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Isolation of Anacardic Acid from Natural Cashew Nut Shell Liquid (CNSL) Using Supercritical Carbon Dioxide

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Solvent extracted cashew nut shell liquid (CNSL), conventionally known as natural CNSL, is a mixture of several alkenyl phenols. One of these alkenyl phenols is anacardic acid, which is present at the highest concentration. In view of anticipated industrial applications of anacardic acid, the objective of this work was to isolate anacardic acid from natural CNSL by supercritical carbon dioxide ($scCO_2$). In this study, the solubility data for natural CNSL in $scCO_2$ under a range of operating conditions of pressure (100, 200, and 300 bar), temperature (40 and 50 °C), and CO_2 flow rate (5, 10, and 15 g min^{-1}) were established. The best $scCO_2$ working conditions were found to be 50 °C and 300 bar at a flow rate of 5 g min^{-1} CO_2. Using 3 g of sample (CNSL/solid adsorbent = 1/2) under these $scCO_2$ conditions, it was possible to quantitatively isolate high purity anacardic acid from crude natural CNSL (82% of total anacardic acid) within 150 min. The anacardic acid isolated by $scCO_2$ was analyzed by different spectroscopic techniques (UV−vis, FT-IR, and ^{1}H NMR) and HPLC analysis, indicating that the anacardic acid isolated by $scCO_2$ has better quality than that obtained through a conventional method involving several chemical conversion steps.

KEYWORDS: Cashew nut shell liquid; anacardic acid; supercritical CO_2; HPLC; FT-IR; NMR

INTRODUCTION

Cashew nut shell liquid (CNSL), a byproduct in cashew nut processing factories has found broad industrial applications such as in brake linings, paints, primers, foundry chemicals, lacquers, cements, and coatings (*1*). Lately, however, CNSL has attracted a great deal of attention as a result of its biological activities that are attributed to its chemical components. The biological activities of CNSL components include molluscicidal activity (*2*), antitumor activity (*3*), prostaglandin synthetase inhibition (*4*), and antimicrobial activity (*5*). Much of these biological activities are associated with anacardic acid, the major component of natural CNSL (*6*). These attributes, allied to its chemical structure, makes anacardic acid a good candidate as a starting material in many reactions to yield a wide variety of useful products. Anacardic acid can participate in many reactions including thermal decarboxylation, hydrogenation of the aliphatic chain (*7*), nitrogenation, and esterification, among others (*8*).

Traditionally, CNSL is obtained during thermal processes of deshelling the cashew nuts for the purpose of obtaining the edible part, the cashew kernels. Hot-oil and roasting techniques, in which CNSL oozes out from the shells, are the most reported methods in the literature. However, the high temperature involved in these thermal methods generally cause decarboxylation of anacardic acid to form cardanol. This is why cardanol is the main component of technical (industrial) CNSL. In addition to the possibility of some of the CNSL polymerizing at elevated temperature, thermal extraction can adversely affect the quality and color of the obtained CNSL (*9*). Likewise, the anacardic rich natural CNSL and anacardic acid, obtained using alternative methods described elsewhere (*10*), have been found to possess almost the same quality weakness including the dark brown color. These drawbacks hamper several industrial applications of the traditionally obtained natural CNSL and their isolates such as anacardic acid.

Recently, carbon dioxide near critical and supercritical states ($scCO_2$) have drawn much attention as solvents, especially in food and in the pharmaceutical industry. This is particularly for the interest of avoiding the use of organic solvents that are economically and environmentally unfriendly, besides the difficulties of completely eliminating organic solvents from the desired end products (*11*). In view of this, few researchers succeeded in obtaining nearly colorless CNSL from cashew nut shells using $scCO_2$ (*12, 13*). Still, the products obtained in these studies were mixtures of all of the phenolic components of CNSL.

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The present study was intended to isolate clean anacardic acid from natural CNSL using $scCO_2$. Since the solubility data for CNSL in $scCO_2$ are not available in the literature, this aspect was also considered by investigating a range of operating conditions (pressure, temperature, and CO_2 flow rate) in order to come up with optimized parameters that can be applied in the process of isolating chemical components from CNSL for various practical applications. Different spectroscopic and chromatography methods were employed to analyze the chemical components of the isolated products. The anacardic acid isolated from CNSL using $scCO_2$ was compared with that obtained by a conventional extraction procedure involving several chemical conversion steps. The results indicate that the simple $scCO_2$ method is able to produce anacardic acid with higher quality and reduces the consumption of organic solvents.

MATERIALS AND METHODS

Materials. Crude natural CNSL was obtained as reported elsewhere (*14*). Celite 521 was purchased from Sigma-Aldrich (Chemie GmbH, Germany), and glass wool (glass fibers, ca. 5−7 μm diameter) was purchased from Tamro MedLab AB. Acetone (99.0%, extra pure), chloroform (99.8%), and acetonitrile (99.9%, HPLC grade) were supplied by Fisher Scientific AB (Västra Frölunda, Sweden), and potassium bromide (99.0%) was acquired from Merck (Darmstadt, Germany). Chloroform-d ($CDCl_3$, 99.8 atom %D) was obtained from Aldrich, and acetic acid (99.8%) was obtained from Fluka (Buchs, Switzerland). All the substances were used as supplied.

Sample Preparation. In a flask, the crude CNSL (50 g) was dissolved in acetone (400 mL) and mixed with 100 g of celite or glass wool. Acetone was removed under reduced pressure using a rotary evaporator at 30 °C, and the celite or glass wool with adsorbed CNSL was kept in vacuo for over 48 h before use.

Equipment for Extraction with $scCO_2$. Extraction experiments were performed in an SFE-2 × 100F system (Thar Technology Inc., Pittsburgh, USA). The apparatus, as described comprehensively in the literature (*15*), was modified by using only one extraction vessel and replacing the two cyclone separators by a single Falcon tube. Before the extraction experiment, the CNSL-loaded celite or glass wool was introduced into the extraction vessel. The Falcon tube was used as a fraction collector.

Influence of Temperature, Pressure, and CO_2 Flow Rate on CNSL Solubility. To establish the influence of these parameters on the solubility of natural CNSL in $scCO_2$, triplicate extraction experiments were carried out, in each case using 3 g of the celite−CNSL mixture. The effect of temperature and pressure was studied using a fixed CO_2 flow rate of 10 g min^{-1}. Extraction experiments were conducted at 40 and 50 °C under the selected pressure (100, 200, and 300 bar). The best conditions of temperature and pressure obtained were then fixed to study the influence of the different flow rates of CO_2. The masses of the extracts were cumulatively recorded after every 10 min for 1 h in each case. The collective mass of extracts was plotted as a function of the cumulative mass of CO_2 consumed. The slopes of these curves in the initial linear portion were then calculated. The mean of the triplicate values was taken as the apparent solubility of CNSL under a given operating condition (i.e., temperature, pressure, and flow rate of CO_2) (*16*).

Time Dependent Extraction Experiment. This experiment was aimed at establishing the time dependent isolation of anacardic acid from natural CNSL. Both CNSL-loaded celite and glass wool (3 g) were introduced into the extraction vessel in these experiments. The extractions were carried out under the optimum condition affording the highest CNSL solubility in $scCO_2$. Effluents were collected every 30 min in separate Falcon tubes, which were kept for further spectroscopic and HPLC analysis.

UV−Vis and FT-IR Spectroscopy. UV−vis analysis was performed using a Beckman Coulter, DU 800 Spectrophotometer. The liquid samples were dissolved in chloroform to a final concentration of approximately 1 μg mL^{-1} before the analysis. FT-IR analysis was performed on a Nicolet Impact 410 spectrophotometer equipped with a QuickIR software (Thermo Fisher Scientific). Approximately 0.2 mg of isolated anacardic acid was thoroughly mixed with 300 mg of KBr to prepare the sample pellet.

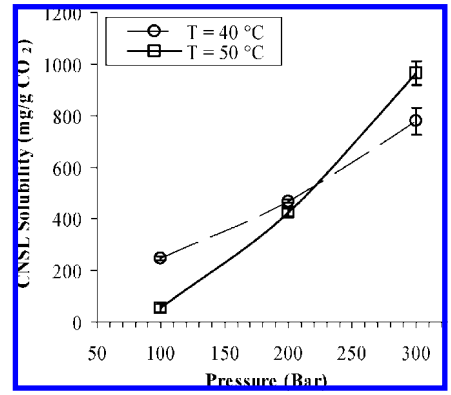

Figure 1. Pressure dependence of CNSL solubility in $scCO_2$ at 40 and 50 °C and CO_2 flow rate = 10 g min^{-1}.

Chromatography Analysis. HPLC analysis was carried out on a Chromolith Performance RP-18e column (Merck, Darmstadt, Germany) connected to a LaChrome system consisting of an L-7100 pump, L-7200 autosampler, L-7455 diode array detector, and a software package D-7000 System Manager (Merck KgaA, Darmstadt, Germany). Solvent A, H_2O/TFA = 100/0.1; solvent B, MeCN/H_2O/HOAc = 80/20/1. Separation of anacardic acid was achieved with isocratic elution using solvent A/B = 10/90 at a flow rate of 0.5 mL min^{-1}. Before HPLC analysis, samples were carefully dried in a vacuum chamber at 20 °C for 48 h. Tandem HPLC-MS analysis was carried out on a SunFire C_{18} column (3.5 μm, 2.1 × 50 mm) mounted on a Waters 2695 Separation Module. Solvent A was 0.1% formic acid in water, and solvent B was MeCN. Anacardic acid was separated with isocratic elution using solvent A/B = 20/80 at a flow rate of 0.3 mL min^{-1} and detected with an electrospray Waters Quattro micro API mass spectrometer in positive mode, using MS scan (ES+, *m/z* 340.0 to 352.0).

RESULTS AND DISCUSSION

Influence of Temperature and Pressure on CNSL Solubility in $scCO_2$. The solubility values plotted as a function of pressure at fixed temperature are presented in **Figure 1**. The results obtained indicate clearly that the highest CNSL solubility can be achieved using an operation condition fixed at 50 °C and 300 bar with a CO_2 flow rate of 10 g min^{-1}. These results are in agreement with predictions from the literature (*17*). At a given temperature, the density of CO_2 increases with pressure, thus boosting its solvent power and consequently increasing the solubility of CNSL. Temperature, however, has an opposite effect. At fixed pressure, the higher the temperature, the lower the density of $scCO_2$ and accordingly the lower the solvent power (*17*). These are in accordance with the results for pressures below the crossover region (P < 220 bar) (**Figure 1**). Above this region, the increase of the solubility of CNSL with the increase in temperature can be attributed to the increase in vapor pressure of some of the components in CNSL.

Influence of CO_2 Flow Rate on CNSL Solubility. The solubility values obtained from these experiments were plotted as a function of CO_2 flow rate, as shown in **Figure 2**. In these experiments, the $scCO_2$ was kept at 50 °C and 300 bar. The graph in **Figure 2** suggests that the solubility of CNSL decreases with increased CO_2 flow rate. The best results obtained at the lowest CO_2 flow rate (5 g min^{-1}) can be explained in terms of the residence time of the fluid in the extraction vessel. In the present study, the volume of the vessel used was 100 mL containing approximately 87 g of CO_2 (density of CO_2, 0.87 g cm^{-3} at 50 °C and 300 bar). Therefore, the CO_2 flow rates of

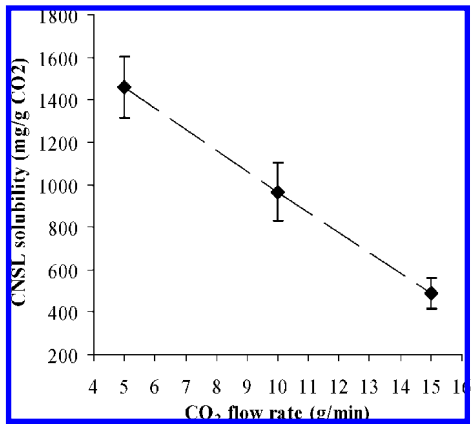

Figure 2. Solubility of CNSL as a function of CO_2 flow rate with $scCO_2$ at 50 °C and 300 bar.

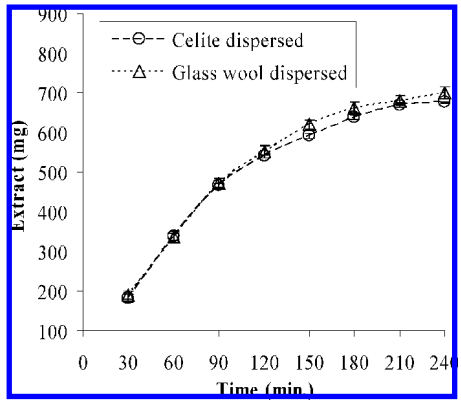

Figure 3. Extraction of CNSL as a function of time at fixed $scCO_2$ conditions (50 °C and 300 bar) and CO_2 flow rate of 5 g min^{-1}.

15, 10, and 5 g min^{-1} correspond to residence times of 5.8, 8.7, and 17.4 min, respectively. It follows that the lower the CO_2 flow rate, the longer its residence time in the extraction vessel, resulting in better mass transfer for CNSL to partition into $scCO_2$.

The combination of these results allow us to conclude that, within the tested range, the optimum conditions giving the highest apparent solubility of CNSL in $scCO_2$ are 50 °C and 300 bar with a CO_2 flow rate of 5 g min^{-1}.

Time Dependent Extraction. In these experiments, both celite and glass wool were tested as solid adsorbents in continuous $scCO_2$ extraction. The mass of extract was recorded cumulatively after every 30 min during a period of 4 h. In each case, the average cumulative mass of the extracts was plotted against time as shown in **Figure 3**. It can be observed that the two different solid adsorbents have similar effect on the recovery of anacardic acid by $scCO_2$. The mass of the extracts obtained after every 30 min interval decreased with time. In the first 30 min, about 185 mg (19% yield) of CNSL was extracted, whereas between 120 and 150 min, the amount of the extract recovered dropped to nearly 60 mg. Accordingly, 13% of the CNSL still remained in the extraction vessel after 120 min of extraction. This observation can be ascribed to the effect of diminished concentration of the solute in the solid adsorbents (celite or glass wool) with time, and the components remaining on the solid adsorbents are believed to be the more polar substances.

Characterization of the $scCO_2$-Extracted Material. The liquid extracted from both celite and glass wool within 150 min had similar visual, UV−vis, FT-IR, MS, and ^1H NMR characteristics. They also had similar chromatogram profiles when separated on a reverse phase column. The $scCO_2$-extracted

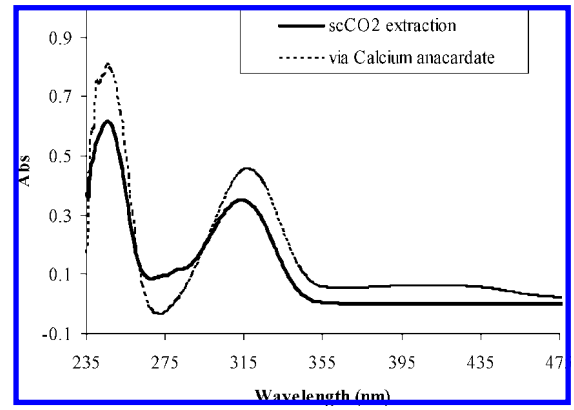

Figure 4. UV−vis spectra of anacardic acid isolated from natural CNSL through $scCO_2$ extraction and via calcium anacardate.

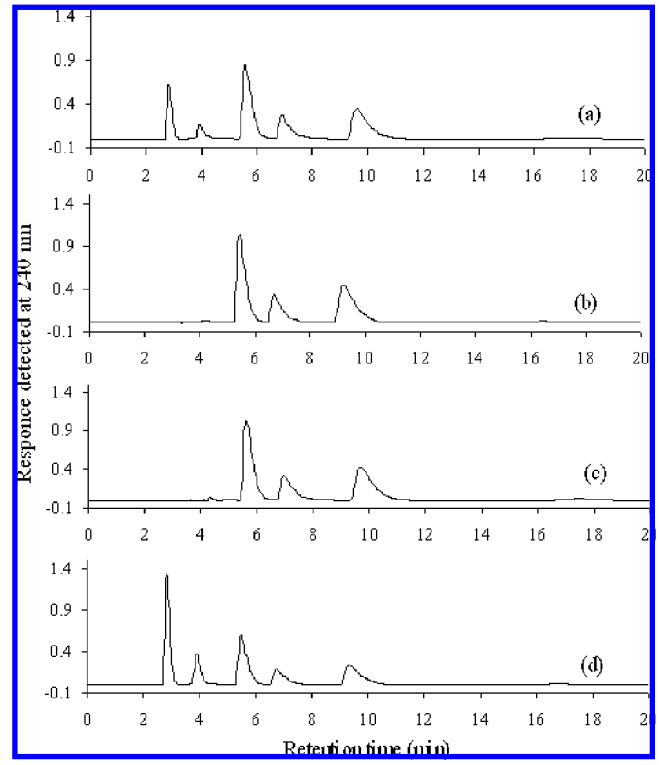

Figure 5. HPLC profile of (**a**) crude natural CNSL, (**b**) anacardic acid isolated by $scCO_2$, (**c**) anacardic acid isolated according to ref *14*, and (**d**) residue remaining on the solid adsorbents.

product has a clear orange color. This is in contrast to the dark brown color of the solvent-extracted CNSL. The UV−vis spectrum of the $scCO_2$-extracted product, shown in **Figure 4**, had two major peaks (λ_{max}) at 314 and 246 nm contributed from the 2-hydroxybenzoic acid substructure (*18*). This is somehow different from the UV−vis spectrum of solvent-extracted anacardic acid via the calcium anacardate procedure, which showed an additional weak and very broad peak ranging from 474 to 374 nm (*14*), possibly due to minor impurities remaining in the sample.

In the HPLC analysis, crude natural CNSL showed five peaks with retention times of 2.83, 3.95, 5.60, 6.91, and 9.60 min (**Figure 5a**), with a ratio of the integrated peak area of 2:1:5:2:4, respectively. The material extracted using $scCO_2$ gave three peaks observed at retention times of 5.47, 6.69, and 9.23 min (**Figure 5b**), with a ratio of the integrated peak area of 3:1:2, respectively. A similar HPLC profile (**Figure 5c**) was shown by anacardic acid isolated from crude natural CNSL via calcium

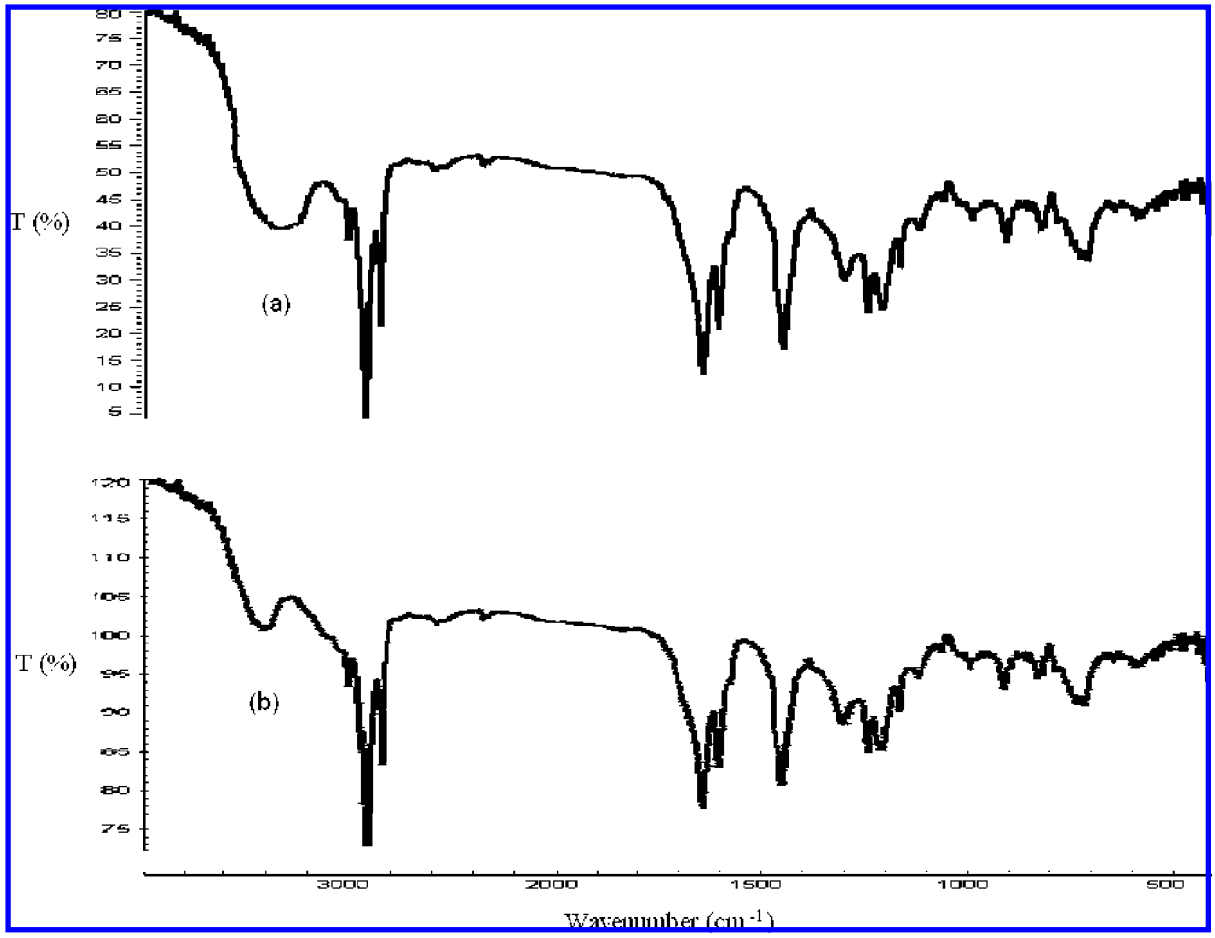

Figure 6. FT-IR spectra of anacardic acid isolated via (**a**) $scCO_2$ extraction and (**b**) the calcium anacardate procedure.

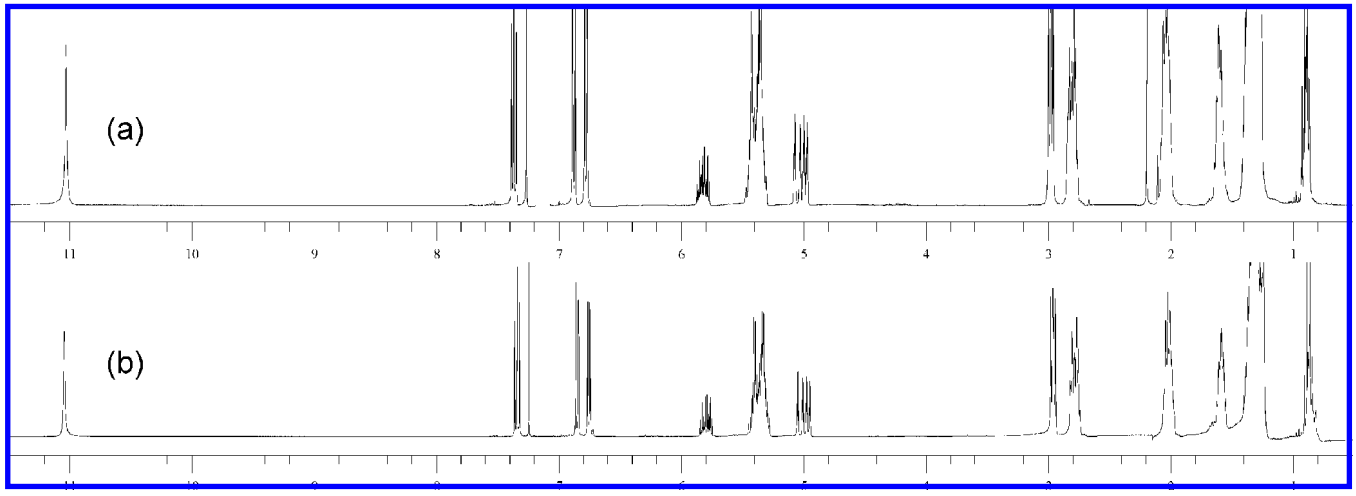

Figure 7. ^1H NMR spectra of anacardic acid isolated via (**a**) $scCO_2$ extraction and (**b**) the calcium anacardate procedure.

anacardate (*10, 14*), with a slight difference in the ratio of the integrated peak area. After $scCO_2$ extraction, the remaining CNSL in the solid adsorbents was washed off with acetone and also analyzed with HPLC. The HPLC profile of this material (**Figure 5d**) had a number of peaks (at 2.83, 3.93, 5.49, 6.75, and 9.33 min) equal to those of the crude natural CNSL, but with a different ratio of the integrated peak area (3:1:2:1:2, respectively). From LC-MS analysis, the $scCO_2$-extracted material (within 150 min) showed three major peaks with molecular ions of *m/e* = 343.284, 345.304, and 347.327, matching the three peaks observed at retention times of 5.47, 6.69, and 9.23 min in the HPLC analysis. The three HPLC peaks correspond to anacardic acid components with tri-, di-, and monoene in their alkenyl side chains with abundances of 50%, 17%, and 33%, respectively. In addition, a relatively weak peak with a molecular ion of *m/e* = 349.324 was observed, which corresponds to the anacardic acid component with a saturated side chain.

The FT-IR spectrum of the $scCO_2$ extract (**Figure 6**) showed all fingerprint peaks for anacardic acid: 3420 and 1304 (Ar-OH), 3400−2400, 1645 and 1304 (-COOH), 3009 (Ar−H and vinyl-H), 2924 and 2849 (aliphatic C−H), 1607 (aliphatic C═C), and 1446 (aromatic C═C). The ^1H NMR results ($CDCl_3$, δ (ppm)) in **Figure 7** clearly showed the substitution pattern of

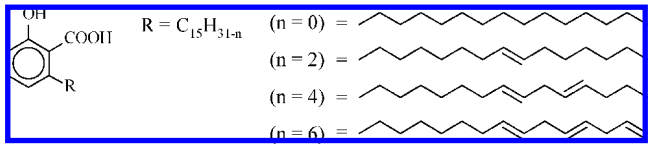

Figure 8. Chemical structure of anacardic acid.

the aromatic ring and therefore the basic structure of anacardic acid. The presence of three aromatic protons observed at δ 6.785 (d), 6.879 (d), and 7.362 (t) indicates that the extracted material contained only the trisubstituted benzene ring constituents of CNSL. The presence of the alkyl side chain (one of the substitutes) was indicated by alkenyl protons at δ 0.862 (m, 3H, -CH_3), δ 1.310, 1.586, 2.026, 2.776, and 2.983 (m, 28H, -CH_2-), δ 5.026 (m, mixed, 1H, -CH═CH-), and δ 5.354 and 5.819 (m, 4H, -CH═CH-). The phenolic and carboxyl (the other two substitutes) protons were assigned at δ 5.026 (m, mixed, 1H) and 11.026 (s, 1H). In this work, the material obtained after 150 min of extraction had ^{1}H NMR characteristics similar to those of the original crude material, indicating coextraction of all the natural CNSL components.

The combination of these spectroscopic and chromatography results, with reference to the findings reported in the literature (*14, 19*), confirmed that the $scCO_2$ extract obtained within 150 min under the present conditions was anacardic acid (**Figure 8**). An average mass of 600 mg of anacardic acid was isolated from 1000 mg of the crude natural CNSL within 150 min, which corresponds to 60% yield. However, since the amount of anacardic acid in natural CNSL is about 73%, this implies that on the laboratory scale of 3 g of sample (CNSL/solid adsorbent = 1/2), up to 82% of anacardic acid in natural CNSL was isolated within 150 min from both celite and glass wool adsorbents, when the supercritical extraction was carried out at 50 °C and 300 bar with a CO_2 flow rate of 5 g min^{-1}. The fact that anacardic acid was eluted before the other phenolic compounds may be explained by the intramolecular hydrogen bond formed between its adjacent phenol and carboxyl groups, resulting in weaker binding to the solid adsorbents compared to the other carboxyl-free phenol compounds.

In this work, we have developed an effective method to isolate anacardic acid from natural CNSL using $scCO_2$. An optimal condition of extraction was established by studying the effect of different operation parameters on the solubility of CNSL. Compared to the traditional method based on chemical conversion and organic solvent extraction, higher purity anacardic acid was obtained with $scCO_2$. The isolated anacardic acid has been thoroughly characterized using different spectroscopic and chromatography techniques, indicating that the product obtained has high purity. We expect that the present laboratory operation can be further scaled up to achieve successful isolation of anacardic acid in large quantities, especially if recycling of CO_2 is introduced into the process. We believe the successful use of nontoxic and environmentally benign $scCO_2$ to isolate high purity anacardic acid should open new opportunities for this interesting agricultural byproduct.

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