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Spondias mombin Seed Oil Compounds Identification by Raman Spectroscopy and NMR

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Abstract: *Spondias mombin* L. has been used in traditional medicine to treat some cases such as infections and inflammations. Some researchers have reported that its biological components, such as carotenoids, carotenes, and phenols, have been characterized primarily by HPLC analysis. Here, we report on the characterization of *Spondias mombin* L. seed oil by Raman spectroscopy, and the profile identification of fatty acids by ^{1}H-NMR and ^{13}C-NMR spectroscopy. The oil was extracted from different weight volumes of seeds using organic solvent, and each batch was characterized. The analysis of the fatty acid profile by NMR indicated that the seed oil is highly unsaturated (monounsaturated: 29.4% and polyunsaturated: 43.5%). Molecular Raman vibrations at 1006, 1158 and 1523 cm^{-1} showed the presence of carotenoids, which in turn performed an antioxidant activity. This was demonstrated by a 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) method. The cell viability in colon cancer cells was promoted in the presence of the oil. The compounds identified in this study from seed oil could be an interesting proposal for food or pharmaceutical applications.

Keywords: vegetable oil; carotenoids; antioxidant activity; cytotoxic effect; fatty acids; cell viability

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1. Introduction

Vegetable oils constitute a major source of nutritional lipids, and supply a great amount of fats and oils consumed worldwide. Several oils have shown to be relevant for medical treatments due to their composition, containing monounsaturated fatty acids and antioxidants which can be used to prevent cardiovascular diseases, osteoporosis and cancer [1,2].

Spondias mombin L. is a tropical fruit that has been widely used for its medicinal and nutritional values. The pulp and leaves of *Spondias mombin* L. are important in food and biomedical industries, and their use in traditional medicine has been reported for the treatment of stomach infection and inflammation [3,4]. In addition, other studies have revealed antibacterial activity against various strains such as *Bacillus cereus*, *Streptococcus pyogenes* and *Mycobacterium fortuitum*. Likewise, it has demonstrated sedative, antipsychotic, diuretic, gastroprotective and antioxidant activity in mammals [4–6]. The essential oils composition of *Spondias mombin* L. shows more than 54 compounds [7]. Particularly, the content of the seed oil is remarkable, as it is present in 31.5% *w/w* yield [8].

Some molecules contained in oils, such as triacylglycerol (TAG) and unsaturated fatty acids, indicate the quality of the sample and its potential for pharmaceutical applications.

Several methods have been developed for the determination of TAG profiles in oils. Here, we use the proton and carbon nuclear magnetic resonance technique (1H-NMR and 13C-NMR, respectively) as an analytical method to determine the structural analysis and quantification of fatty acids and their derivatives. The analysis focuses on characterizing several specific signals such as those of the olefinic, allylic and bis-allylic, methylene and the terminal methyl group protons [9,10].

Raman spectroscopy (RS) is an analytical method that provides many advantages compared to traditional analysis methods, as it is precise and easy to use while not requiring sample preprocessing. It has been used in chemical, medical, biological, environmental and food applications, sometimes in combination with chemometric methods [11–15]. This method has been used to provide an accurate identification of compounds present in oils and is therefore useful to identify purity or possible adulterations [16–20]. RS has been used for the identification of carotenoid compounds of different vegetable oils such as goji berries, buriti and passionflower, as well as the petals of some flowers [21–23]. Furthermore, in samples of fruit and vegetable juices such as tomatoes, carrots, oranges, spinach and black grapes, they have been analyzed to identify main carotenoids such as lycopene, lutein, β-carotene, α-carotene, β-cryptoxanthin and zeaxanthin [24].

This work is intended to contribute to the development of feasible and accurate methods to characterize natural product composition, and relate them with biological activities and biocompatibility. We performed RS and NMR (^{1}H- and ^{13}C-) as noninvasive and non-destructive analytical techniques to identify molecule profiles through the characterization of molecular vibrations and resonant frequencies associated with the carotenoids and fatty acid profile of *Spondias mombin* L. seed oil.

2. Materials and Methods

2.1. Seed collection and Oil Extraction

Spondias mombin L. fruits were collected in Sierra de Otontepec, located in the northern region of Veracruz State, Mexico. The seeds were separated from the fruit and oven-dried at 65 °C for 72 h. Different amounts of seeds (40, 80, 120 and 160 g) were submitted to hexane Soxhlet extraction and the yield was calculated. Each extract was evaporated under reduced pressure in a rotary evaporator (Büchi Rotavapor RII) followed by oven evaporation (Universal oven, Memmert UN30) at 60 °C to extract the solvent excess.

2.2. Acquisition of the Raman Spectral Block

The spectral recording was carried out using an integrated RS system (DXR Thermo Scientific) The excitation source was a 780 nm fiber laser operating at 20 mW average optical power. For each batch, 10 spectra were recorded in a range from 100 to 3000 cm^{-1} with 15 s of exposure time (focusing on different points of the sample through a 20× resolution objective). A total of 120 spectra were recorded and averaged for each batch for their comparative analysis regarding molecular vibrations.

2.3. NMR Experiments

^{1}H-NMR and ^{13}C-NMR spectra were obtained from a Varian 500 MHz instrument. Experiments using samples (approx. 50 mg) in $CDCl_3$ with TMS as internal standard were carried out. Chemical shifts were recorded in ppm, using the solvent proton signal as standard. Data were analyzed by using the equipment software.

^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.87–0.92 (m, terminal CH_3), 1.25–1.41 (m, CH_2), 1.59–1.66 (m, CH_2–CH_2–COO), 2.00–2.09 (m, CH_2–CH = CH), 2.31–2.37(t, J = 6.0 Hz, CH_2–COO), 2.77–2.80 (t, J = 3 Hz, HC = HC–CH_2-CH = CH), 4.14–4.19 (dd, J_1 = 9 Hz, J_2 = 6 Hz, glyceryl CH_2O), 4.29–4.33 (dd, J_1 = 9 Hz, J_2 = 3 Hz, glyceryl CH_2O), 5.25–5.29 (m, glyceryl CHO), 5.30–5.41 (olefinic H).

^{13}CNMR (150 MHz, $CDCl_3$): δ 14.02 (α-CH_3), 22.54 (β-CH_3), 24.82 (C-3), 25.62 (C-11, diallylic), 27.16 (overlapping signals: C8-11 (oleyl), C8-14 (linoleyl)), 29.07-29.68 (CH_2n), 31.51 (C-16, linoleyl), 34.01 (α- C-2), 34.17 (β-C-2), 62.09 (glyceryl CH_2O), 68.91 (glyceryl

CHO), 127.88 (C-12, linoleyl), 128.06 (C-13, linoleyl), 128.06 (C-13), 129.68 (C-9, oleyl), 129.95 (C-9, linoleyl, 129.98 (α-C-10, oleyl), 129.99 (β-C-10, oleyl), 130.20 (C-10, linoleyl), 172.79 (α-C-1), 173.25 (β-C-1), 173.21 (β-C-1).

To determine the relative percentage of saturated and unsaturated fatty acids in the oil using ^{1}H-NMR, we followed the methodology described by Thoss et al. [25], who established the following equations using the integrals of different proton environments: polyunsaturated fatty acids: *PUFA = F/E*; monounsaturated fatty acids: *MUFA = [C/2E]-PUFA*; saturated fatty acids: *SFA = 1-[D/2E]*, where *D*: α-allylic proton environment at δ 2.05 ppm; *E*: integrations of the acyl group at δ 2.34 ppm; *F*: protons attached to the bis-allylic carbon at δ 2.79 ppm.

2.4. ABTS Radical Scavenging Activity Assay

The radical cation scavenging capacity of *Spondias mombin* L. seed oil was examined against 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) radical cation (ABTS•+). A stock solution was prepared by reacting 7 mM ABTS (Sigma-Aldrich, Saint Louis, MO, USA) solution with 2.5 mM potassium persulfate as a final concentration, followed by 16 h incubation at room temperature in the dark. The ABTS• + stock solution was diluted with ethanol to an absorbance of 1.0 at 734 nm. Then, antioxidant activity was evaluated by incubating 150 µL of ethanol-ABTS• + with 50 µL of oil solution in ethanol at different concentrations (25–500 µg mL^{-1}). After 30 min, the percentage inhibition of ABTS• + at 734 nm was calculated with the equation *% Inhibition = [(AC—AO)/AC] × 100*, where *AC* is the absorbance of the control (ABTS• + solution), and *AO* is the absorbance of the oil. Trolox and catechol were used as positive controls.

2.5. Sample Preparation and Cell Culture

A stock solution (100 mg mL^{-1}) of *Spondias mombin* L. seed oil solubilized with ethyl alcohol (1:4) was prepared. Prior to the cell experiments, these samples were diluted to final concentrations of 1, 10, 50, 100, and 500 µg/mL using culture medium. HTC 116 cells (obtained from American Type Culture Collection, ATCC) were grown in a 100 mm cell culture dish at 37 °C with 5% CO_2 in DMEM medium, supplemented with 10% fetal bovine serum. The cells were placed on a 96-well cell culture plate for cell proliferation assay. The oil was free of any solvent except for the alcohol used for the final dilution, in a concentration range from 0.00075% to 0.375%. These concentrations did not interfere with the assay. Standard protocols contain ≈2% alcohol concentration as previously reported [26,27]. Effects on cell viability by ethyl alcohol have been reported at a concentration of 5% and above [28].

2.6. Cell Proliferation Assay

The analysis of cell proliferation in cultured cells was performed by a water-soluble tetrazolium salt (WST) assay, following the manufacturer's instructions (Abcam). Briefly, 5×10^4/well cells were cultured in a 96-well microtiter plate in a final volume of 100 µL per well 24 h prior the experiment. Cells were incubated with oil sample solutions for 24 h at the five different concentrations described previously. WST-1 reagent was added to each well and incubated in standard culture conditions for 1 h. Absorbance of samples was measured using a microtiter plate reader at 420–480 nm. Nontreated cells were the reference of 100% viability; each treatment was compared to this control.

3. Results and Discussion

Spondias mombin L. has a variety of applications; indeed, previous reports demonstrated that the leaf and stem bark extracts have been used in traditional medicine [3,4]. In this study, we described the molecular vibrations and functional groups contained in the oil extracted from the seed of *Spondias mombin* L. identified by Raman spectroscopy, ^{1}H-NMR and ^{13}C-NMR.

Figure 1a shows the Raman spectrum for *Spondias mombin* L. oil seed obtained from different weights (40, 80, 120 and 160 g of seeds, processed separately). Figure 1b indicates a linear correlation between mass and yield. The corresponding yield is 2.58%.

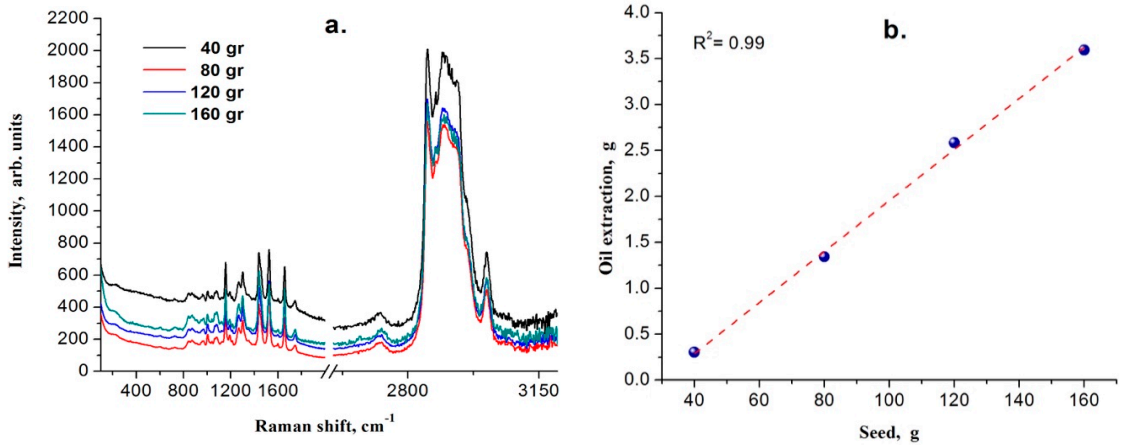

Figure 1. Raw Raman spectra recorded. (**a**) Average Raman spectra recorded for each batch of oil extracted with different amount of seed. (**b**) The weight ratio between the processed seed versus extracted oil shows a linear tendency.

Different amounts of extracted oil exhibit slight changes in peak intensities in the Raman spectrum; however, no significant changes are shown in the Raman frequencies' positions. *Spondias mombin* L. oil measurements were carried out, which allowed a qualitative analysis through the identification of the characteristic Raman frequencies associated with carotenoids [29] (Figure 2).

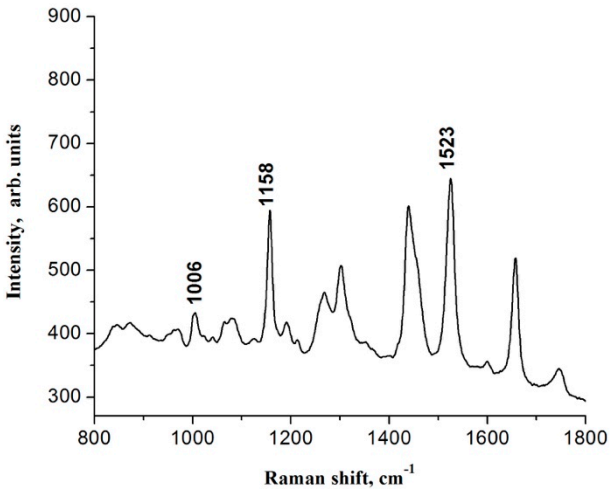

Figure 2. Region of the Raman spectrum of an oil sample from *Sondias mombin L.* where Raman frequencies are located at 1006, 1158 and 1523 cm^{-1}, which are associated with carotenoids compounds.

Indeed, no literature on the characterization of *Spondias mombin* L. seed oil using the RS technique was found. The characteristic frequencies identified in the Raman spectra of *Spondias mombin* L. oil are shown in Table 1. The frequencies are related to previous reports from other vegetable oils [30–34].

Raman spectra recorded from *Spondias mombin* L. vegetable oil samples showed signals associated with the vibrational modes C-C (ν), C-H (δ), C = O (ν), C = C (ν), as well as bands assigned to functional groups such as CH_2 and CH_3 [32,35]. Registered Raman spectra showed very strong characteristic bands in the range of 2850 and 2900 cm^{-1}, which are associated with lipids, lignins and the functional groups C-CH_3, CH_2 and OH [34]. Raman frequency region between 1006 and 1300 cm^{-1} showed characteristic

shifts corresponding to alkyl aryl ether; in particular, at the frequency of 1006 cm^{-1}, it showed a stretching vibration corresponding to a C-O group. On the other hand, these shifts can also be attributed to vibrations of groups CH_3, HC-CH_3, aromatic rings and υ C-C aliphatic chains [35]. In addition, the level of unsaturation of the fatty acids can be determined by the vibrational band at 1300 cm^{-1} by the group =CH- [30]. The relatively weak band at 1080 cm^{-1} is characteristic of vegetable oils, attributed to the presence of the terpene 1,8-cineole, aromatic rings or υ C-C aliphatic chains [35,36]. Bands at 1006 (bending), 1158 (stretching) and 1523 cm^{-1} (stretching) correspond to CH_3, C-C and C=C, respectively, and can also be attributed to carotenoids [34,37]. CH_3 and CH_2 are associated with the vibrational band at 1440 cm^{-1} and are related to modes of inflection between both groups and phenolic compounds. The shift at 1657 cm^{-1} was associated with the stretching of the functional group C = C assigned to monocyclic monoterpenes and showed relative intensity in relation to the level of unsaturation of fatty acids. The band located at 1746 cm^{-1} was assigned to the stretching of group C = O in triglycerides structures and the presence of polyesters, pectins and lignins [30,38]. The vibrational frequency located at 3010 cm^{-1} was associated with unsaturated fatty acids and stretching groups CH = CH, C-H aromatic, =CH_2 and =C-H [39]. In each batch of fruit seed oil, the frequencies associated with carotenoids were observed (Lutein, α-carotene and β-carotene) at 1006, 1158 and 1523 cm^{-1} [30,40,41].

Table 1. Assignment of Raman frequencies that identify the vegetable oil of *Spondias mombin* L. Abbreviations: vs, very strong; m, medium; w, weak, mw, medium weak; sh, shoulder; ν, stretching; δ, bending.

Raman Frequency (cm^{-1})	Functional Group	Vibrational Mode
871 sh, w	-(CH_2)n-	C-C, ν
968 sh, w	Trans RHC = CHR	C = C, δ
1006 w	HC-CH_3	CH_3, δ
1080 sh, w	-(CH_2)n-	C-C, ν
1158 m	-(CH_2)n-	C-C, ν
1267 mw	*cis* RHC = CHR	=C-H, δ
1300 mw	-CH_2	C-H, δ
1440 m	-CH_2	C-H, δ
1523 m	RHC = CHR	C = C, ν
1657 m	*cis* RHC = CHR	C = C, ν
1746 w	RC = OOR	C = O, ν
2850 vs	-CH_2	C-H, ν
2900 sh, vs	-CH_3	C-H, ν
3010 m	*cis* RHC = CHR	=C-H, ν

NMR is a powerful and widely analytical method used in structure elucidation protocols. NMR spectroscopy constitutes an accurate, sensitive method to supplement chemical information for structural identification. Here, we obtained the 500 MHz ^{1}H-NMR spectra of the crude oil, showing a characteristic signal pattern found in other seed oils [42] (Table 2).

The olefinic protons for all unsaturated fatty acids appeared at δ 5.30–5.41 ppm. In the low-field region of the spectra, a multiplet at δ 5.25–5.29 ppm was assigned to the proton attached to C-2 of the glycerol. The chemical shifts and multiplicities of coupling constants for the signals at δ 4.14–4.19 ppm (J_1 = 9 Hz, J_2 = 6 Hz, dd) and δ 4.29–4.33 ppm (J_1 = 9 Hz, J_2 = 3 Hz, dd) are characteristic for H-1 and H-3 protons of glycerol system. Bis-allylic and allylic proton signals appeared as a triplet at δ 2.77–2.80 ppm (J = 3 Hz) and a complex multiplet at δ 2.00–2.09 ppm. A triplet at δ 2.31–2.37 ppm (J = 6.0 Hz) was designated to methylene H-2 protons adjacent to the acyl group, a multiplet at δ 1.59–1.66 ppm was assigned to H-3, while the other methylene protons appeared between δ 1.25 and 1.41 ppm. The terminal methyl protons appeared at δ 0.87–0.92 ppm. The downfield-shifted proton signal at δ 6.25 indicated the presence of linoleic acid [43]. The relative % of *SFA*, *MUFA* and

PUFA present in the sample was calculated following the method described by Thoss et al. (Figure 3) [25].

Table 2. Chemical shifts and assignments of the characteristic resonances in the ^{1}H-NMR spectrum of crude seed oil.

Chemical Shift (ppm)	Proton	Functional Group
0.87–0.92	–C$\underline{H}_3$	Terminal methyl protons of saturated and unsaturated chains
1.25–1.41	C$\underline{H}_2$	Protons of methylene envelop
1.59–1.66	C$\underline{H}_2$–CH$_2$–COO	H-3 protons of acyl moieties in triacylglycerols
2.00–2.09	C$\underline{H}_2$–CH = CH	Allylic methylenes
2.31–2.37	C$\underline{H}_2$–COO	H-2 protons of acyl moieties in triacylglycerols
2.77–2.80	C = C–C$\underline{H}_2$-C = C	Protons attached to bis allylic carbon
4.14–4.19 4.29–4.33	C$\underline{H}_2$O	H-1 and H-3 protons of glycerol
5.25–5.29	C$\underline{H}$O (β)	H-2 of the glycerol backbone
5.30–5.41	C$\underline{H}$ = C$\underline{H}$	Olefinic protons of unsaturated Fatty acids

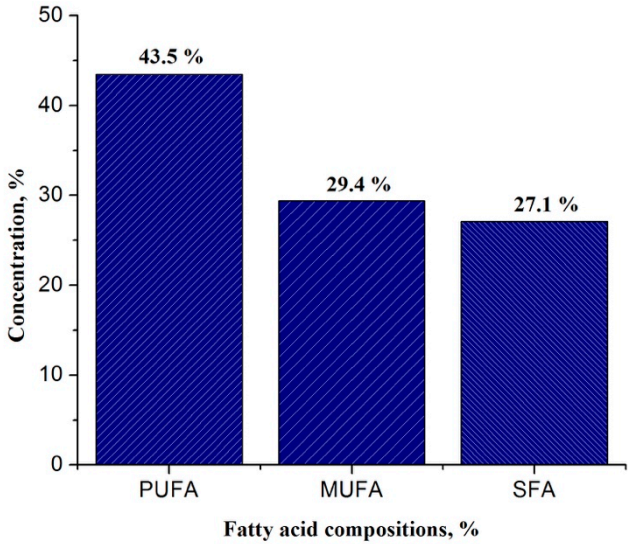

Figure 3. Relative % of saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) as determined using ^{1}H-NMR.

The chemical shift differences between some fatty acids depend on substitution on glycerol backbone. The ^{13}C NMR spectra (Table 3) showed signals at δ 173.25 and 173.21 ppm assigned to carbonyl carbons attached to 1,3-glycerol positions, and a signal at δ 172.79 ppm corresponding to a fatty acid esterified at position two of glycerol, while no signal was observed for free fatty acids (δ 177.0–182.0 ppm). The C-1 and C-3 terminal carbons for the triglyceride moiety were found at the same chemical shift, 62.09 ppm, whereas signal for C-2 was present at 68.91 ppm. The olefinic carbons appeared in the range from δ127.88 to 130.20 ppm. For most components, such as oleic acid, α and β carbon signals could be distinguished, such as C-10 at δα = 129.98 and δβ = 129.99 ppm. As expected, most of the signals were observed at the low field region (δ 14.02–34.17 ppm), corresponding to methyl, methylene and allylic carbons. All of the above NMR signals were in agreement with the structure of a triacylglycerol bearing unsaturated fatty acid chains.

Table 3. Chemical shifts and assignments of the characteristic signals in the carbon nuclear magnetic response (^{13}C-NMR) spectrum of crude seed oil.

Chemical Shift (ppm)	Carbon	Assignment
14.02	α-CH_3	All acyl chains
22.54	β-CH_3	All acyl chains
24.82	C-3	All acyl chains
25.62	C-11	Diallylic
27.16	C8-11 (oleyl), C8-14 (linoleyl)	Allylic
29.07–29.68	CH_{2n}	All acyl chains
31.51	C-16	Linoleyl
34.01	α-C-2	All acyl chains
34.17	β-C-2	All acyl chains
62.09	α-C$\underline{H}_2$O	Glycerol (triacylglycerols)
68.91	β-C$\underline{H}$O	Glycerol (triacylglycerols)
127.88	C-12	Linoleyl
128.06	C-13	Linoleyl
129.68	C-9	Oleyl
129.95	C-9	Linoleyl
129.98	α-C-10	Oleyl
129.99	β-C-10	Oleyl
130.20	C-10	Linoleyl
172.79	α-C-1 Glycerol	Triacylglycerols 2xC, oleoyl and linoleoyl
173.25 and 173.21	β-C-1 Glycerol	Triacylglycerols

It has been demonstrated that antioxidant compounds are present in natural products such as vegetables, fruits, and oil seeds [44]. Several investigations have determined the antioxidant activity of carotenoids [45,46], and it has been possible to state the carotenoid content as responsible for the antioxidant activity. On the other hand, it has been shown that there is a high carotenoid content in plants from the *Anacardiaceae* family [46,47], such as Mexican plum (*Spondias purpurea*), ambarella (*Spondias dulcis*) and yellow mombin (*Spondias mombin* L.). Chemical composition in these plants varies according to the season of the year, where it is harvested, state of maturation, etc. [48].

Some authors have shown antioxidant capacity for *Spondias mombin* L. pulp under certain conditions [49,50], and in this study we demonstrated a higher efficiency. *Spondias mombin* L. seed oil had concentration-dependent antioxidant activity as shown in Figure 4a, with a maximum ABTS• + radical inhibition of 45%.

Our data showed a free radical scavenging effect in *Spondias mombin* L. oil, suggesting a protective activity against alterations in oxidant/antioxidant imbalance, promoting an increase of cell viability in the presence of higher oil concentration. Previous reports support this finding; pomegranate seed oil demonstrated an antioxidant effect on a diabetes model in kidney, heart and mitochondria from rats and H9c2 cell line [51]. The antioxidant capacity has been highly correlated with cell viability. Several studies demonstrated that carotenoids promote a protective effect resulting in cell viability against different insults such as UV light [52,53]. Other reports indicated that safflower seed oil increased significantly the viability and proliferation of embryonic neural stem cells [54]. Antioxidant compounds promote cell viability by several pathways. Some studies involved antioxidants in the maintenance of the genomic integrity (DNA) during proliferation stages [55]. Other reports have demonstrated that antioxidants prevent cells from oxidant-mediated cell death, through reducing membrane lipid peroxidation [56] and oxidative damage to cellular organelles [57].

The content of compounds in crude extracts depends on the extraction solvent used. In this case, we used hexane to extract mainly nonpolar molecules, including carotenoids and fatty acids, as it was demonstrated by the applied spectroscopy technics. In general, these compounds constitute only a few necessary sources of nutrients for cell proliferation, as cells mainly need carbohydrates. Indeed, different research groups report antiproliferative

and apoptotic effects for oils extracted from plants evaluated on different cell lines [58–60]. As a result, the proposed molecular mechanism associated with increased cell viability has a cytoprotective effect. Moreover, it has been demonstrated by Giusti et al. that molecules in natural product oils increase cell viability revealed by proteomic analyses [61].

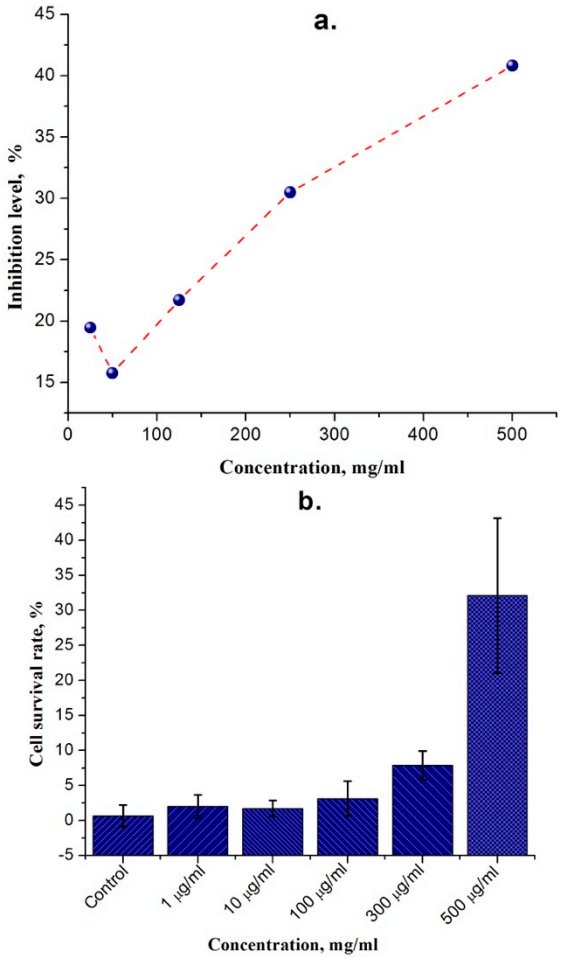

Figure 4. Antioxidation and cell viability tests. (**a**) *Spondias mombin* L. seed oil shows concentration-dependent antioxidant activity. It was measured by 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) antioxidant activity assay incubated for 30 min. (**b**) *Spondias mombin* L. seed oil increases cell viability. HTC 116 cells were cultured and incubated with different concentrations of vegetable oil. After 24 h, a water-soluble tetrazolium salt (WST) assay showed the increase of cell viability according to oil concentration. Nontreated cells were used as control referring 100% viability.

Previous reports have demonstrated the *Spondias mombin* L. cytotoxic effect. Indeed, the antimicrobial activity has been reported from herbalists through ethnobotanical surveys [62]; a study demonstrated antibacterial capacity from leaf and bark extracts, comparable to antibiotics. Supporting this evidence, another study of aqueous and ethanolic leaf extracts revealed vibriocidal and antimicrobial activities [63]. An in vitro study on rat red blood cells indicated an increase in hemolysis in the presence of the aforementioned extracts [64]. In this study, the seed oil of *Spondias mombin* L. was tested for cell viability, where, surprisingly, the treated cells incubated with oil showed more viability than the control cells without treatment. Likewise, Figure 4b shows an increment in cell viability according to oil concentration.

4. Conclusions

In the present work, we demonstrated the use of Raman and ^{1}H- and ^{13}C-NMR spectroscopy as noninvasive and nondestructive analytical techniques, which allowed for the characterization of the oil extracted from the seed of *Spondias mombin* L. Through the use of these two techniques, the characteristic vibrational frequencies of the *Spondias mombin* L. seed oil were identified, as well as its fatty acid profile. Raman vibratory frequencies at 1006, 1158 and 1523 cm^{-1} were identified and assigned to carotenoid compounds with antioxidant activity. On the other hand, NMR experiments proved to be a valuable tool to obtain the fatty acid profile of crude oil from the seed. Selected peak integrals were used to calibrate the composition of MUFA, PUFA and SFA. Applying this approach to a *Spondias mombin* L. seed oil sample, we obtained values for MUFA and PUFA contents of 29.4 and 43.5%, which indicated that the oil is highly unsaturated (>70%). The oil showed high biocompatibility and promoted cell viability in the HTC 116 cell line.

Due to its properties, the seed oil of this tropical fruit could have a potential application in cosmetic and pharmaceutical products as an antioxidant agent. Finally, the combination of Raman spectroscopy and the NMR technique are very powerful and useful tools for the characterization of organic compounds, particularly in natural vegetable oils.

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Conflicts of Interest: The authors declare no conflict of interest.

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