MATERIALS AND METHODS

In this section, the experimental procedure for laboratory-scale solvent extraction of anacardic acid from cashew nut shell is described. Details of all components include the type of reagent, equipments, and analytical method for identifying chemical species.

Materials

1. Erlenmeyer flask
2. Stand and clamps
3. Beaker
4. Glass funnel
5. Separatory funnel
6. Condenser
7. Thermometer
8. Hotplate stirrer (model HTS-1003)
9. High Performance Liquid Chromatography (HPLC; Thermo Sprectra System Isocratic Pump)
10. HPLC columns LC-18 Phenomenex ® (size 150x4.60mm i.d., 5 µm particle size)
11. Hot air oven (model E53)
12. Centrifuge (model Jouan B3.11)
13. Digital weigh scales (model Mettler Toledo CB203)
14. Cross Beater mill (model Retsch muhle)

Chemicals

1. Cashew nut shell
2. Deionize Water (DI)
3. Acetic acid 100% supplied by Merck Germany

4. Ethanol 100% supplied by Merck Germany
5. Acetonitrile (HPLC grade) supplied by Burdick& Jackson
6. Methanol (HPLC grade) supplied by Burdick& Jackson
7. n-Hexane (AR grade) supplied by Burdick& Jackson
8. Calcium hydroxide supplied by Merck Germany
9. Anhydrous sodium sulfate supplied by Merck Germany
10. Hydrochloric Acid 37% supplied by Merck Germany
11. Ethyl acetate supplied by Ajax chemical

Analytical Instrument

Cashew nut shell liquid (CNSL) and anacardic acid obtained from solvent extraction experiments were analyzed by High performance liquid chromatography (HPLC; Thermo Sprectra System Isocratic Pump) (Figure13)

Column : LC-18 Phenomenex ® (size 150x4.60mm i.d., 5 µm particle size)
Filter : Nylon filter 0.45 µm
Mobile phase : Acetonitrile/Water/Acetic acid (80:20:1, v/v)
Flow rate : 1.8 mL/min
Wavelength (nm) : 280nm
Injection volume : 3 µL

Figure 13 High performance liquid chromatography instrument (HPLC)

High performance liquid chromatography analysis was done on a modular HPLC instrument comprising of P4000 pumps, a UV6000 variable-wavelength detector, and a Rheodyne injector, all from Thermo Scientific. A Supelcosil LC-18 Phenomenex ® (150 mm x 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 C18 Sep-Pak cartridge (Phenomenex®), and injecting a 3-µL sample.

Methods

1. Preparation of cashew nut shell

Grinding the cashew nut shell to small particles by using a cross-beater mill was followed by drying at 60ºC for 12 hours in order to reduce moisture content prior to experimentation.

2. Solvent extraction

2.1 Weighing the cashew nut shell 10 g into the erlenmeyer flask

2.2 Putting the n-hexane solvent 40 ml

2.3 Extracting by using hotplate stirrer at 500 rpm and 30°C for 1 hour

2.4 Filtrating the solution and then spinning it by using a large capacity centrifuge at 3000 rpm for 20 minutes

2.5 Collecting the resultant solution for analyzing and using in the next step

2.6 Repeating by varying volume from 40 ml to 100 ml

2.7 Repeating steps 1-6 by increasing the extraction temperature to 50°C

2.8 Repeating steps 1-7 by changing the organic solvent from n-hexane to methanol, and ethanol respectively

3. Preparation of Calcium anacardate from CNSL

3.1 Dissolving the sample obtained from solvent extraction into 5% aqueous methanol 30 ml

3.2 Adding calcium hydroxide 2.5 g in the portions under stirring. After complete addition of calcium hydroxide, the temperature of the reaction mixture was raised to 50°C

3.3 Stirring the mixture for 3 hours

3.4 After completion of the reaction, the precipitated calcium anacardate was filtered and washed thoroughly with methanol 10 ml

3.5 Drying the calcium anacardate at 45-50°C for 2 hours

3.6 Collecting and analyzing data

4. Separation of Anacardic acid from calcium anacardate

4.1 Suspending calcium anacardate 10 g in distilled water 40 ml and 11 M HCl 5.45 ml and stirring for 1 hour

4.2 Extracting the resultant solution with ethyl acetate (2x14 ml)

4.3 Washing the organic layer with deionized water (2x10 ml)

4.4 Drying the organic layer with anhydrous sodium sulfate and concentrating under reduced pressure to obtain the mixture of anacardic acid (monoene, diene, and triene)

4.5 Collecting and analyzing the sample by using High performance liquid chromatography (HPLC)

5. Decarboxylation of Anacardic acid

5.1 Weighing the cashew nut shell 10 g into the erlenmeyer flask

5.2 Putting the ethanol solvent 80 ml

5.3 Extracting by using hotplate stirrer at 500 rpm and 30°C for 1 hour

5.4 Filtrating the solution and then spinning it by using a large capacity centrifuge at 3000 rpm for 20 minutes

5.5 Collecting the resultant solution for evaporation solvent

5.6 Evaporating the solvent until the remaining was CNSL

5.7 Heating the CNSL at 200°C for 30 minutes

5.8 Collecting and analyzing the sample by using High performance liquid chromatography (HPLC)

6. Acid–Base reaction

6.1 Dissolving the sample obtained from solvent extraction into 60 ml of 5% aqueous methanol

6.2 Adding 5 g of sodium hydroxide in the portions under stirring. After complete addition of sodium hydroxide, the temperature of the reaction mixture was raised to 50°C

6.3 Stirring the mixture for 3 hours

6.4 After completion of the reaction, the precipitated sodium anacardate was filtered and washed thoroughly with methanol 20 ml

6.5 Drying the sodium anacardate at 45-50°C for 2 hours

6.6 Collecting and analyzing data