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Separation and purification of cardol, cardanol and anacardic acid from cashew (*Anacardium occidentale* L.) nut-shell liquid using a simple two-step column chromatography

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ABSTRACT

Long chain phenols contained in cashew nut shell liquid have important applications. Anacardic acids and cardols are extensively used in the pharmaceutical industry, while cardanols find use in resins, coating and polymer industries. In this work, a two-step reversed-phase silica gel column chromatography was employed to purify these major phenols in cashew nut shell liquid (CNSL). 6-Pentadecyl salicylic acid ($C_{22}H_{36}O_3$, 98.8% purity and 82.4% recovery), 5-pentadecyl resorcinol ($C_{21}H_{36}O_2$, 99.3% purity and 80.7% recovery) and 3-pentadecyl phenol ($C_{21}H_{36}O$, 99.2% purity and 81.8% recovery) were successfully obtained and identified by ^1H and ^{13}C NMR, FTIR, mass spectrometry and elemental analysis.

1. Introduction

Cashew (*Anacardium occidentale* L.) nut shell (CNS) is a by-product of cashew production. It is a source of unsaturated long-chain phenols, such as anacardic acids, anacardols, cardols and their isomers. Both anacardic acids and cardols were reported to have antitumor [1–3], antimicrobial [2], urease inhibitory [4], lipoxygenase activities [5] as well as uncoupling ability [6]. On the other hand, cardanols are widely used in coating and resin industry due to their outstanding resistance to softening action of mineral oil and high resistance to acids, alkalis, microbe, termite and insect. Anacardic acids are thermally labile and easily degraded to their corresponding cardanol by decarboxylation at high temperature [7].

Several studies have been done on the extraction of phenols from CNS. According to Tyman et al. [8], around 6.1 wt.% (based on dry weight of CNS) of cashew nut shell liquid (CNSL) was extracted by soaking CNS in light petroleum for a week and then subjecting the half-processed shell to soxhlet extraction at 40–60 °C for 12 h with light petroleum as the solvent. They also reported that the yield increased to 25.7% (based on the total weight of cashew nut with shell) when shells were macerated and re-extracted again for another 10 h. CNSL obtained consists of 19.72% cardols, 58.14% anacardic acids, 1.92% cardanols and 20.22% polymeric materials. Yuliana et al. [9] reported that CNSL extracted for 30 h by using methanol contains 28.64% cardanols, 19.06% anacardic acids, 45.27% cardols, 1.86% β-sitosterol, 3.41% triacontene and a trace amount of polymeric materials.

Due to the different uses of anacardic acids, anacardols and cardols, it is important to separate and purify these compounds in order to identify their properties for specific applications. Microanalysis of phenolic acids is an important aspect in the biochemistry of many natural products [10]. Tyman et al. [8] showed that the polarities of compounds detected in CNSL were similar. Therefore, purification of individual compound is difficult to achieve. To date, only anacardic acid isomer (15:0) and (15:3) are available in the purified form.

This study proposed a facile method to separate these 3 major phenolic compounds from CNSL using column chromatography.

Abbreviations: CNS, cashew nut shell; CNSL, cashew nut shell liquid; TLC, thin layer chromatography; HPLC, high performance liquid chromatography; RP, reversed-phase.

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This method has been known as the mainstay of modern chromatography for both analytical and preparative separations for the last four decades. Compared to high performance liquid chromatography (HPLC), column chromatography has several advantages such as low cost, simple, more flexible in application, has higher sensitivity and ability to detect the co-elution, a phenomenon in which two compounds with similar structures and polarities can exit the column at the same time. Column chromatography offers flexibility in adjusting column length, particle size of beads, column dimension (packing height to packing diameter ratio) and capping chemistry used without purchasing different columns.

Reversed-phase (RP) column chromatography is by far the most widely used technique because it is applicable to most compounds with low and moderate polarities. Majors [11] stated that octadecylsilane (C18) silica gel is the most commonly used phase in this method due to its stability over ionization that might occur in separation process. Due to the nature of target compounds (long chain phenols), separation using RP octadecylsilane silica gel column chromatography was employed in this work. Conditions such as column dimension, silica gel to sample ratio and mobile phase composition were investigated to obtain optimal separation of the three phenols.

2. Materials and methods

2.1. Materials and chemicals

CNSs were obtained from the waste of cashew (variety *Venguria-4*) nut production in a factory in Solo, Indonesia. They were grounded, sieved, and stored at −4 °C. CNSL was obtained by subjecting CNS to soxhlet extraction for 10 h. The extraction solvent was methanol, petroleum ether or diethyl ether.

All solvents used in this study were of HPLC grade. Petroleum ether was purchased from Acros Organics (New Jersey, USA) while methanol (99.5% purity), diethyl ether (99.0% purity) and acetone (99.9% purity) were obtained from Echo Chemical (Miao Li, Taiwan). Deionized water was provided from a Mixed Bed Deionizers model MB18-PVN/m9060 (Pure Aqua Inc., Santa Ana, CA). Chloroform (99.9% purity) was supplied by Sigma Aldrich (St. Louis, MO).

RP silica gel, Siliabond C18, was provided by Silicycle (Quebec, Canada), while pre-coated thin layer chromatography (TLC) sheets ALUGRAM RP-18 W/UV_{254} were obtained from Macherey-Nagel (Düren, Germany). Fast Blue B salt as reagent for TLC analysis was purchased from Sigma Aldrich (St. Louis, MO).

Both NMR sample tubes (model 1000 5 mm 400–500 MHz L7in) and the dissolving solvent, deuterated chloroform ($CDCl_3$), as well as potassium bromide used to prepare the blank pellet in the FTIR analysis were purchased from Sigma Aldrich (St. Louis, MO).

2.2. Dewaxing and degumming of CNSL

Degumming was performed by mixing hot distilled water with crude CNSL (1:1, v/v) in a 50 cm^3 beaker. The beaker was then placed in a water bath (60 °C) equipped with a magnetic stirrer (750 rpm) for 1 h. After degumming, the mixture was cooled to room temperature and centrifuged (2000 × *g*, 30 min). The degummed CNSL was decanted, dried and weighed.

Wax was separated by adding acetone (−5 °C) to the degummed CNSL (1:1, v/v). The mixture was stored at −5 °C for 24 h before subjected to centrifugation (4000 × *g*, 30 min). The wax formed was separated by decantation, dried in an oven and weighed. The dewaxed and degummed CNSL will be referred to as CNSL hereafter.

2.3. Purification of phenols by RP silica gel column chromatography

RP silica gel Siliabond C18 was activated overnight at 100 °C under vacuum. Three columns with different column height to column diameter ratio (4:1, 6:1 and 8:1) were prepared. The activated silica gel was placed in a column with two cylindrical polyethylene frits placed at both ends. The top of the column was then gently filled with methanol. Around 30 cm^3 methanol was passed through the column bed in order to clean the column before use.

Fig. 1 shows the general procedure of column chromatography of phenols. Three columns with sample to silica gel ratio of 1:20, 1:50 and 1:100 (w/w) were prepared. After placing sample at the top of the column, the column bed was filled with methanol and it was let to pass through the column by gravity. Ten fractions (0.5 cm^3 each) were collected. After completing the elution, the column was washed by chloroform. All fractions collected were then subjected to TLC analysis (Section 2.4). Fractions that contained more than one phenol were subjected to another column chromatography. This process was repeated until pure compounds were obtained. The isolated compounds were then carefully kept in a tight vial at −20 °C before further analyses.

2.4. TLC analysis

A specific reagent was used for detecting phenols in TLC analysis. This reagent was prepared by dissolving 300 mg Fast Blue B in 25 cm^3 acetone–water mixture (9:1, v/v). The desired spot will show red–violet color. Methanol was used as the mobile phase. The reagent was sprayed uniformly until the desired spot appeared.

2.5. HPLC analysis

Purity and recovery of phenols obtained from column chromatography were determined using a 5 μm Phenomenex Luna C18 column (240 mm × 4.6 mm; Phenomenex, Torrance, CA), with a flow rate of 1 cm^3/min and an injection volume of 2×10^{-2} cm^3. Peaks were detected with a Jasco UV-2077 multi-wavelength detector at 254, 278 and 300 nm. The eluting solvent (methanol) was delivered using a Jasco PU-2089 pump for 20 min. Peaks were identified by using elemental analysis, nuclear magnetic resonance (NMR) spectroscopy, FTIR and mass spectrometry.

2.6. Compound identification analyses

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker AVANCE III 500 MHz Fourier-transform NMR spectrometer using tetramethylsilane (TMS) as the reference substance and deuterated chloroform as the solvent. Distortionless enhancement by polarization transfer (DEPT) spectra was also measured to identify the structure of phenols. The IR spectra were recorded on a Bio-Rad FTS-40 FTIR Spectrophotometer with KBr pellets as the blank. An average of 32 scans per sample was made. Mass spectra were obtained by using a Finnigan LCQ Mass Spectrometer. A Perkin Elmer 240 C elemental analyzer was used for elemental analysis.

3. Results and discussion

3.1. Purification of three major phenolics

Preliminary TLC analysis of CNSL (Fig. 2) showed that the polarities of the 3 major phenols in CNSL are close to each other. This indicates that it is difficult to separate these 3 major phenolics. According to their polarity, compounds A, B, and C are tentatively identified as cardanol, cardol and anacardic acid, respectively.