Novel Method for Isolation of Major Phenolic Constituents from Cashew (*Anacardium occidentale* L.) Nut Shell Liquid

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Commercially available cashew (*Anacardium occidentale* L.) nut shell liquid (CNSL) mainly contains the phenolic constituents anacardic acid, cardol, and cardanol. These phenolic constituents are themselves heterogeneous, and each of them contains saturated, monoene, diene, and trienes in the fifteen-carbon side chain. This communication describes the separation of anacardic acid, cardol, and cardanol for industrial application. Anacardic acid was selectively isolated as calcium anacardate. The acid-free CNSL was treated with liquor ammonia and extracted with hexane/ethyl acetate (98:2) to separate the mono phenolic component, cardanol. Subsequently, ammonia solution was extracted with ethyl acetate/hexane (80:20) to obtain cardol.

Keywords: *Anacardic acid; cardol; cardanol; calcium salt; cashew nut shell liquid; Anacardium occidentale L.*

INTRODUCTION

Cashew (*Anacardium occidentale* L.) is one of the well-known species of the Anacardiaceae family. India is the largest producer and exporter of cashew kernel, accounting for almost 50% of world exports. In 1998−1999 alone 75,000 metric tons of cashew kernel was exported to different countries such as the U.S., Netherlands, U.K., Japan, U.A.E., and France. This amount is almost 100 times the amount of 1988−1989 exports (courtesy: Cashew Export Promotion Council of India, Cochin). The other important cashew nut producing countries are Brazil, Mozambique, Tanzania, and Kenya. Cashew nut shell liquid (CNSL), a byproduct obtained during the processing of cashew nuts, is used in the manufacture of industrially important products such as cement, specialty coatings, primers, etc. (*1*). The major phenolic constituents of CNSL are cardol (Figure 1, **1**−**4**), anacardic acid (Figure 1, **5**−**8**), and cardanol (Figure 1, **9**−**12**). Anacardic acid inhibits enzymes such as prostaglandin synthase (*2*), tyrosinase (*3*), and lipoxygenase (*4*). It is also known to exhibit antitumor (*5*), antimicrobial (*6*), and antiacne (*7*) properties. Cardanol finds many applications in the form of phenol−formaldehyde resins in varnishes, paints, and brake linings (*8*). Even though cardol was reported to be toxic (*9*), recent studies on rats show that there is tolerance of up to 5 g/kg body weight (*10*). Cardol is also active against the filarial parasite of cattle S*etaria digitata* (*10*). In view of its biological and industrial applications it was considered necessary to develop a simple and efficient method for the isolation of all the major phenolic constituents of CNSL.

Because of the thermolability of the carboxylic group of anacardic acid (tendency to get converted to cardanol), CNSL constituents cannot be separated by fractional distillation (*11*). General Foods Corporation (Rye, NY) (*12*) was the first to report a method to isolate anacardic acid as alkaline earth metal salt. Although this method is efficient, the purity of the product was not supported by any modern chromatographic or spectral data. Later, Nagabhushana et al (*13*) reported a column chromatographic method using triethylamine treated silica gel for medium-scale isolation of anacardic acid from CNSL. However, this process is not suitable for large-scale isolation. At nearly same time, Tsunetaro et al (*14*) isolated anacardic acid on ion-exchange resin using organic solvents (nonaqueous) as mobile phase. This method is not ideal for industrial isolation as use of nonaqueous solvent affects the life of ion-exchange resins. Moreover, all the above methods focused on the isolation of anacardic acid only and not much attention was given for the isolation of cardol and cardanol which are equally important for industrial application. We

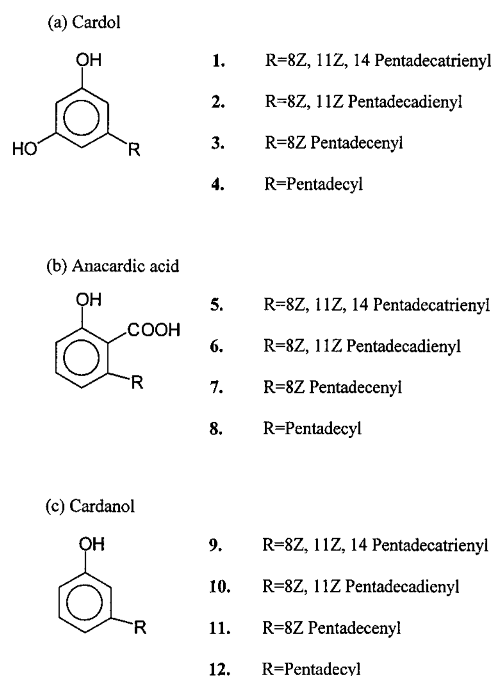

Figure 1. Structures of major phenolic constituents of CNSL.

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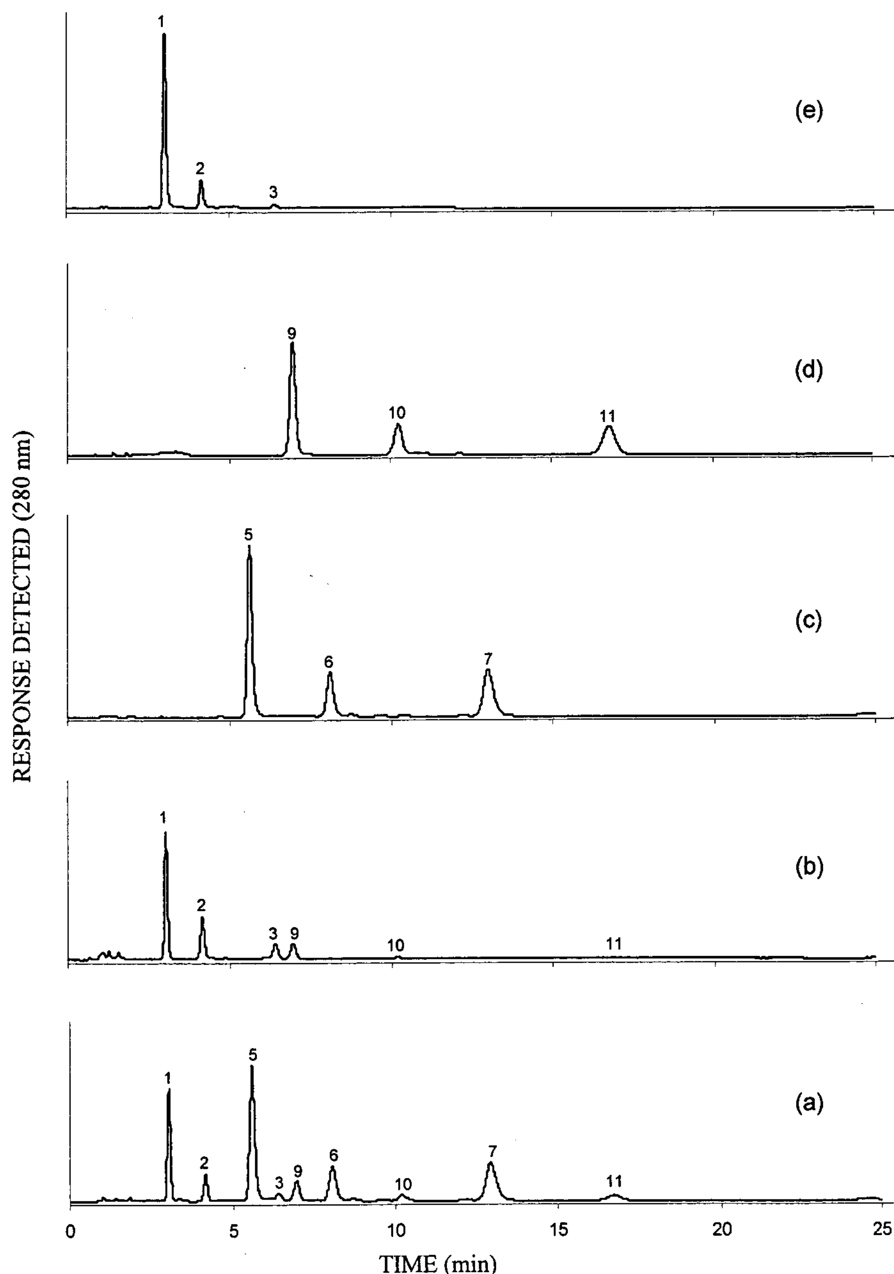

Figure 2. HPLC profiles of (a) CNSL, (b) anacardic acid free CNSL, (c) anacardic acid, (d) cardanol, (e) cardol; using Supelcosil, LC-18 and acetonitrile/water/acetic acid (80:20:1) as mobile phase at 280 nm.

report a novel method for isolation of major phenolic constituents of CNSL based on (a) the ability of anacardic acid to form a stable salt with calcium, and (b) the difference in the acidity of cardol and cardanol.

MATERIALS AND METHODS

Solvent-Extracted CNSL. Solvent-extracted cashew nut shell liquid (CNSL) containing a mixture of (saturated, monoene, diene, and triene in the fifteen-carbon side chain) 63% anacardic acid (2-hydroxy-6-pentadecyl benzoic acid), 22% cardol (5-pentadecyl resorcinol), and 10.5% cardanol (3-pentadecyl phenol) was obtained from the cashew nut processing industry, Mangalore, India.

Chemicals and Solvents. All organic solvents, chemicals, and TLC plates (silicagel GF254) were obtained from Merck (India).

High-Performance Liquid Chromatography. High- performance liquid chromatography analysis was done on a modular HPLC instrument comprising two 510 reciprocating pumps, a 481 variable-wavelength detector, and a Rheodyne injector, all from Waters Corporation (Milford, MA). A Supelcosil LC-18 (150 mm × 4.6 mm i.d., 5 μm particle size) column was used, and the mobile phase was acetonitrile/water/acetic acid (80:20:1) at 1.80 mL/min; absorbance was monitored at 280 nm. Each analysis was carried out by dissolving 25 mg of sample in 5 mL of acetonitrile, passing that through a C_{18} Sep-Pak cartridge (Waters Associates, Milford, MA), and injecting a 20-μL sample (*15*).

Isolation of Anacardic Acid from CNSL. Commercially available solvent-extracted CNSL (100 g) was dissolved in 5% aqueous methanol (600 mL), and calcium hydroxide (50 g) was added in portions under stirring. After complete addition of calcium hydroxide, the temperature of the reaction mixture was raised to 50 °C and stirring was continued for 3 h. The supernatant solution was monitored by TLC for the absence of anacardic acid. After completion of the reaction, the precipitated calcium anacardate was filtered and washed thoroughly with methanol (200 mL), and the cake was dried under vacuum at 45−50 °C for 2 h (dry weight 110 g). The

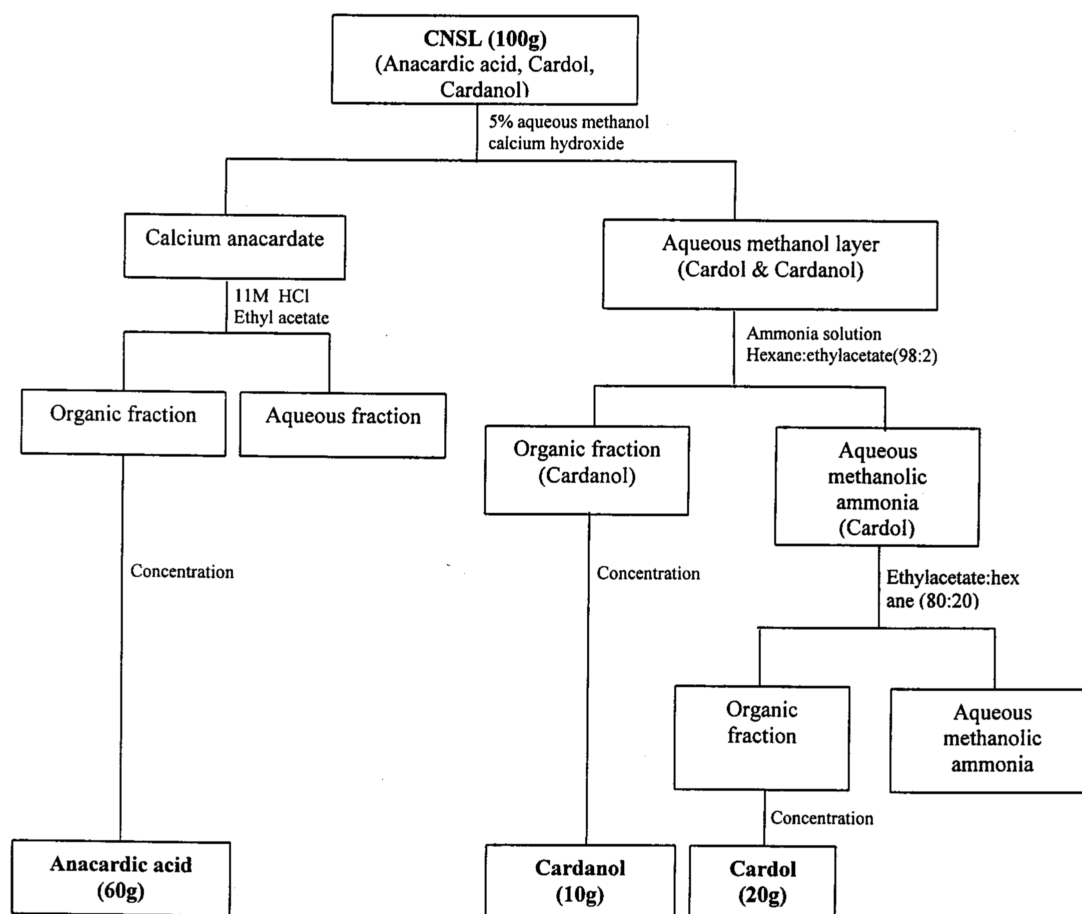

Figure 3. Flow diagram for separation of anacardic acid, cardol, and cardanol from CNSL.

filtrate was preserved for subsequent isolation of cardol and cardanol. Calcium anacardate (110 g) was suspended in distilled water (440 mL) and 11 M HCl (60 mL) was added and stirred for 1 h. The resultant solution was extracted with ethyl acetate (2 × 150 mL). The combined organic layer was washed with distilled water (2 × 100 mL), dried over anhydrous sodium sulfate, and concentrated under reduced pressure to yield 60 g of mixture of anacardic acid (monoene, diene, and triene). The identity of the compound was confirmed by HPLC (Figure 2c) and comparison with standard samples (*15*).

Separation of Cardol and Cardanol. The methanolic solution obtained after filtration of the calcium anacardate (described above) was concentrated to 200 mL under reduced pressure. Liquor ammonia (25%, 200 mL) was added and stirred for 15 min. This solution was then extracted with hexane/ethyl acetate (98:2) (3 × 100 mL). The combined organic layer was washed with NaOH solution (2.5%, 200 mL) followed by 5% HCl solution (100 mL) and distilled water (100 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated to get pure cardanol (10 g). Methanolic ammonia solution was extracted with ethyl acetate/hexane (80:20) (200 mL). The organic layer was washed with 5% HCl (100 mL) followed by distilled water (100 mL), dried over anhydrous sodium sulfate, and concentrated to yield pure cardol (20 g). The identity of cardanol and cardol was confirmed by HPLC (Figure 2d,e) and comparison with standard samples (*15*).

RESULTS AND DISCUSSION

In the initial step, during which the anacardic acid was precipitated from CNSL as calcium anacardate, the composition of aqueous methanol was found to be very crucial in obtaining anacardic acid in high purity and in good yield. When the water content in aqueous methanol was increased beyond 5% the yield and purity decreased. It was also observed that during precipitation of the calcium anacardate, excess of $Ca(OH)_2$ had to be added in order to get the product as a free flowing powder. The optimum reaction temperature for the formation of calcium anacardate was found to be 50 °C. At this temperature the salt formation was completed in 3 h and recovery of anacardic acid from CNSL was good (95%). At room temperature (27 °C) the reaction takes 24 h to complete, and at reflux temperature (70 °C), hard lumps were formed resulting in >10% loss in the yield of calcium anacardate.

The mother liquor obtained after the filtration of calcium anacardate contained primarily the other two major phenolic constituents cardol and cardanol of CNSL. It was stirred with liquor ammonia and then extracted with a mixture of hexane/ethyl acetate (98:2). Cardol remained in the ammonical solution while the cardanol was extracted into the organic layer. Subsequently, extraction of the ammonical solution with a mixture of ethyl acetate/hexane (80:20) yielded cardol in high purity. Use of the hexanes−ethyl acetate mixture instead of ethyl acetate gave proper partitioning of aqueous and organic layers. Purity of all compounds was confirmed by HPLC (Figure 2, panels c, d, e). The results reported above are averages of four experiments performed at 100 g, 200 g, 500 g, and 1 kg scale. The yield and purity are consistent in all batches.

The procedure described above and summarized in Figure 3 is superior to existing methods, as it accounts for the complete isolation of the major phenolic constituents in quantitative yield. To our knowledge this

is the first method to separate mono- and biphenolic components chemically, on the basis of their differences in acidity. This process is particularly suitable for industrial scale separations and it avoids more tedious chromatographic methods.

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Supporting Information Available: HPLC chromatograms of (a) CNSL, (b) anacardic acid free CNSL, (c) anacardic acid, (d) cardanol, and (e) cardol. HPLC chromatograms of spiked experiments (a) CNSL, (b) anacardic acid, (c) cardol, (d) cardanol, (e) anacardic acid spiked with 1% cardol, (f) anacardic acid spiked with 1% cardanol, and (g) anacardic acid spiked with 1% cardol and 1% cardanol. This material is available free of charge via the Internet at http://pubs.acs.org.

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