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Full Length Research Paper

Gas chromatography–mass spectrometry (GC-MS) analysis of the constituents of the fixed oils obtained from the barks, leaves and stems of *Spondias tuberosa* Arruda (Anacardiaceae)

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***Spondias tuberosa* Arruda (Anacardiaceae) is an endemic tree in the Caatinga Brazilian Biome, known locally as "umbuzeiro" and used for various purposes, especially as food and folk medicine. In this study, it was realized for the first time, the extraction and characterization by gas chromatography-mass spectrometry (GC-MS) of the fixed oils of barks, leaves and stems of *S. tuberosa*. The constituents of the oils were identified by comparing the mass spectra obtained with those of the equipment database. The major component of fixed oil obtained from barks was the 3-*n*-Pentadecylphenol (44.91 %), in the leaves and stems was observed presence of the tetratetracontane (38.17 and 28.57%, respectively). Antioxidant activity was evaluated by using 2,2-diphenyl-1-picrylhydrazil (DPPH) radical scavenging and β-carotene-linoleic acid bleaching test. The fixed oil obtained from the leaves presented the best antioxidant activity (51.13%±11.8) in β-carotene-linoleic acid bleaching test. This study described for the first time identification and quantification of the components present in the fixed oils of the bark, leaves and stems of *Spondias tuberosa*.**

Key words: Natural products, fatty acids, gas chromatography-mass spectrometry (GC-MS), antioxidant.

INTRODUCTION

Brazil has the largest diversified forest reserve on the planet, which allows the search of new medicinal plants and their active principles with pharmaceutical and food applicability (Santos and Torres, 2012). In view of the great diversity of the Brazilian flora, in recent years there is a growing interest in the lipidic properties of non-

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conventional vegetable oils, as these oils present themselves as a complex mixture of bioactive lipophilic substances, as a rich source of functional components and nutrients such as fat soluble vitamins. Previous studies described interesting pharmacological activities of fixed oils derived from plants and herbs used in traditional medicine, including antimutagenic, antiinflammatory, antiviral, antioxidant and antimicrobial properties (Ashaala et al., 2010; Oliveira et al., 2010; Singh et al., 2007; Lv et al., 2012).

Among the main causes of food quality deterioration is lipid peroxidation and the result of this peroxidation is the formation of reactive oxygen species and free radicals (Roby et al., 2013). One way to combat such oxidizing agents is to use antioxidant agents in foods to retard the formation of toxic products formed in the oxidation process, thereby maintaining nutritional quality and increasing the shelf life of food products (Maisuthisakul et al., 2007).

Many secondary metabolites that present as natural antioxidants, for example, tannins, phenolic compounds and terpenoids are found in various plant products (fruits, leaves, seeds and oils) (Jeong et al., 2004). Therefore, there is a growing interest in the search for plant species that present these natural antioxidants that may have been used as an example in the food industry as a deterrent to the effects and consequences of food oxidation.

Species of Anacardiaceae family has been quite promising in the search for bioactive substances. On the few existing studies can be seen that two classes of substances are characteristic in the family: flavonoids and phenolic lipids. The phenolic lipids, are considered non-isoprene natural products, which exhibit amphipathic behavior, since it has aliphatic and aromatic groups in its structure, such a feature facilitates the incorporation of these lipids in cell membranes, these common metabolites in the family Anacardiaceae species and many reported biological activities and this has proven that these species are assigned to this class of substances (Correia et al., 2006).

Spondias tuberosa Arruda (Anacardiaceae) is an endemic tree in the Caatinga Brazilian Biome, known locally as "umbuzeiro" and used for various purposes, especially as food and folk medicine (Neto et al., 2010). This species is used in folk medicine with the following therapeutic applications conjunctivitis, venereal diseases, digestive problems, colic, diarrhea, diabetes, menstrual disturbances, placental delivery, renal infection, throat afflictions, antiemetic and tonic (Albuquerque et al., 2007).

The present study was carried out to analyze the constituents of the fixed oils by gas chromatography-mass spectrometry (GC-MS) from the bark, leaves and stems of the species *S. tuberosa*. In addition, the *in vitro* antioxidant activity of the fixed oils was evaluated using the free radical bleaching test (DPPH) and β- carotene.

MATERIALS AND METHODS

Plant material

The botanical material used (bark, leaves and stems) of *Spondias tuberosa* were collected in Petrolina city (Coordinates: S 09°19'53,90"; W 040°32'47,60"), State of Pernambuco, Brazil, in June of 2013. The samples were identified by a botanist from Centro de Recuperação de Áreas Degradadas da Caatinga (CRAD). A voucher specimen (18319) was deposited at the Herbarium of San Francisco Valley (HVASF) of the Federal University of San Francisco Valley (UNIVASF).

Extraction

The botanical material dried and pulverized of the leaves (7.4 g), barks (82.3 g) and the stems (16.9 g) was subjected to extraction with petroleum ether in a Soxhlet apparatus for 2 h. After distillation of the solvent obtained were the fixed oils leaves (770.0 mg), barks (126.1 mg) and stems (310.0 mg). For achievement, the analysis of fatty acids present in the oil composition derivatization process of the fatty acid methyl esters by saponification followed by methylation of the fatty acids was performed (Matos et al., 1992).

Saponification

The samples of each oil was saponified in a system reflux containing methanol and potassium hydroxide (KOH) for 30 min. Then, the methanol was removed by distillation, after distilled water was added. The unsaponifiable material was extracted from the alkaline mixture with ethyl ether.

Methylation of saponified fraction

After the saponification, the alkaline solution was acidulated to pH 2 with 10% hydrochloric acid and fatty acids extracted with ethyl ether. The fatty acids methylated according to the semi-micro technique and the samples were refluxed for 2 mins with concentrated hydrochloric acid and methanol. After adding water to the reaction medium, the methylated fatty acids were extracted with hexane. Then, methylation of the methyl esters of leaves, barks and stems were analyzed.

GC-MS analysis

The compounds presents in fixed of the bark, leaves and stems of *S. tuberosa* were investigated on a Shimadzu QP-2010 gas chromatograph interfaced to a mass spectrometer (GC-MS). The following conditions used were: ZB-5MS column Phenomenex Zebron (30 m x 0.25 mm x 0.25 µm); helium (99.999%) carrier gas at a constant flow of 1.1 mL/min; 1 µL injection volume; injector split ratio of 1:40; injector temperature 240°C; electron impact mode at 70 eV; ion-source temperature 280°C. The oven temperature was programmed from 100°C (isothermal for 5 min), with an increase of 10°C/min to 250°C (isothermal for 5 min) and 10°C/min to 280°C (isothermal for 15 min). A mixture of linear hydrocarbons (C_9H_{20}–$C_{40}H_{82}$) was injected under the same experimental conditions as samples, and identification of the constituents was performed by comparing the spectra obtained with those of the equipment's database (Wiley 7 lib and Nist 08 lib).

DPPH free radical scavenging assay

The first test of antioxidant activity followed the methodology of free

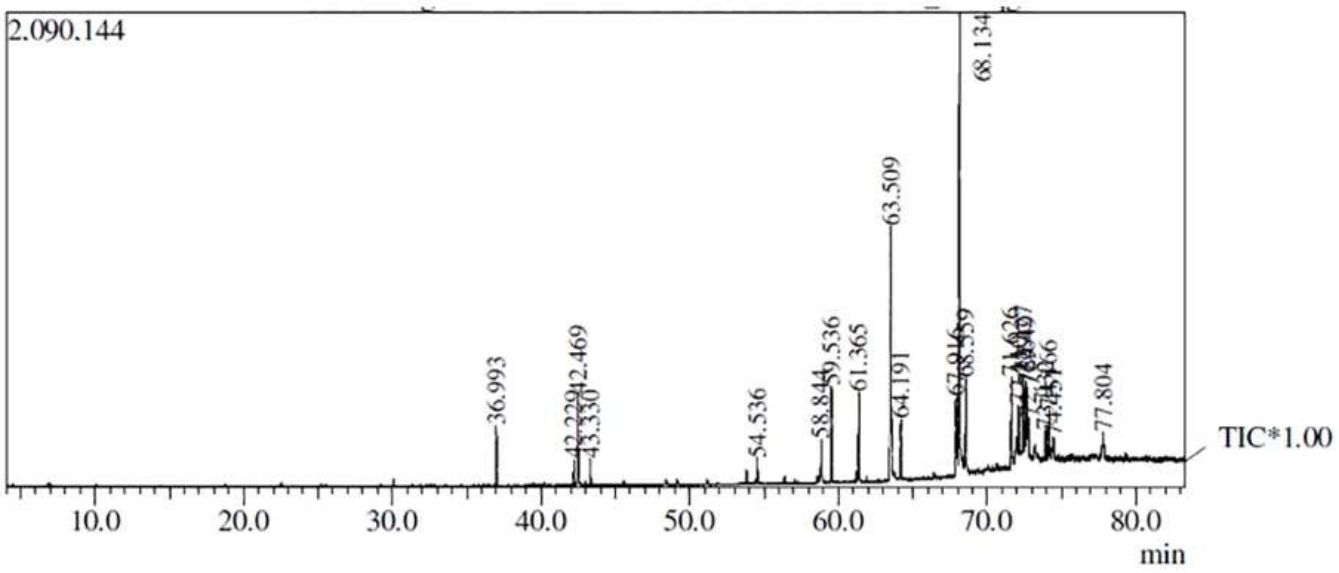


Figure 1. Chromatogram of the fixed oil of the bark (FOB).

radical scavenging activity which was measured using the 2,2-diphenyl-1-picrylhydrazil (DPPH) assay (Santana et al., 2012). A solution of DPPH at the concentration of 50 µg/ml was prepared in ethanol. 1 ml of DPPH solution was added to 2.5 ml of sample solutions (1 to 243 µg/mL), and allowed to react at room temperature. Using a spectrophotometer (NOVA INSTRUMENTS®, UV-1600, Piracicaba, Brazil), the absorbance values were measured at 518 nm after 30 min at room temperature. The positive controls (ascorbic acid, BHA and BHT) were those using the standard solutions. Assays were carried out in triplicate. The IC_{50} values were calculated by linear regression using by GraphPad Prism 5.0 program.

Inhibition of auto oxidation of β-carotene

The second method utilized was the method of inhibition of co-oxidation of β-carotene / linoleic acid system which is based on the loss of yellow color of β-carotene due to its reaction with the radicals formed by oxidation of linoleic acid by aeration of the reactional medium (Santana et al., 2012). The emulsion (3.0 ml) was added to a tube containing 0.12 ml of solutions (1.0 mg/ml) of reference compounds and fixed oils. The absorbance was immediately measured at 470 nm and the test emulsion was incubated in a water bath at 50°C for 120 min, when the absorbance was measured again. Ascorbic acid, BHA and BHT were used as positive control. For negative control, the samples were substituted with an equal volume of ethanol. The results are expressed as percentage of antioxidant activity (% AA). Tests were carried out in triplicate.

Statistical analysis

All determinations were conducted in triplicates, and the data are expressed as mean ± SD. Values were considered significantly different at $p < 0.05$. The IC_{50} values were obtained by interpolation from linear regression analysis with 95% of confidence level. IC_{50} is defined as the concentration sufficient to obtain 50% of a maximum effect estimate in 100%. Values are given as mean ± SD (n=3).

RESULTS AND DISCUSSION

The constituents of the oils were identified by comparing the mass spectra obtained with those of the equipment database. Table 1 shows the retention times (min) and total peak area (%) in the chromatogram of each constituent that were identified in the fixed oil obtained from the species *S. tuberosa*.

Figure 1 shows the chromatograms corresponding to the chromatographic analysis (GC-MS) of the fixed oil the barks (FOB) and allowed the identification of 19 different compounds of which the phenolic compound 3-pentadecylphenol (44.91%) was the major constituent. The triterpene spinacene (4.72%), steroids γ-sitosterol (4.95%), stigmast-4-en-3-one (2.75%) and 5α-stigmastane-3,6-dione (1.54%) were also identified.

From the chromatogram analysis of the fixed oil obtained from the leaves (FOL) were observed (Figure 2) the presence of 16 different constituents. The main constituents of the oil derived from the leaves was hydrocarbons *n*-tetratetracontane (38.17%) and 2,2-Dimethyl-3-vinyl-bicyclo[2.2.1]heptane (13.53%). Also were identified in this sample the following substances 3-pentadecylphenol (10.1%), triterpeno lupeol (4.25%), triterpenoids β-amyrin (1.00%), friedelan-3β-ol (2.90%) and friedelin (1.95%).

In the third chromatogram (Figure 3), analysis of the fixed oils obtain from stems, shows the presence of 20 components among them are hydrocarbon *n*-tetratetracontane (28.57%) the major constituent, steroid γ-sitosterol (11.35%), 3-pentadecylphenol (2.46%), lupeol (9.92%), β-amyrin (1.51%), friedelan-3β-ol (1.06%) and friedelin (1.06%).

In the three, fixed oils of *S. tuberosa* were identified the

Table 1. Chemical composition of fixed oils of the bark (FOB), leaves (FOL) and stems (FOS) of *S. tuberosa.*

Constituent	Amount (%)			Retention time (min)
	FOB	FOL	FOS	
Acid methyl ester				
Arachidic	-	-	1.31	49.15
Cerotic	2.86	-	-	64.19
Docosanoic	1.06	-	0.94	54.53
Lignoceric	4.59	-	1.76	59.53
Linoleic	-	-	6.20	42.23
α-Linolenic	-	3.42	4.00	42.39
Octacosanoic	4.96	-	-	68.56
9,12-Octadecadienoic	1.04	-	-	42.23
Octadecanoic (stearic)	1.25	2.20	2.23	43.33
11-Octadecenoic	4.38	-	-	42.47
Oleic	-	-	2.83	42.46
Palmitic	2.77	5.05	6.41	36.99
Tetradecanoic	-	3.17	2.70	30.07
Triacontanoic	4.60	1.21	0.74	72.65
Triterpenes				
Lupeol	-	4.25	9.92	73.25
Spinacene	4.72	-	-	61.36
Triterpenoids				
β-amyrin	-	1.00	1.51	72.31
Friedelan-3β-ol	-	2.90	1.06	75.63
Friedelin	-	1.95	1,06	76.03
Steroids				
γ-Sitosterol	4.95	-	11.35	71.63
Stigmast-4-en-3-one	2.75	-	-	74.17
5α-Stigmastane-3,6-dione	1.54	-	-	77.80
Hydrocarbons				
2,2-Dimethyl-3-vinyl-bicyclo[2.2.1]heptane	-	13.53	3.30	57.91
Decane	-	1.20	-	4.61
Tetradecane	-	3.54	-	6.95
n-Tetratriacontane	1.95	2.18	-	58.84
Tetratetracontane	-	38.17	28.57	67.90
Phenolic constituents				
3-pentadecylphenol	44.91	10.1	2.46	58.31
Other constituents				
Octatriacontyl pentafluoropropionate	4.34	-	-	67.92
Hexacosan-1-ol	2.92	-	-	72.11
4α,14-Dimethyl-9β,19-cyclo-5α-ergost-24(28)-en-3β-ol acetate	3.69	-	-	73.35
1,2,4- Benzenetricarboxyilic acid, 1,2,4- tris (2-ethyl-hexil) ester	0.72	4.14	1.93	74.45
Hexyl hexanoate	-	-	9.71	17.10
Total	100.0	98.01	99.99	-

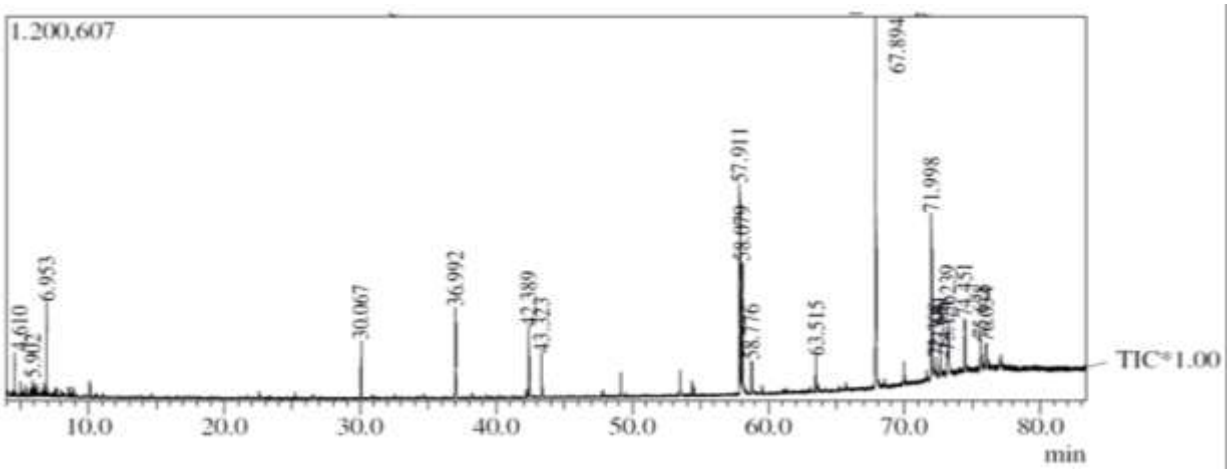


Figure 2. Chromatogram of the fixed oil of the leaves (FOL).

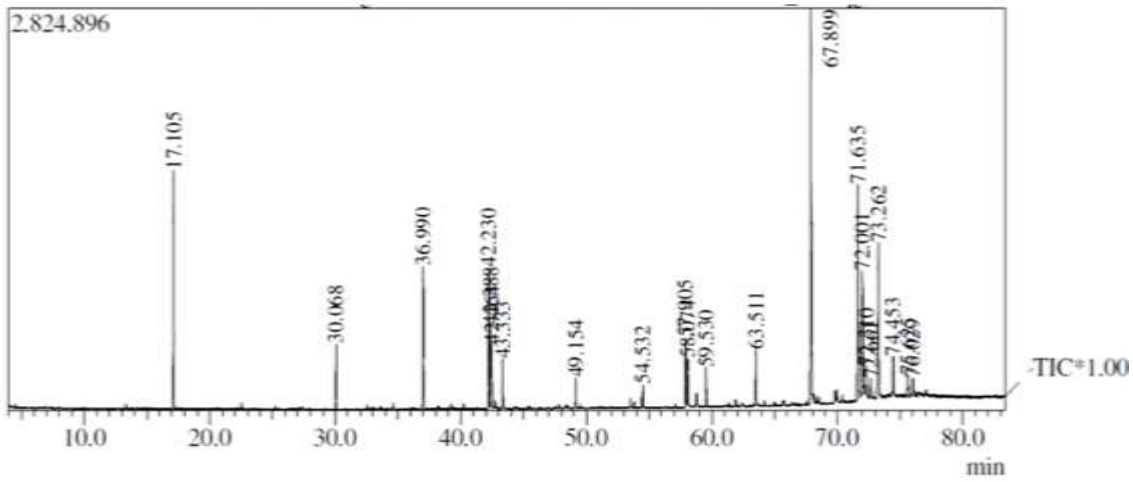


Figure 3. Chromatogram of the fixed oil of the stems (FOS).

methyl esters long-chain following fatty acids palmitic, stearic and triacontanoic. Palmitic acid and stearic acid have also been identified in the seed of the fruit “umbuzeiro” (Borges et al., 2007). These acids can be linked to the source of the alkyl chains present in the phenolic lipids, such as the 3-pentadecylphenol that has been isolated from the specie *Anacardium occidentale*, species belonging to the family Anacardiaceae (Correia et al., 2006).

The 3-n-pentadecylphenol was also identified in the three oils, it is a phenolic lipid, a alkylphenol or phenol long chain. The phenolic lipids have many proven biological activities, because it has hydrophilic and hydrophobic regions. The amphipathic character of the phenolic lipids favors the incorporation into the cellular membranes, causing changes in the structure and properties of these. The interaction by means of hydrogen bonds between the aromatic ring hydroxyl groups and the membrane phospholipids causes a stabilizing effect between the phenolic lipids and derivatives in the membranes. On the other hand, the biological activity undergoes a significant influence of the alkyl chains since with the increase of the solubility of the phenolic portions in the lipidic regions, in which protection against biological degradation or chemical oxidation is required (Correia et al., 2006).

In addition to the phenolic lipids identified among the compounds present in fixed oils, stand out from the others, constituents that also exhibit biological activity as the steroid γ-sitosterol present antihyperglycemic activity (Balamurugan et al., 2011). The triterpene lupeol has several pharmacological activities, such as anti-inflammatory and antimicrobial activities (Santos and Torres, 2012). Also highlights the β-amyrin and friedelin

Table 2. Antioxidant activity of fixed oils from bark, leaves, stems and standards (ascorbic acid, BHA and BHT).

Sample	DPPH (IC_{50}, µg/ml)	β-carotene (% AA)
FOB	Nd	17.43±1.30
FOL	91.72±6.29	51.13±7.10
FOS	522.5±112.0	37.94±9.10
Ascorbic acid	3.75±0.45	5.23±2.41
BHA	3.95±0.11	85.34±1.34
BHT	9.4±0.64	88.29±0.77

*Nd: not determined.

terpenoids which have antinociceptive effect in the writhing test induced by acetic acid (Filho, 2000; Noldin et al., 2006).

Table 2 shows the results of the antioxidant evaluation. In the DPPH method can be observed that the fixed oil from leaves showed the lowest IC_{50} value (91.72±6.29) compared to the other oils tested, but since when compared with standard ascorbic acid (IC_{50} 3.75±0.45), it can be observed that the result was not satisfactory, this result is possibly related to low amount of compounds capable of donating a proton easier to stabilize DPPH at the same time remain stabilized by resonance since the samples these are oils and present nonpolar character.

Many studies have shown that β-carotene inhibits the auto-oxidation of lipids in biological tissues and food products, but few details of the kinetics and mechanisms of these reactions have been revealed. The co-oxidation method using the β-carotene / linoleic acid system is a simple test that allows the determination of the antioxidant activity of thermosensitive substances, since it does not use high temperature and is more reproducible for organic substrates of lipophilic character (Alves et al., 2010).

In this method, the fixed oil of the leaves also showed better activity (51.13%±2.43) compared with other fixed oils tested and standard ascorbic acid (5.23%±2.41). The presence of a greater amount of some triterpenes and triterpenoids in the fixed oils of the leaves and stems may justify the significant result in the antioxidant activities of these oils, for example, lupeol that can influence in the antioxidant property of the plant (Santiago and Mayor, 2014). Although this is the first report of compounds identified in the fixed oils of aerial parts and barks of the species *S. tuberosa* (Table 2).

Conclusion

This study described for the first time identification and quantification of the components present in the fixed oils of the bark, leaves and stalks of *S. tuberosa*. The following metabolites were found in leaf oil: lupeol, β-amirin and friedelin, which may be related to the popular use of its leaves as anti-inflammatory, to combat diarrhea, dysentery and worms, since these substances have several pharmacological activities. The moderate result of the antioxidant activity in the co-oxidation of the β-carotene / linoleic acid system of the oils allied with the presence of important fatty acids, such as stearic and palmitic acids that have the property of being good emollients when incorporated into the dermis-formulations, shows the possibility of the fixed oils of *S. tuberosa* species being used in the food and pharmaceutical industries. The presence of these compounds in the studied plant is important because it contribute to the chemical knowledge of this specie.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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