RESULTS AND DISCUSSION

Solvent extraction of anacardic acid directly from cashew nut shell was performed by following the procedure previously described. Results are discussed in the following sections. Details include the preparation of cashew nut shell, solvent extraction, acid-base reaction followed by the effects of organic solvent and operating temperature.

1. Sample preparation

Cashew nut shell as received was put in a cross-beater mill where the shell structure was broken down to clumps of oily and sticky tiny solid particles. This presumably would help to substantially reduce the equilibration time during solvent extraction. The obtained crushed CNS was dried in a vacuum oven at 60ºC for 12 hours and determined the moisture content by comparing the initial and final weights (eq1).

$$\text{moisture content} = \left(\frac{\text{initial weight - final weight}}{\text{final weight}} \right) \times 100\% \qquad \text{(eq1)}$$

It was found that the moisture content was approximately less than 2%, the level of which its interference on the experimentation and analysis should be negligible.

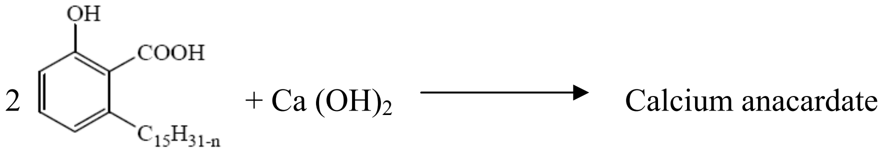
2. Solvent Extraction and Acid-Base reaction

This part elaborates the observations and chemistry involved in the solvent extraction and acid-base reaction. When 10 g of dried CNS was dissolved in a specific solvent and vigorously stirred at 30°C and 50°C for 1 hour, the color of the solution became reddish brown or dark brown or transparent depending on the kind of organic solvent used as shown in Figures 14. It is probable that chemical constituents other than polyphenols were also extracted from the CNS. Despite the kind of solvent, solid particles at the bottom of the flask appeared alike as very fine cake. The color, however, was lighter when the solution was darker.

The solution then underwent acid-base reaction upon mixing with 5% MeOH aqueous solution and a stoichiometric amount of $Ca(OH)_2$. This chemical transformation is described in eq.2. Once formed, calcium anacardate promptly separated from the bulk solution by precipitation.

N-Hexane Methanol Ethanol

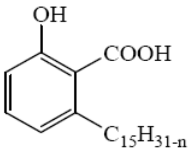
Figure 14 The solutions after completing acid-base reaction



(eq 2)

According to the separation process proposed by Paramashivappa *et al.*, (2001), MeOH was used as an emulsifier for CNSL and aqueous solution of $CaCl_2$. To verify whether the use of MeOH is necessary for the case of CNSL dissolved in organic solvent, the same experimental procedure was followed except that 5% MeOH was not added. The mixture was homogeneous and yielded the same amount of calcium anacardate. Therefore, the application of MeOH in the step of acid-base reaction is not required.

In the attempt to find an alternative to $Ca(OH)_2$, a similar acid-base reaction was tested with a common salt, sodium hydroxide (NaOH). The solution turned dark (Figure 15) as the reaction proceeded for three hours (eq 3). However, the anacardate salt did not precipitate. This result suggested that sodium hydroxide can not serve as a substitute for $Ca(OH)_2$ in the separation of anacardic acid from other constituents extracted from cashew nut shell.



$$+ \text{NaOH} \longrightarrow \text{Sodium anacardate} \qquad \text{(eq 3)}$$

Figure 15 The solution after completing acid-base reaction by using sodium hydroxide as a base

Accordingly, the obtained cake of fine particles (calcium anacardate) was filtered, thoroughly washed out with methanol, and dried in an oven at 45-50ºC for two hours in order to remove any solvent retained within the cake. The resulting dried cake appeared in light purple (Figure 16). It was weighted in order to determine the percent yield of anacardic acid from solvent extraction.

Figure 16 Dried calcium anacardate

Knowing the molecular structure of anacardic acid, it was possible to estimate the amount of anacardic acid from the weight of calcium anacardate. There are four structures of striking similarity as depicted in Figure 17. Their molecular weights differ slightly due to the number of unsaturated bonds. By mean calculation from different structure of anacardic acid as shown in Figure 17.

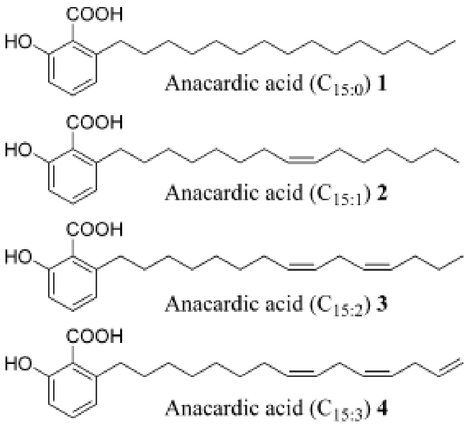


Figure 17 Structure of anacardic acid

Where number 1 is saturated form of anacardic acid (MW= 348 gram mole)

number 2 is monoene form of anacardic acid (MW= 346 gram mole)

number 3 is diene form of anacardic acid (MW= 344 gram mole)

number 4 is triene form of anacardic acid (MW= 342 gram mole)

The number of moles of calcium anacardate obtained from each experiment was linked to four resulting acid molecules assuming complete substitution of hydrogen over calcium atoms. Their corresponding molecular weights were used to estimate the amount of ene mix anacardic acids and the percent yield as described in the following equation (eq 4-5).

$$\left\{\begin{array}{c}\text{Ene mix}\\ \text{form of}\\ \text{anacardic acid}\end{array}\right\} = 0.25 \times \frac{\text{Weight of calcium anacardate}}{\text{MW of calcium anacardate}} \left\{\begin{array}{l}\text{MW of saturated form}\\ +\text{ MW of monoene form}\\ +\text{ MW of diene form}\\ +\text{ MW of triene form}\end{array}\right\} \quad \text{(eq4)}$$

$$\%\ \text{yield of anacardic acid} = \frac{\text{Ene mix form of anacardic acid}}{\text{weight of cashew nut shell}} \times 100\ \% \quad \text{(eq5)}$$

The calculated yield of anacardic acid revealed a marked improvement of 2.48 times the yield reported by Paramashivappa *et al.* (2001). The technique of extraction was slightly different regarding the starting material (CNSL versus CNS). The comparison was made on the basis that CNSL contained in the shell about 25–30% by weight in India (Das *et al.,* 2003).

3. Isolation of anacardic acid from calcium anacardate

In this section, a conversion of anacadate salt previously obtained through precipitation into its acid form is considered. Concentrated hydrochloric acid (11 M) was used to remove calcium in the form of calcium chloride ($CaCl_2$) while protons combined with the anacadate ions. However, it was a homogeneous solution containing both anacardic acid and $CaCl_2$. Liquid-liquid extraction with ethyl acetate was applied in order to separate the anacardic acid from the rest. Being soluble in water, calcium chloride stayed in the aqueous phase. On the other hand, anacardic acid moved into the organic phase appeared as brown oil floating on top of the aqueous phase as shown in Figure 18. A separating funnel was used to take apart the two phases. Rinsing with DI water was followed by the use of anhydrous sodium sulfate to get rid of traces of water dissolved in ethyl acetate. The solvent was removed through evaporation leaving a concentrated anacadic acid. Small samples were taken for analysis using high-performance liquid chromatography (HPLC) as previously described.

Figure 18 Isolation of anacardic acid from calcium anacardate

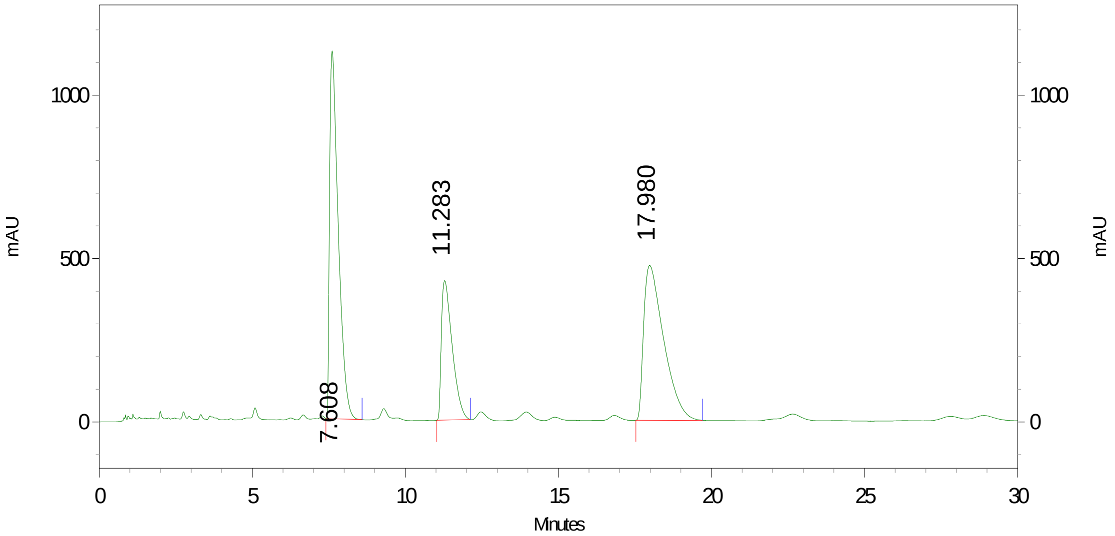


Figure 19 Chromatogram of anacardic acid produced from calcium anacardate by using ethanol as a solvent (HCl as acid)

Name	Retention Time
Anacardic acid (triene)	7.608
Anacardic acid (diene)	11.283
Anacardic acid (monoene)	17.980

Figure 19 is a chromatogram of the anacardic sample. There are three distinct peaks at 7.608, 11.283, and 17.980 corresponding to triene-, diene-, and monoene anacardic acid respectively. It is evident that other constituents of cashew nut shell was not appreciably present in the sample. By comparing this with a chromatogram of the extracted CNS (Figure 22), it is obvious that the organic solvent extracted a variety of chemical species simultaneously including many forms of anacardic acid, cardanol, and cardol. Note that chromatograms of liquid samples from extraction experiments by the use of other solvents also exhibit the same features.

Sulfuric acid was also considered as an alternative reagent to conc hydrochloric acid for the regeneration of anacardic acid. In this way, white powder of calsium sulfate (Figure 20), a by-product from this step, was simply separated by filtration. The filtrate contained ethyl acetate and anacardic acid, which was purified by evaporation of solvent afterward. Figure 21 is a chromatogram of the anacardic sample in the case of using sulfuric acid instead hydrochloric acid. There are three distinct peaks at 7.467, 11.028, and 17.617 corresponding to triene-, diene-, and monoene anacardic acid respectively.

Figure 20 Isolation of anacardic acid by using sulfuric acid

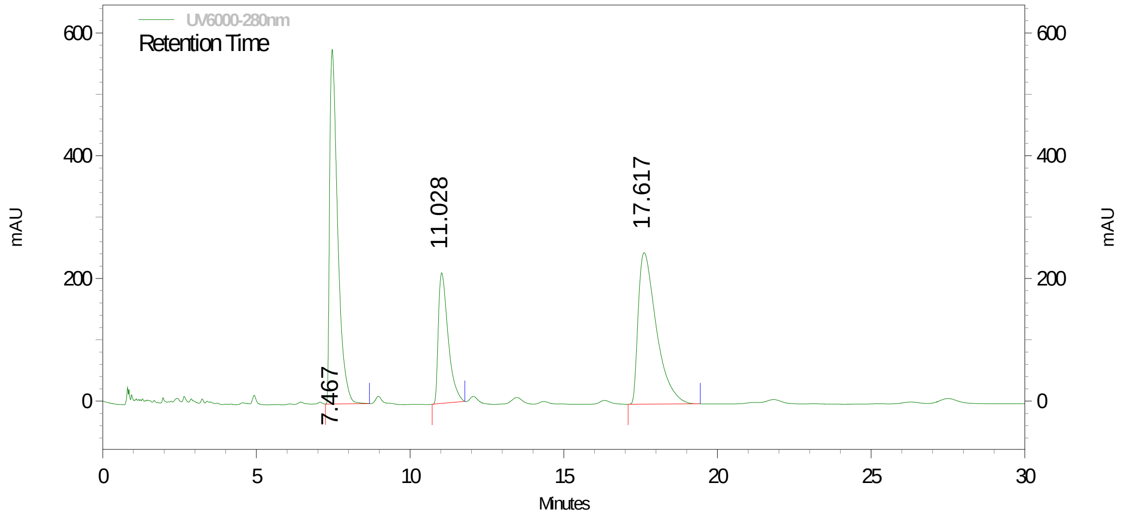


Figure 21 Chromatogram of anacardic acid produced from calcium anacardate by using ethanol as a solvent (H_2SO_4 as acid)

Name	**Retention Time**
Anacardic acid (triene)	7.467
Anacardic acid (diene)	11.028
Anacardic acid (monoene)	17.617

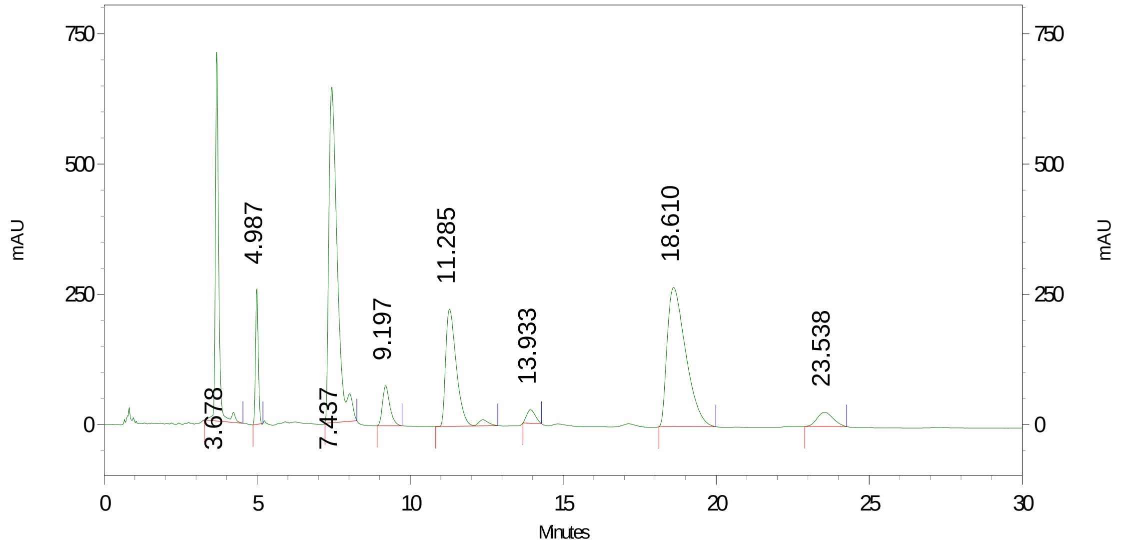


Figure 22 Chromatogram of CNSL dissolved in ethanol at 60 minutes

Name	Retention Time
Cardol (triene)	3.678
Cardol (diene)	4.987
Anacardic acid (triene)	7.437
Cardanol (triene)	9.197
Anacardic acid (diene)	11.285
Cardanol (diene)	13.933
Anacardic acid (monoene)	18.610
Cardanol (monoene)	23.538

4. Equilibration time

For each solvent extraction experiment in this study, it was necessary to establish an equilibrium state. Therefore, a series of experiment were carried out by focusing on the effect of extraction time, which was varied from 5-120 minutes. The determination was based on the weight of calcium anacardate obtained after the precipitate was filtered and dried. For all solvents used, Figure 23 shows that 5 minutes of extraction period did not completely remove anacardic acid from the cashew nut shell. Apparently, allowing longer extraction periods increased the amount of calcium anacardate until they reached plateaus at 60 minutes indicating complete extraction. Consequently, the extraction period of 60 minutes was applied for all subsequent experiments.

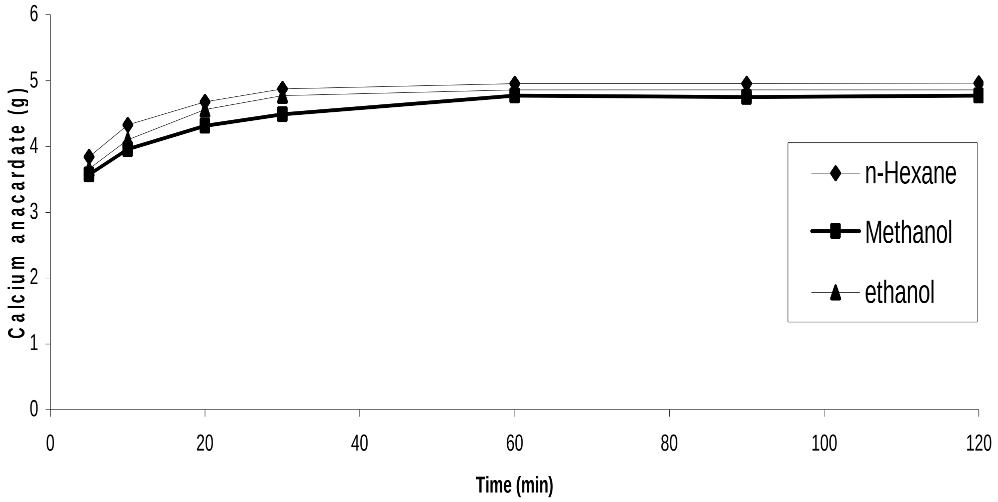


Figure 23 Equilibrium time for solvent extraction

5. Effect of solvent and solvent-to-CNS ratio

In order to determine the suitable operating conditions for solvent extraction of anacardic acid from cashew nut shell, different types of solvent, extraction temperature, and their proportion to that of the crushed cashew nut shell were varied. Results were compared in terms of anacardic acid yield as shown in Figures 24 and 25. For extraction at 30°C, at solvent-to-CNS ratio of 40:10 (ml/g), the estimated anacardic acid from the weight of calcium anacardate was lowest compared to other ratios especially for n-hexane. This indicated that some amount of anacardic acid was left in the CNS. Increasing the volume of solvent to 60 ml while the initial weight of CNS was kept at 10 g increased the yield of anacardic acid to around 41% for all types of solvent. At low ratios of solvent-to-CNS, 80:10 and 100:10 did not significantly improve the yield. Therefore, at 30°C, solvent-to-CNS ratio of 80:10 is sufficient to extract essentially all of anacardic acid from the crushed cashew nut shell. Note that additional amounts of $Ca(OH)_2$ were also applied to make certain that the solution above the cake did not contain anacardic acid.

For the selection of solvent, it is necessary to take into account not only the yield of the desired product, but also the associated health risks for handling along with the price of a particular solvent. N-hexane and methanol are considered more toxic compared with ethanol. Consequently, it is suggested that ethanol can be chosen as an effective solvent without sacrificing the extraction performance. The volume of solvent may be substantially reduced by the use of recycled solvent obtained by distillation of the final solution that is separated from the anacardic salt. Further investigation may also be followed by using 95% ethanol instead of anhydrous ethanol.

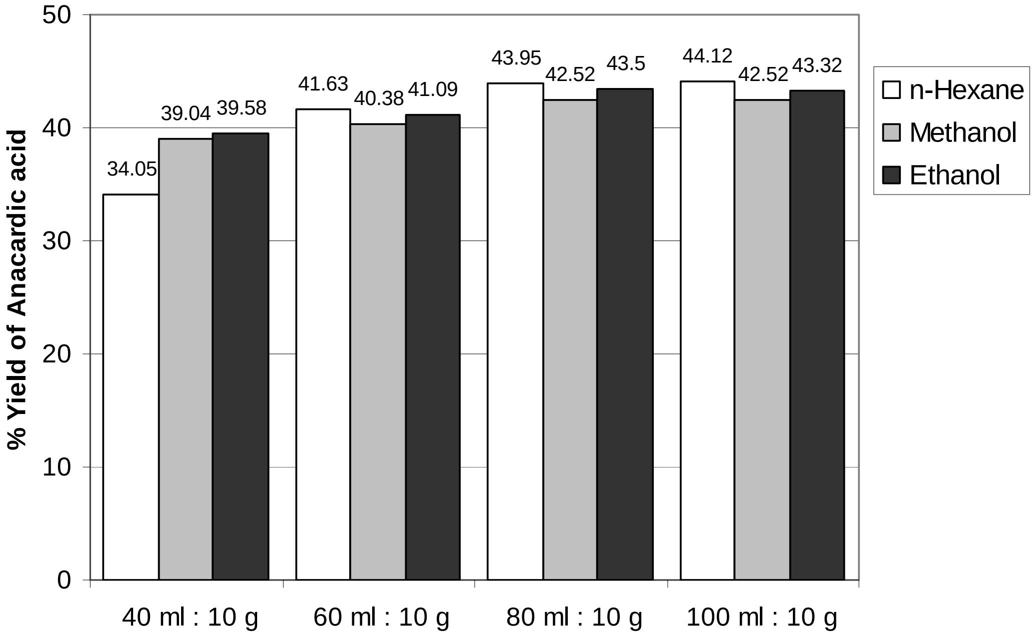


Figure 24 Effect of solvent-to-CNS ratio on extraction of anacardic acid at 30°C

6. Effect of temperature on extraction

The same procedure was followed for a series of experiments carried out at 50°C. Despite the type of solvent used, it was found that the yield of anacardic acid increased marginally as shown in Figure 25. The trend of these results is virtually the same as those of solvent extraction at 30°C. This suggests that the cost of energy required for operation at 50°C possibly outweighs the slight improvement. The extraction temperature may be raised to a much higher level in order to enhance the extraction efficiency. However, anacardic acid is decarboxylated at temperatures above 150°C.

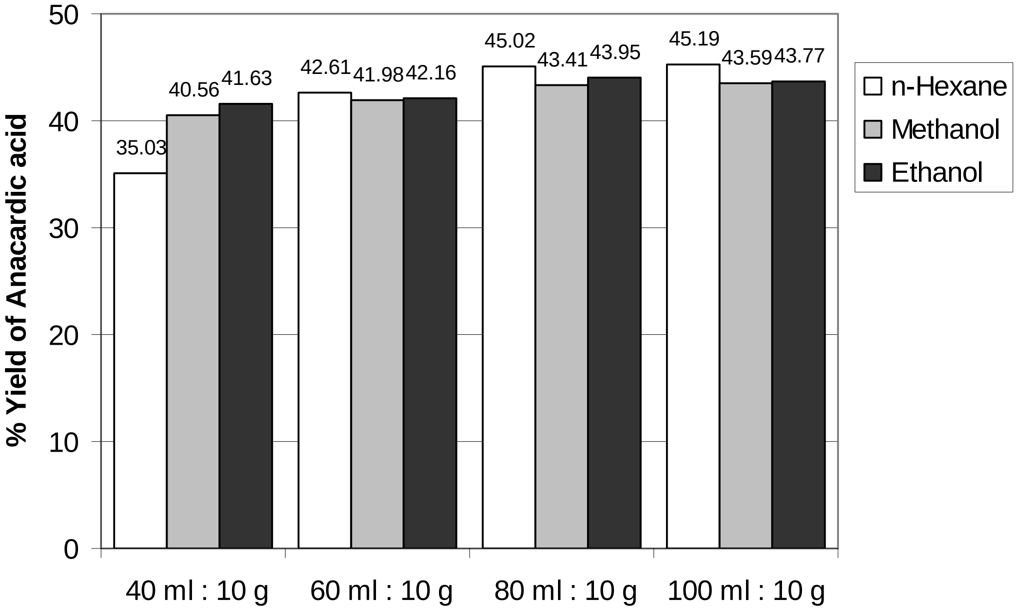


Figure 25 Effect of solvent-to-CNS ratio on extraction of anacardic acid at 50°C

7. Solvent extraction by soxhlet extractor

To compare extraction results with that from the standard procedure using soxhlet extractor, 200 ml of ethanol was used to dissolve 10 g of dried CNS placed inside the thimble. Allowing multiple refluxes for 5 hours, the final solution was taken for acid-base reaction treatment. Note that the last reflux also gave light brown solution indicating that more CNSL could be extracted. Following the same procedure as previously described, calcium anacardate was obtained. Figure 26 shows the estimated amount of anacardic acid is compared with previous result. Less amount of anacardic acid obtained from soxhlet extraction was due to poor mixing of ethanol and CNS inside the thimble

Figure 26 Effect of type on extraction performance

8. Effect of ethanol concentration on extraction performance

It is conceivable that the use of anhydrous ethanol provides the highest amount of anacardic acid compared to less concentrated ethanol. Yet, the cost of solvent is one of the key factors affecting the profitability of the process. Diluted with deionised water, 50%, 75, and 95% ethanol were prepared for solvent extraction. A series of steps including solvent extraction (80 ml: 10 g), filtration, acid-base reaction, filtration, and regeneration of anacardic acid were carried out. Figure 27 shows that the anacardic acid yield for the case of 95% ethanol was slightly lower than the case of anhydrous ethanol. To maintain the same %yield, the solvent-to-CNS ratio was increased to 100 ml: 10 g for 95% ethanol and was further increased to 120 ml: 10 g for 75% ethanol. Low ethanol concentration (50%), on the other hand, gave poor extraction performance regardless of the solvent-to-CNS ratio (up to 140 ml: 10 g). Therefore, care should be taken when using low purity ethanol as solvent.

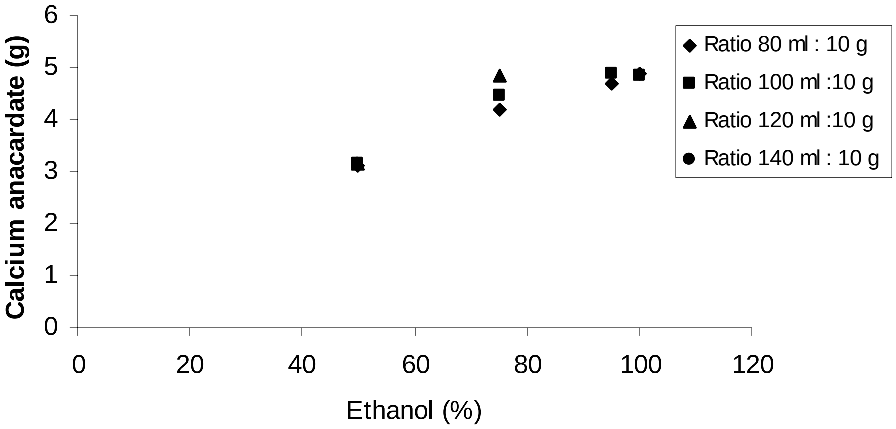
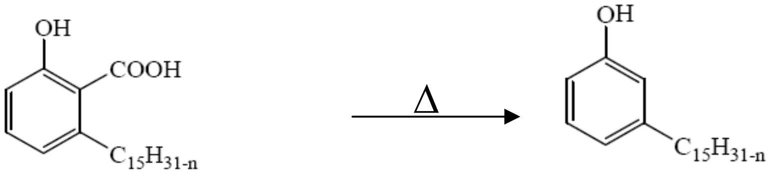


Figure 27 Effect of ethanol concentration on extraction performance

9. Decarboxylation of Anacardic acid

The decarboxylation of anacardic acid was demonstrated. First, CNSL was prepared by dissolving CNS in 80 ml of ethanol. The solution was evaporated in order to remove the solvent. The collected CNSL was then heated to 200°C for a period of 30 minutes. This high temperature caused the carboxyl group to detach from the ortho as depicted in eq 6. The sample was analyzed by HPLC. Figure 28 shows a much different chromatogram compared with Figure 22. Peaks corresponding to anacardic acid of various forms completely disappeared. Therefore, solvent recovery by distillation should be considered in the step after anacardic acid has already been isolated from the solution.



(eq 6)

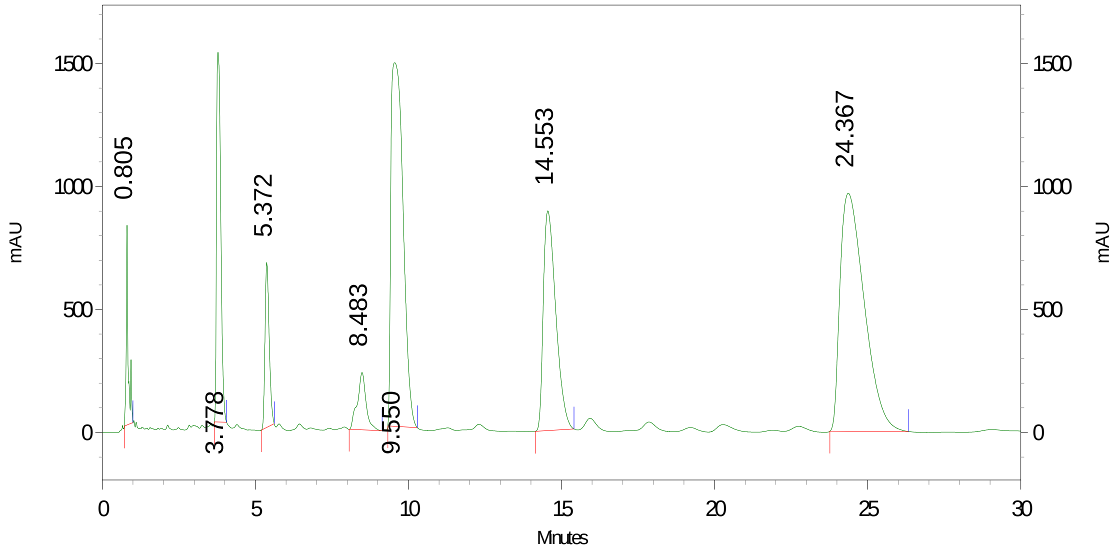


Figure 28 Chromatogram of CNSL after heated above 200ºC for 30 min.

Name	Retention Time
Cardol (triene)	3.778
Cardol (diene)	5.372
polymeric	8.483
Cardanol (triene)	9.550
Cardanol (diene)	14.553
Cardanol (monoene)	24.367

8. Process flow diagram

According to these experimental results and analysis of product, a process flow diagram for extraction of anacardic acid from cashew nut shell is proposed (Figure 29). It begins with size reduction of the raw cashew nut shell. Solvent extraction using ethanol removes CNSL from the crushed CNS followed in filtration. The filtrate then undergoes acid-base reaction producing calcium anacardate in the form of fine solid particles. After filtration, the cake is subject to liquid-liquid extraction while converting the anacardate salt back to anacardic acid. The bulk solution obtained after calcium anacadate is removed still contains significant amount of ethanol, which can be recycled via distillation.

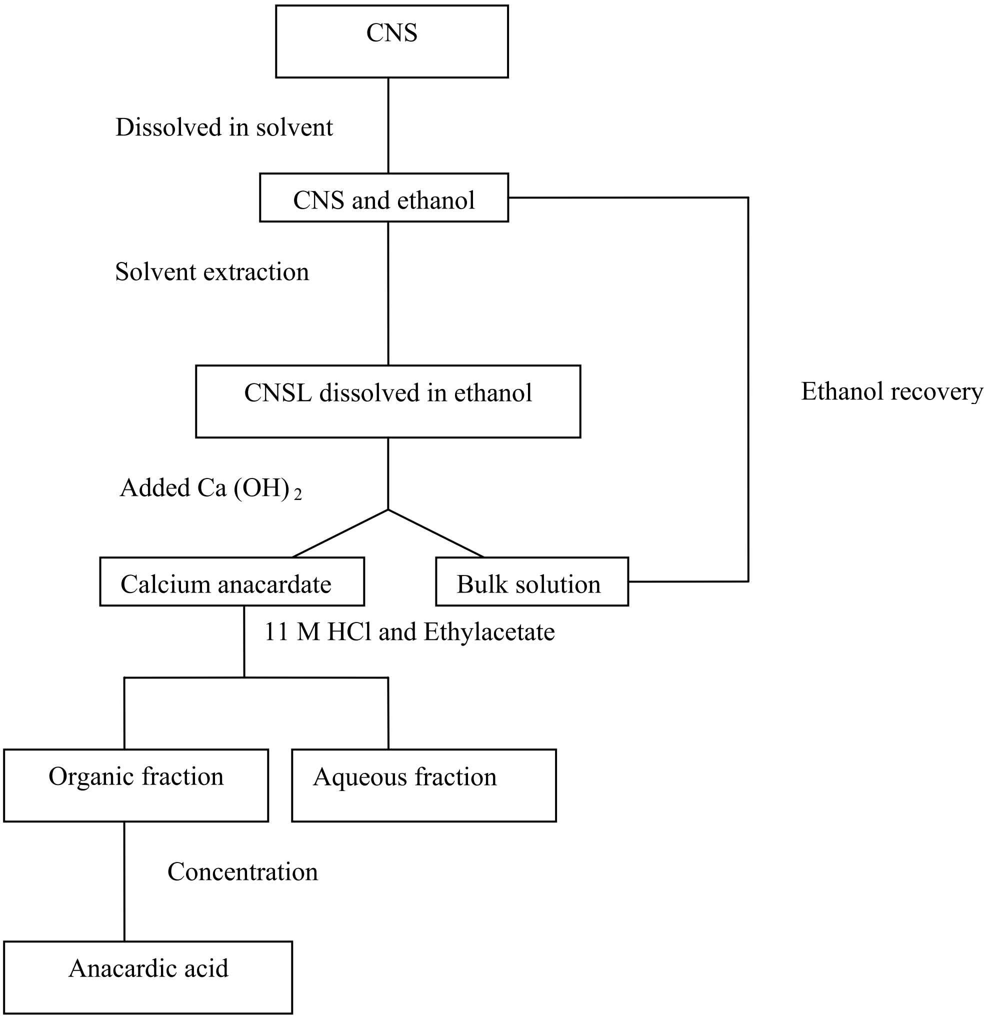


Figure 29 Process flow diagram for separation of anacardic acid