i>)	0.02	0.33
C17:0 (<i>heptadecanoic acid</i>)	35.81	0.24
C17:1 (<i>cis-10-heptadecanóico acid</i>)	-	0.10
C18:0 (<i>stearic acid</i>)	2.85	7.83
C18:1ω9c (<i>oleic acid</i>)	7.40	19.96
C18:2ω6c (<i>linoleic acid</i>)	24.09	55.18
C18:3ω3 (<i>linolenic acid</i>)	0.48	1.48
C20:0 (<i>arachidic acid</i>)	0.10	-
SFA	43.04	20.88
MUFA	7.42	20.39
PUFA	24.57	56.66
PUFA/SFA	0.57	2.71

* Percentage of acids determined in chromatograma 75,08 %. SFA=saturated fatty acid; MUFA=monounsaturated fatty acid; PUFA=polyunsaturated fatty acid.

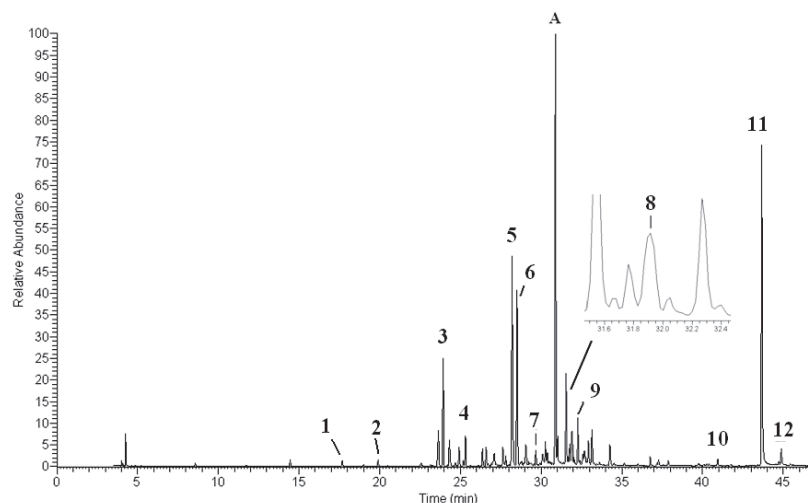
Fonte: Dados do autor

Table 4 – Essential oil composition of leaves *J. gossypifolia* L. by GC-MS.

	RT	Compounds	Formule	*KI	KI	(%)
1	17.68	α -terpineol	C ₁₀ H ₁₈ O	1188	1186	0.22
2	19.89	geraniol	C ₁₀ H ₁₈ O	1253	1249	0.25
3	23.93	α -copaene	C ₁₅ H ₂₄	1374	1374	4.52
4	25.30	β -caryophyllene	C ₁₅ H ₂₄	1417	1417	1.49
5	28.20	<i>p</i> -cresol, 2,6-di-tert-butyl-	C ₁₅ H ₂₄ O	1511	1514	10.18
6	28.50	cadinene	C ₁₅ H ₂₄	1538	1537	7.28
7	29.66	nerolidol<(E)>	C ₁₅ H ₂₆ O	1561	1561	0.73
A	30.89	humulane-1,6-dien-3-ol	C ₁₅ H ₂₆ O	1621	ND	21.28
8	31.93	Muurolol <epi- α ->	C ₁₅ H ₂₆ O	1640	1640	3.62
9	32.27	cadinol< α ->	C ₁₅ H ₂₆ O	1652	1652	2.36
10	40.94	hexadecanoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	1991	1993	0.43
11	43.67	phytol	C ₂₀ H ₄₀ O	2033	2133	15.58
12	44.88	linolenic acid, ethyl ester	C ₂₀ H ₃₄ O ₂	2188	2194	1.55
					Σ Total	69.49

*KI – calculated Kovats indict; KI – literature indict; RT – Retention Time (min); ND – not detected.

Fonte: Dados do autor.

Figure 1 – The total ions chromatograms (TIC) of essential oil compounds of *J. gossypifolia* by GC-MS.

Fonte: Dados do autor

Table 2 – Cytotoxic assay on *A. salina* for the oils extract from *Jatropha gossypifolia* L.

Oils Samples	DL ₅₀ μ g mL ⁻¹ *
Leaves	78.66 \pm 0.25
Albúmen	74.25 \pm 0.21
Seeds	397.76 \pm 0.45

*Avarage \pm standard deviation for three measures.

Fonte: Dados do autor.

Chromatographic analysis

The fatty acids chromatographic profile obtained for albumen and seeds are equivalent between themselves. However, the chromatographic profile for the oil extracted from the seeds showed a high amount of the chromatographic peak not identified by comparison to the standard of methyl ester analyzed by GC-FID. In Table 3 are showed the

fatty acid composition. For the oil seeds, the fatty acids present in major percentage are palmitic (C16:0), stearic (C18:0), oleic (C18:1n9c) and linoleic (C18:2n6c). In this case, no difference was observed in the composition of the analyzed oil of the albumen. For the seeds oils, the heptadecanoic acid was found in major percentage among all acids, with 35.81 %. The composition was lower than the other acids (C16:0; C18:0; C18:1n9c and C18:2n6c) with varying from 2.85 to 24.09 % percentages. The fatty acids determined in the *J. gossypifolia* extracts are recognized as those who perform important function in our body. Palmitic acid is important for infant nutrition (RADMANN; COSTA, 2008), and stearic acid acts as important precursor of the oleic acid (YONG, 2002). Oleic acid is considered essential to our metabolism, acting in hormone synthesis and as a w-9 fatty acids precursor (YOUNG, 2002; VIANNI; BRAZ-FILHO, 1996). The PUFA/SFA ratio was adequate for consumption of oils analyzed, being above 0.45 as determined by the English health department. However, the albumen oil presents high toxicity against the *A. salina* microcystean ($LD_{50} 74,25 \pm 0,21 \mu\text{g mL}^{-1}$).

Essential oils extract of the leaves were analyzed by gas chromatography coupled to mass spectrometry. The total ions chromatogram (TIC) is showed in Figure 1. Twelve compounds were identified by GC-MS analysis (Table 3). The identification was made by NIST and Adams (2007) library. The compounds determined in the TIC correspond to the 69.49% of identified compounds. However, the major peak (A) of TIC was not identified by Kovatz index and Adams (2007) library. The oil compositions present too a natural antioxidant in large percentage, the butylated hydroxytoluene (10.18%). This indicates that these extracts may exhibit antioxidant properties.

Conclusion

The potential of fruits and leaves the *J. gossypifolia* as raw material for fatty acids and essential oil production has been evaluated. The albumen show high content of fatty material that can be utilized to as source for biofuel production. Although the acidity index and saponification are unsuitable for consumption, the PUFA/SFA ratio was higher than that indicated by the English health department (HMSO, 1994). The ratio was the 2.71 and 0.57 for albumen and seeds, respectively. Cytotoxic activity was observed for the oil leaves and albumen extract in the *A. salina* assay. A total 13 compounds were identified to the essential oil of the leaves when compared to the IK literature.

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