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Comprehensive Characterization of Cinnamomum cassia Stem Extracts by NMR and Spectroscopic Techniques for Food and Medical Applications

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ABSTRACT

Cinnamon (Cinnamomum cassia) has long been valued for its health-promoting properties, particularly its antioxidant, anti-inflammatory, and antimicrobial effects. In this study, essential oil was extracted from the stem of C. cassia using hydrodistillation via a Clevenger apparatus. The resulting oil, predominantly composed of cinnamaldehyde (92%), was characterized using a multidisciplinary set of techniques, including Nuclear Magnetic Resonance (NMR), Thermogravimetric Analysis (TGA), Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), and X-ray Diffraction (XRD). NMR, in both time-domain and solution modes, was highlighted as a powerful analytical tool for evaluating chemical composition, molecular structure, and purity. The integration of these techniques provided detailed insights into both the liquid extract and the residual solid material, revealing structural and molecular reorganization after oil extraction. The findings reinforce the potential application of cinnamon oil in food, pharmaceutical, and dental products, especially due to its high cinnamaldehyde content and purity.

Keywords

Cinnamomum cassia, Essential oil, Cinnamaldehyde, Nuclear Magnetic Resonance, Food analysis, Hydrodistillation, Functional ingredients.

Introduction

Cinnamon oils are generally mined in minimal quantities. They are extracted by distilling the plant's leaves or bark, producing essential oil [1].

It is known that cinnamon bark oil has a delicate spice aroma, is a sweet spice taste, and its main constituent is cinnamaldehyde. Therefore, other minor components are extracted and can transmit their characteristic aroma and flavor. Normally this oil is mainly used in the flavoring industry, where it is used in seasonings for meats, sauces and pickled vegetables, bakery products, confectionery, cola-type drinks, and flavors in cigarettes and dental and pharmaceutical preparations. Cinnamon leaf oil has a warm, spicy, but rather harsh aroma. Its main constituent is eugenol, which is used as a flavoring agent for seasonings and crackers. Its fragrance is added to soaps and insecticides. When cassia oil is distilled from a mixture of leaves, twigs, and stem bark, cinnamaldehyde is the main constituent and this mixture is principally used to flavor cola-type drinks, and in smaller quantities in bakery products, sauces, confectionery, and liqueurs [1,2].

Studies developed by the American Heart Association showed that cinnamon can decrease the risk of cardiovascular damage caused by a high-fat diet [3]. It activates antioxidant and anti-inflammatory molecules in the body, demonstrating one of the benefits of cinnamon for humans [4-7]. Other cinnamon applications, such as antimicrobial, antioxidant, bactericidal, antidermolytic, and antifungal actions, have also been studied [6,8-11].

The constituents extracted from cinnamon were analyzed using several techniques: Scanning Electron Microscopy (SEM) was employed to evaluate the morphology of the ground cinnamon particles and Energy Dispersive Spectroscopy (EDS) was applied to identify the chemical elements in the sample; X-ray diffraction (XRD) was used to determine the chemical compounds present in the nutrients found in the soil used for cinnamon cultivation; Thermogravimetric Analysis (TGA) assessed the degradation temperature of cinnamon compounds and oils; Fourier Transform Infrared (FTIR) identified functional groups through the vibrations of chemical bonds, and finally, Nuclear Magnetic Resonance (NMR) was employed to identify and quantify organic compounds. NMR was chosen because it gives more detailed information than other techniques, complementing the results.

It is known that solution nuclear magnetic resonance is a powerful technique to evaluate oils and a great variety of food samples [12-14], due to the capability to investigate the samples in detail. The solid-state NMR through the time domain relaxation analyses is also a technique used in food samples to obtain information on sample dispersion and molecular movements by the type of food sample [15-17]. This study aimed to extract and characterize cinnamaldehyde and other minor components from Cinnamomum cassia stem using hydrodistillation, followed by comprehensive analysis through NMR, FTIR, TGA, SEM, and XRD, to evaluate its potential for food, medical, and dental applications.

Materials and Methods

Sample Preparation

The commercial stem of cinnamon cassia was used as raw material; obtained from China, and acquired in 2022 in the national market by the manufacturer "Kitano". The stem was dried in an oven at 60 °C for 24h and transformed into powder using a knife mill from Solab Cientifica, model GL30, series 0021, 300 w. The grains were sieved using two Bertel Indústria Metalúrgica LTDA grain fractionation sieves with openings of 80 mesh (6.35 mm) and 35 mesh (13.5 mm). Thermogravimetric Analysis, Scanning Electron Microscopy, Energy Dispersive Spectroscopy, X-ray diffraction, and Time-Domain Nuclear Magnetic Resonance characterized cinnamon powder.

Essential Oil Extraction

After characterizing the cinnamon powder, the essential oil was extracted by hydrodistillation using the Clevenger apparatus. The Clevenger apparatus consists of a 500 ml flask heated by a thermal blanket (Fisatom, model 52E, series 1595523, 200 W), where the steam generated is conducted through the system to a vertical condenser. The steam with the extracted oils is condensed and the liquid products are stored in a separator funnel with a valve. The oil sample extracted by the Clevenger apparatus will be designated as “Cinnamon Oil extracted by Clevenger”.

The major component extracted from the cinnamon stem is cinnamaldehyde. For this reason, an aliquot of Cinnamaldehyde P.A. was analyzed with 98% of the molecular weight (designated by sample “Cinnamaldehyde Standard”). Aiming to compare the result of the cinnamon extracted by hydrodistillation with a commercial sample, an aliquot of Cinnamon cassia essential oil from the manufacturer “Via Aromas” was also analyzed (known as the “Commercial Cinnamon Oil” sample). The extracted oils were characterized by FTIR, TGA, and Time-Domain Nuclear Magnetic Resonance (TD-NMR).

Characterization by Scanning Electron Microscopy

For the characterization of the initial material by Scanning Electron Microscopy, equipment from the manufacturer TESCAN model MIRA 4th generation was used, equipped with a field emission gun (FEG - Schottky), secondary electron detector (SE), electron detector backscatter (BSE) and Energy Dispersive Spectroscopy (EDS) detector.

Characterization by X-ray diffraction

The X-ray diffraction characterization of the cinnamon powder a Rigaku diffractometer, Ultima IV model, operating in the Bragg Brentano configuration was used. The power of 40 kV and 40 mA, tube with copper anode, incidence slit (2/3)°, was applied to time per 5-second step with 2 sweeps from 5° to 100°.

Characterization by Thermogravimetric Analysis

The thermogravimetric analysis was carried out in the “TA Instruments” equipment, model Q500. The analysis was conducted in the temperature range from 20 °C to 700 °C, with a mass of approximately 10 mg/sample in a nitrogen atmosphere, with a variation of 10 °C/min, to verify the degradation profile of the sample. The analyses were performed in triplicates and the results were represented by statistical average and standard deviation.

Characterization by Infrared Spectroscopy with Fourier Transform

Fourier transform infrared spectroscopy was used to identify the main chemical bonds that characterize the compounds extracted from cinnamon. The spectrometer used in the work was the Thermo Scientific Nicolet iS5, with the iD5 Total Reflectance Attenuation (ATR) accessory in the wavelength range from 500 to 4000 cm^{-1}.

Characterization by Time-Domain Nuclear Magnetic Resonance

Time-domain NMR analysis was acquired to determine nuclear relaxation measurements using the Oxford Instruments MARAN Ultra equipment, operating at 0.54 T (23.4 MHz for the ^{1}H) equipped with an 18 mm probe, at 30°C. Three pulse sequences were applied, and their description of them listed below:

- To determine the longitudinal relaxation times of hydrogen for the sample, the pulse sequence of Small-Angle Flip-Flop (SAFF) was applied [15]. The parameters used to acquire the experiment were: 90° pulse (duration 7.5 µs), number of scans 16, and receiver gain 42 dB.
- The transverse relaxation times of hydrogen (with a time constant T_2) were obtained from the Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence using the following parameters: 90° pulse (duration 7.5 µs), time between echoes 600 (2t, µs), number of echoes 8192, points per echo 1, number of scans 4, recycle time 1, and receiver gain 40 dB.

- The transverse relaxation times of hydrogen (with a time constant T_2*) for solid samples, the Solid Eco pulse sequence was applied [15], using the parameters: 90˚ pulse (duration 7.5 µs), number of 256 points, the interval between each point 0.5 µs, number of scans 512, recycle time 1 and receiver gain 40 dB.

Characterization by Solution Nuclear Magnetic Resonance Spectroscopy

Nuclear magnetic resonance measurements in solution were done using 300 MHz Varian Mercury VX equipment with a 5-mm-diameter probe. Samples were prepared using dimethylsulfoxide deuterated (DMSO). NMR spectra of hydrogen-1 and carbon-13 were acquired using the equipment's standard pulse sequence. The conditions of measurements for the ^{1}H nucleus were: probe temperature of 40°C; 16 scans were accumulated; the relaxation time was 10 seconds; pulse width was 3.53; an acquisition time of 2 seconds with a spectral width of 4800 Hz was established. The acquisition parameters for the ^{13}C nucleus were: probe temperature of 40°C; 1024 number of transients obtained; the relaxation time between pulses was 2 seconds; pulse width was 6.95; an acquisition time of 1.5 seconds with a spectral width of 18868 Hz was established. Spectrum processing was performed using the MestreNova version 12.0 (MestreLab – Spain).

Result and Discussion

Extracting essential oils

Cinnamon extraction was performed with 40 g of grains larger than 35 mesh (13.5 mm) and 300 ml of distilled water. After 3 h of distillation, 1 ml of essential oil was produced. The viscous oil extracted was yellow.

Figure 1 shows the scanning electron microscopy and energy dispersive spectroscopy of cinnamon powder before the extraction of essential oil. It exhibits the distribution of cinnamon grains with a backscattered electron detector, magnification of 200x, and acceleration of 15 keV.

The SEM micrograph reveals particles with irregular morphologies and sizes, which are characteristic of samples ground in knife mills, presenting a great variation in grain size, even having been sieved at 80 mesh (6.35 mm).

Figure 2 shows a comparison of the grain morphology of cinnamon grains before (A) and after (B) the extraction of essential oils. Both images were performed with a secondary electron detector, magnification of 2000x, and acceleration of 5 keV.

In Figure 3, the SEM micrograph with the minimum size of the grains before and after the extraction of the oils is carried out with a secondary electron detector, acceleration of 5 keV, and magnification of 30000x and 5000x, respectively. This Figure shows an increase in pore size on the cinnamon grain surface from approximately (2 – 5) µm to (16 – 18) µm, indicating the leakage of oils/water from the molecular structure of the grain, indicating that the oil was inside the structure. And its extraction promoted changes in the molecular organization.

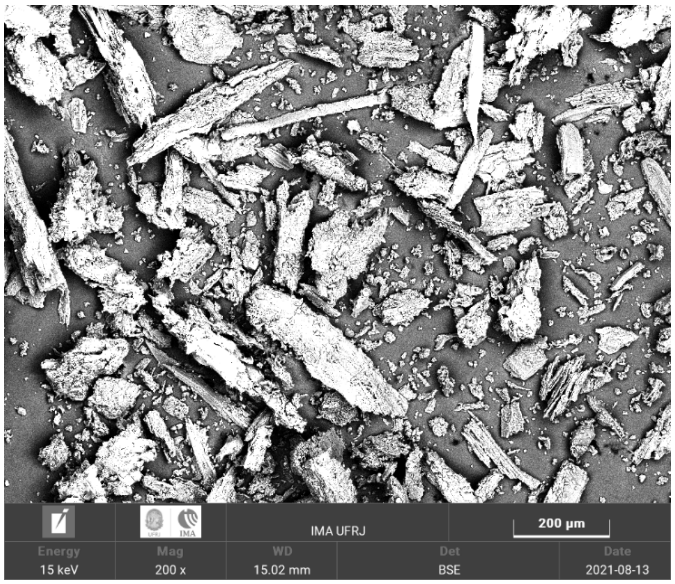

Figure 1: Morphology of cinnamon grains.

In addition to the increase in the pore size of cinnamon grains, in Figure 3 can be seen that the minimum grain size also increased from approximately 3 µm to 27 µm for the same reason mentioned before.

The EDS analysis of cinnamon grains, conducted before and after essential oil extraction, was used to identify the main chemical elements present in the samples. The results are presented in Table 1. The analysis was performed under the following conditions: 15 keV and 1000x magnification.

The major elements present in the samples were carbon, oxygen, nitrogen, calcium, potassium, and other minor elements were identified in the cinnamon grain. In the characterization of the grain in the cinnamon powder sample, after extracting the essential oils, they did not show considerable differences, which is important not to destroy the cinnamon powder sample structure; only the amounts of calcium and potassium had smaller amounts, and this is probably due to the procedure for extracting the essential oil from the cinnamon grains which solubilize some chemical components in water.

Table 1: Elements identified in the energy dispersion spectrum.

Chemical	Weight %	
	Before extracting oil	After extracting oil
Carbon	58.30	57.20
Oxygen	35.70	38.10
Nitrogen	4.00	4.10
Calcium	1.40	0.60
Potassium	0.50	0.01
Sulfur	0.03	0.01
Sodium	0.01	0.01
Magnesium	0.01	0.01
Silicon	0.01	0.01
Phosphorus	0.01	0.01

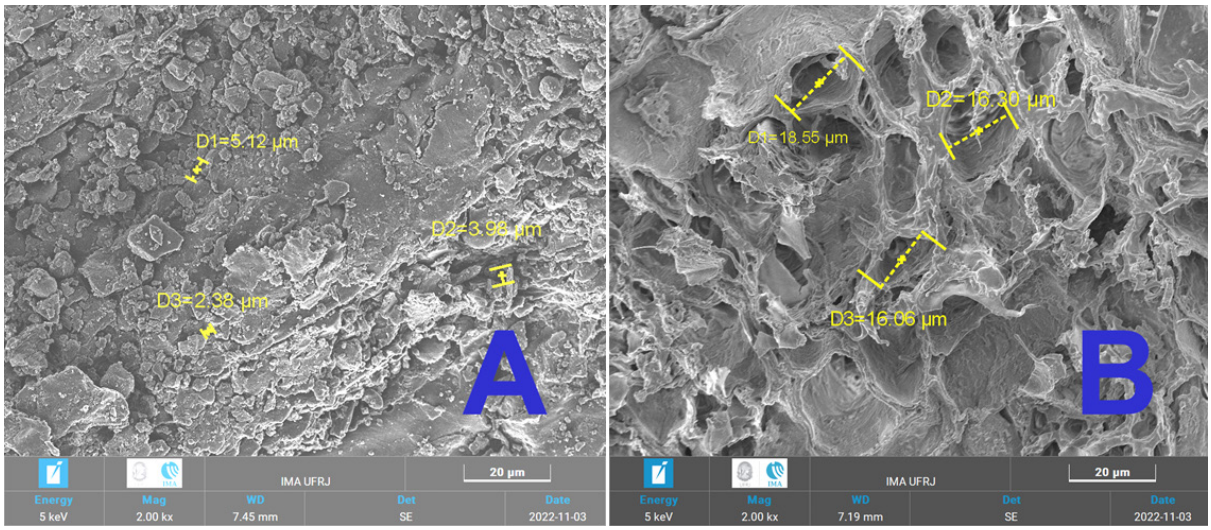

Figure 2: Morphology of cinnamon grains (a) before and (b) after essential oil extraction.

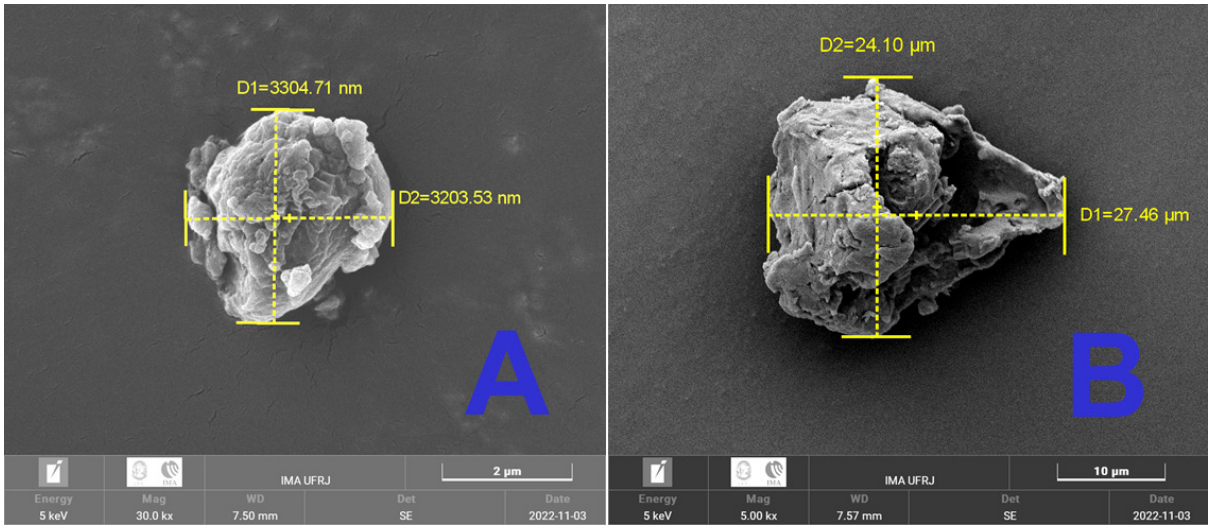

Figure 3: Minimum size of cinnamon grains (a) before and (b) after essential oil extraction.

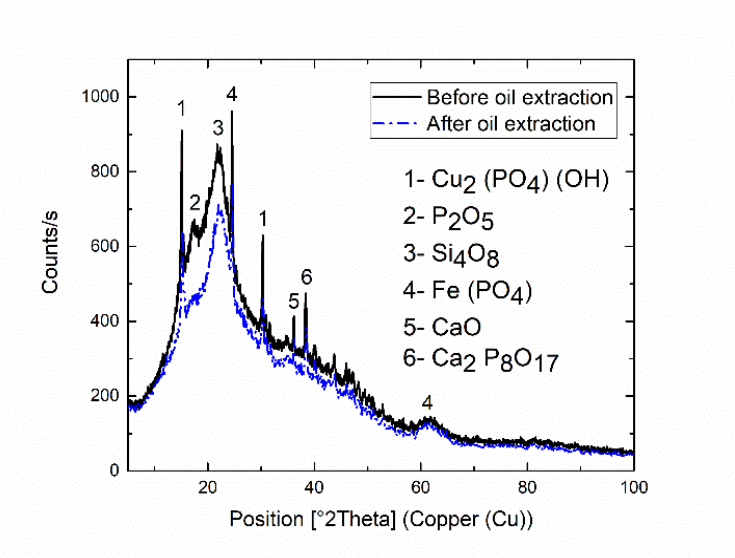

Figure 4: Diffractogram of cinnamon powder before and after essential oil extraction.

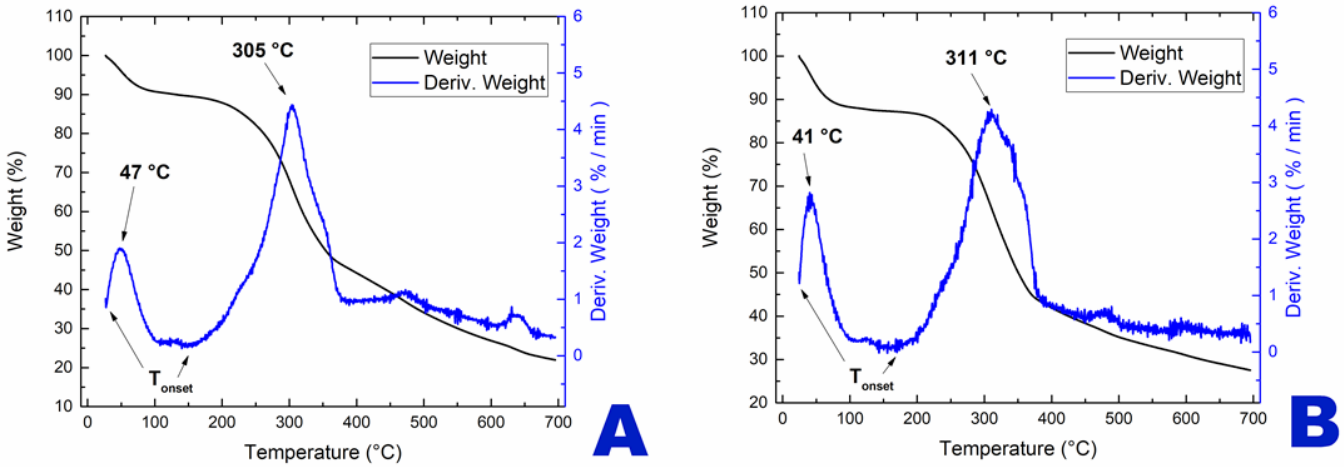

Figure 5: TGA and DTG of cinnamon powder (a) before and (b) after essential oil extraction.

X-ray diffraction result of cinnamon powder

X-ray diffraction analysis was performed using 5 grams of cinnamon powder with sizes smaller than 80 mesh. The diffractogram resulting from cinnamon before and after the extraction of essential oils is shown in Figure 4.

The diffractograms in Figure 4 determined the crystallinity index as 3% for the sample before essential oil extraction and 4% for the sample after extraction. The crystallinity index of the sample after extraction slightly increased, which indicates the removal of non-crystalline organic materials from the cinnamon powder.

The qualitative analysis compared the experimental results with established crystallographic standards JCPDF (Joint Committee on Powder Diffraction File) published by the COD database (Crystallography Open Database). The qualitative analysis of the cinnamon diffractogram indicates a correlation between the crystal planes of the phases listed in Table 2.

Table 2: Crystalline phases identified in cinnamon powder.

COD Reference	Component	Chemical Formula
96-900-1580	Cristobalita	Si_4O_8
96-152-8173	Cupric base phosphate	$Cu_2(PO_4)$ (OH)
96-431-8791	Iron Phosphate	Fe(PO4)
96-231-1014	Phosphorous oxide (V)	$P2O_5$
96-810-4401	Calcium Phosphate	Ca_2 (P6 O17)
96-152-0967	Iron (III) Phosphate	$Fe(PO_3)_3$
96-900-6741	Calcium Oxide	CaO

The crystalline compounds identified in cinnamon powder correspond to fertilizers commonly used in cinnamon cultivation and components of the planting soil, aligning with findings previously reported in the literature [1]. The amorphous signals refer to the non-crystalline organic materials of the sample (oils/water) [16-18]. In the same way, the powder was characterized after the extraction of essential oils and the results did not change. The diffractogram referring to the cinnamon powder sample after extracting the essential oils does not show the planes referring to the P2O5 phase, this may be due to the solubility of this phase in water, which in the extraction process was rejected and not analyzed.

Thermogravimetric Analysis

The results of the thermogravimetric analysis and the derivative of the TGA (DTG) of the cinnamon powder before and after the extraction of essential oils are represented by Figure 5, where "Tonset" is the initial time of the event.

The result indicates two peaks in the DTG curve, generated by the cinnamon powder sample: before the extraction of essential oils, a peak at 47°C and the other centered at 305°C with a total mass loss of 78%; after extracting the essential oils, one peak at 41°C and the other at 311 °C with a total mass loss of 73%. The first events of each sample refer to the loss of volatiles and the second events represent the total mass loss of the sample due to degradation. The small differences between the samples found in the temperatures of the peaks of the DTGs of the second event may indicate a change in the molar mass of the cinnamon grains due to the departure of the essential oils.

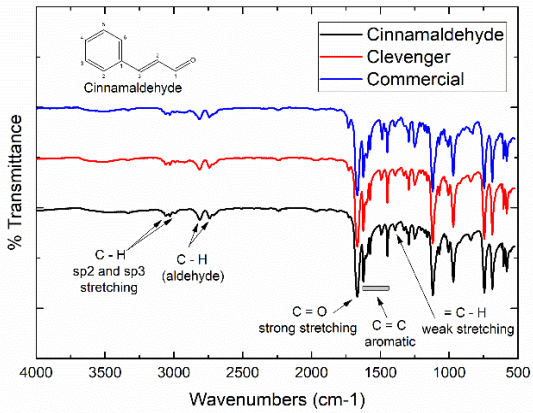

Figure 6: T_1H values and domain relaxation curves of cinnamon stem powder before oil extraction; cinnamon stem powder after oil extraction.

The TGA and DTG curves were created for the other cinnamon samples and the results of the beginning of the events, Tonset, end of the events, Tendset, and the peak of the event, Tpeak, referring to the DTG curves which are listed in Table 3. The temperatures Tonset indicates the starting point of sample degradation.

Table 3: Temperatures of each DTG event of the analyzed samples.

Sample	(Tonset)	(Tendset)	(Tpeak)
	± 2°C	± 2°C	± 2°C
Cinnamaldehyde Standard	46	175	146
Commercial Cinnamon Oil	45	211	168
Cinnamon Oil extracted by Clevenger	40	221	145
Cinnamon stem powder before oil extraction 1st peak	24	103	47
Cinnamon stem powder before oil extraction 2st peak	150	380	305
Cinnamon stem powder after oil extraction 1st peak	21	105	41
Cinnamon stem powder after oil extraction 2st peak	182	385	311

The results of the TGA curves indicated that the cinnamon oils started to degrade at a temperature of 40°C, which is the critical temperature for applications with this material.

Result of Infrared Spectroscopy with Fourier Transform

Figure 6 shows the result of comparing the cinnamon samples from the FTIR analysis.

The three samples showed vibrations at the same wavelength positions, indicating agreement with the analyzed reference material, the Cinnamaldehyde Standard. The position of the wavelengths of the main vibrations is shown in Table 4.

Table 4: Result of the main wavelengths found in the samples.

Wavelength (cm^{-1})	Bond Type
1667.33	C = O (aldehyde)
2811.29	C – H (aldehyde)
2740.86	
1630.00	C = C (aromatic ring)
1445.00	
1250.00 – 950.00	C – H (vibrations in the plane)
900.00	C – H (out-of-plane vibrations)
650.00	

The main absorption peaks of aldehydes appear at 1667.33 cm^{-1} referring to the (C = O) bond; 2811.29 cm^{-1} and 2740.86 cm^{-1} are peaks indicative of (C – H) bonding. The absorption peaks of the aromatic ring appear from 1630.00 to 1445.00 cm^{-1}, these peaks refer to (C = C) bonds; 1250.00 to 950.00 cm^{-1} are vibrations in the plane of the (C – H) bonds with several intense bands; and between 900.00 and 650.00 cm^{-1} with one or more bands with strong out-of-plane vibrations, referring to the (C – H) bonds of the aromatic ring [19,20]. These results are consistent with those studied by Weiwei Shi, 2021; Hu, 2018; Kuan Yang, 2021 [11,21,22].

Time-Domain Magnetic Resonance

Cinnamon powder samples were analyzed before and after the extraction of essential oils, the "Cinnamaldehyde Standard", the "Commercial Cinnamon Oil" and the "Cinnamon Oil extracted by Clevenger". Figure 7 shows the curves referring to the longitudinal relaxation times and the values of T1H of cinnamon stem powder before oil extraction, and cinnamon stem powder after oil extraction. Figure 8 exhibits the T1H values and domain relaxation data of cinnamaldehyde standard, commercial cinnamon oil, and cinnamon oil extracted by Clevenger.

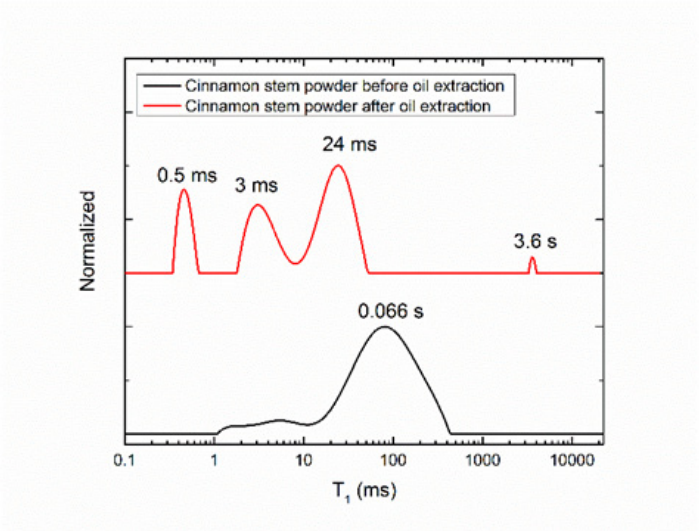

Figure 7: T_1H values and domain relaxation curves of cinnamon stem powder before oil extraction; cinnamon stem powder after oil extraction.

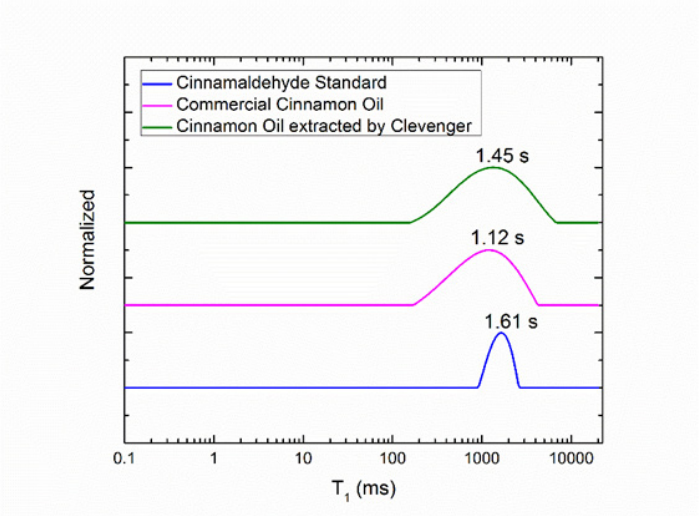

Figure 8: T_1H values and domain relaxation curves of cinnamaldehyde standard; commercial cinnamon oil; cinnamon oil extracted by Clevenger.

The curves of the relaxation domain from the stem powder before and after oil extraction are large and heterogeneous, respectively. The large domains in the sample before extraction are due to a mix of components and arrangements. Due to the low molecular mobility, the rigid domain presents a high value of relaxation

times and controls the relaxation processing through the dipole-dipole intermolecular interactions. Looking for the relaxation domains for the sample after oil extraction, change in the domain curves and consequent relaxation values are due to the molecular reorganization of the sample and the formation of new and multiple intermolecular interactions and water absorbed.

The curves referring to the standard cinnamon oil are homogeneous due to the sample purity; the commercial sample and the oil extracted by Clevenger in this work are larger than the pure oil due to other small components and absorbed water. The small differences come from their chemical composition which generates differences in the intermolecular interaction forces, which is due to the molecular distribution.

To obtain more information on the sample molecular organization due to the intermolecular interactions the samples in the investigation were analyzed by proton spin-lattice relaxation data and proton spin-spin relaxation data. The solid samples have T_2*H (solid echo) determined, while the liquid samples and the spin-spin were analyzed by CPMG. Table 5 exhibits the comparison of values of T_1H relaxation data and T_2*H from solid eco related to cinnamon stem powder before oil extraction, and cinnamon stem powder after oil extraction. And Table 6 lists the values of T_1H relaxation data and T_2H transverse relaxation cinnamaldehyde standard; commercial cinnamon oil; cinnamon oil extracted by Clevenger.

Table 5: T_1H and T_2*H values for cinnamon stem powder before oil extraction, and cinnamon stem powder after oil extraction.

Sample	T_1H (SAFF)	T_2*H (Solid Echo)	
		Rigid fraction	Mobile fraction
Cinnamon powder before extraction	66.0 ms	10.0 µs	82.0 µs
Cinnamon powder after extraction	9.8 ms	15.0 µs	223.0 µs

Table 6: T_1H and T_2H values for cinnamaldehyde standard; commercial cinnamon oil; cinnamon oil extracted by Clevenger.

Sample	T_1H (SAFF)	T_2H (CPMG)
Cinnamaldehyde Standard	1.6 s	1.4 **s**
Commercial Cinnamon Oil	1.1 s	0.9 **s**
Cinnamon Oil extracted by Clevenger	1.5 s	1.2 **s**

All relaxation data shows that after the extraction the mobile fraction changes more than the rigid phase, which can indicate that the oil and the other components extracted were in the mobile region. That may explain the biggest molecular reorganization of the sample after the oil is extracted. Certainly, the new molecular arrangements promoted new intra and intermolecular interactions. The relaxation times of the oil sample extracted by Clevenger also maintained the same range (T_1H = 1.5 s and T_2H = 1.2 s)

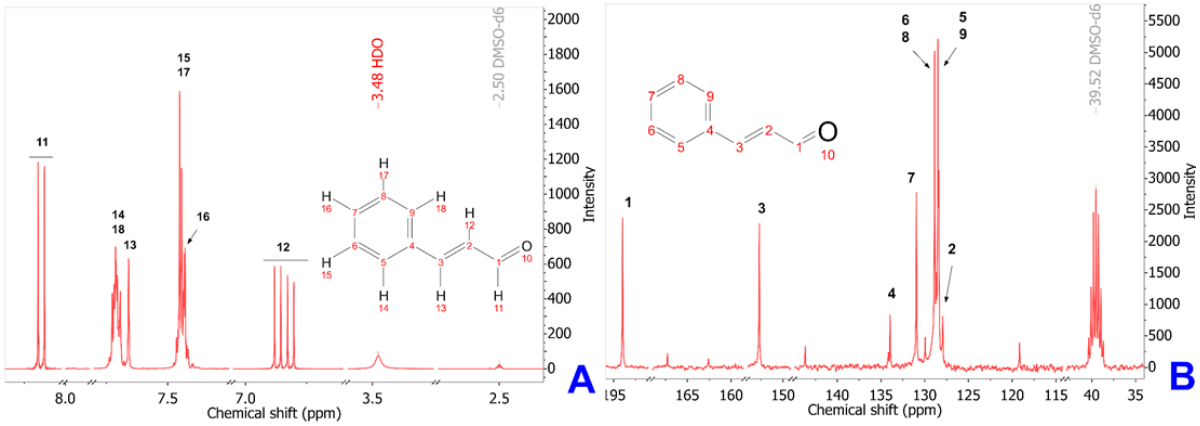

Figure 9: Cinnamaldehyde standard with DMSO-d6 solvent. (a) H-1 nucleus; (b) C-13 nucleus.

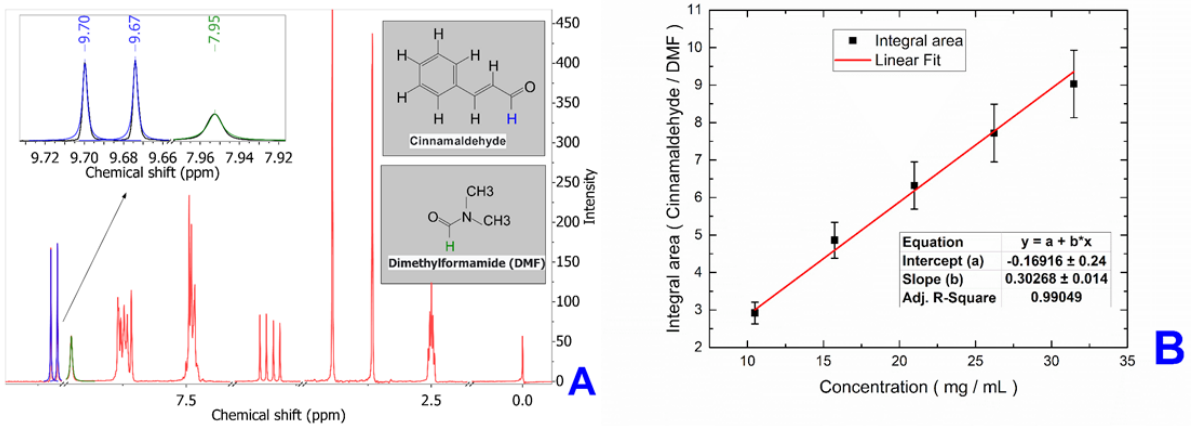

Figure 10: Cinnamaldehyde standard with DMSO-d6 solvent. (a) H-1 nucleus; (b) C-13 nucleus.

the relaxation values are like those of cinnamaldehyde standard (T_1H = 1.6 s and T_2H = 1.4 s), indicating a good purity in the oil extracted by Clevenger and composition in comparison to the commercial oil (T_1 = 1.1 s and T_2 = 0.9 s). The value of transverse relaxation time, T_2H, maintains the same behavior as T1H, this behavior indicates the efficiency of oil extraction.

Nuclear Magnetic Resonance Spectroscopy

Figure 9 shows the results of the nuclear magnetic resonance spectra of ^{1}H or ^{13}C nuclei of the Cinnamaldehyde Standard sample and its chemical shift assignments.

The cinnamaldehyde purity of this reference sample, calculated from the ^{1}H NMR, was 95% of the molecular weight, where 5% refers to the solvent used (water and DMSO). Through NMR it was also possible to calculate the amount of cinnamaldehyde present in the commercial cinnamon oil sample, being 80%. To calculate the exact concentration in 1 ml of cinnamon oil extracted by the Clevenger apparatus, the analytical methodology by NMR based on the internal standard was applied. The internal standard used for the analysis was N, N- Dimethylformamide – DMF (99.8%) from the manufacturer due to its non- reactivity with cinnamon extract. The methodology generates five samples of known concentrations of the standard Cinnamaldehyde P.A. from the manufacturer.

The calibration curve was generated from the ^{1}H NMR result, through the doublet (δ 9.67 ppm and δ 9.70 ppm) of the aldehyde hydrogen of the Cinnamaldehyde P.A. in comparison with the singlet (δ 7.95 ppm) of the aldehyde hydrogen of the DMF internal standard molecule, as shown in Figure 10(a). The calibration curve is represented in Figure 10(b). The quantification of the cinnamaldehyde concentration present in the cinnamon extract sample was calculated based on the linear equation of the calibration curve, represented in Figure 10(b).

After preparing a new sample, containing an aliquot of cinnamon extract extracted by Clevenger with DMF (40%) and performing a new ^{1}H NMR, the cinnamaldehyde concentration was calculated, obtaining approximately 2 g/ml of cinnamaldehyde in the extract. The quantity of cinnamon oil extracted by Clevenger was 92% of cinnamaldehyde, which was higher them the value found for the commercial sample, which is 80%. The use of Clevenger extraction process was an effective method to obtain this oil, and it can be used for other oils.

Conclusions

The extraction of essential oil from *Cinnamomum cassia* stem by hydrodistillation yielded a highly pure product containing 92% cinnamaldehyde, surpassing commercial formulations. Advanced characterization techniques, especially NMR spectroscopy, proved essential for elucidating the molecular composition, purity, and structural changes in the samples. The integration of spectroscopic and morphological analyses confirmed the efficiency of the extraction process and highlighted the potential applications of cinnamon oil in food, medical, and dental fields due to its high cinnamaldehyde content and known antimicrobial properties.

Acknowledgements

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