Indian Journal of Natural Products and Resources
Vol. 3(1), March 2012, pp. 131-134

A simple and improved method for isolation of karanjin from *Pongamia pinnata* Linn. seed oil

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Received 14 February 2011; Accepted 16 November 2011

Study was undertaken with the objective to develop a simple method for isolation of karanjin from Karanja (*Pongamia pinnata* Linn.) seed oil. The seed oil was subjected to liquid–liquid extraction with methanol. The extract was further subjected to solvent partitioning followed by crystallization to get karanjin, purity of which was ascertained by HPLC. The purity of isolated karanjin was found to be 98%. Mass spectrum for the compound in ESI^+ mode showed signals at 293 $[M+H]^+$, which confirmed the molecular weight to be 292. From IR, ^1H and ^{13}C NMR spectral data, structure elucidation was done and the structure was conformed as karanjin.

Keywords: *Pongamia pinnata*, Karanjin, Karanja oil, Medicinal oil, HPLC.

IPC code; Int. cl. (2011.01)—A61K 36/00

Introduction

Pongamia pinnata (Linn.) Pierre syn. *P. glabra* Vent., belonging to family Fabaceae, is a medium sized glabrous tree, found throughout India up to an altitude of 1200 m. The plant, known as Karanj or Karanja, grows in forest from which many tonnes of seeds are collected every year in India[1].

Different parts of this plant have been used as a source of traditional medicine. *P. pinnata* seeds contain 33-36% oil which is mainly used in tanning industry for the dressing of leathers and to some extent it is used in soap industry. Oil is employed in scabies, herpes, leucoderma, sometime as stomachic and cholagogue in dyspepsia and sluggish liver[2]. Karanjin is an active principle responsible for the curative effects of the oil in skin diseases[1]. Seed extract inhibits growth of herpes simplex virus and also possesses hypoglycaemic, antioxidative, anti-ulcerogenic, anti-inflammatory and analgesic properties[3]. The seed oil contains 5-6 % flavonoids[1,4] in which the main constituent is karanjin, a furano-flavonoid (Figure 1).

During the course of exploration of new compounds from *P. pinnata* seed oil several workers[5-11] have identified some new components of its seed oil apart from karanjin. Karanjin possess pesticidal[12] and insecticidal[13] activity, promising anti-hyperglycemic, anti-ulcer activity in experimental animals[14] and also anti-inflammatory activity[15].

Considering the role of karanjin in different areas and from reported work on the extraction, isolation and purification of karanjin gives an impression that there is a need to develop simple method for the isolation of karanjin in large quantities. Present study was aimed to develop the simple procedure for the isolation of karanjin from seed oil which will assist in evaluation, formulation development and can be employed to develop the natural pesticide for food and agricultural use.

Materials and Methods

Seed oil

HPLC-grade methanol and AR grade petroleum ether (40-60°C) were sourced from Merck (India) and *P. pinnata* seed oil was procured from local market, Mumbai, India. Distilled water was filtered through 0.45 µm filter.

Extraction and isolation

In the present study, method for isolation of karanjin has been attempted (Flow Chart) with a view to minimizing the processing steps and to avoid tedious processes. Purification of karanjin by silica gel chromatography involves greater losses of karanjin resulting in poor yield[11,16]. Though the steps

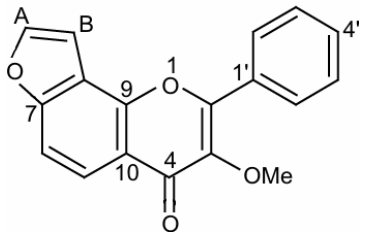

Figure 1—Chemical structure of karanjin

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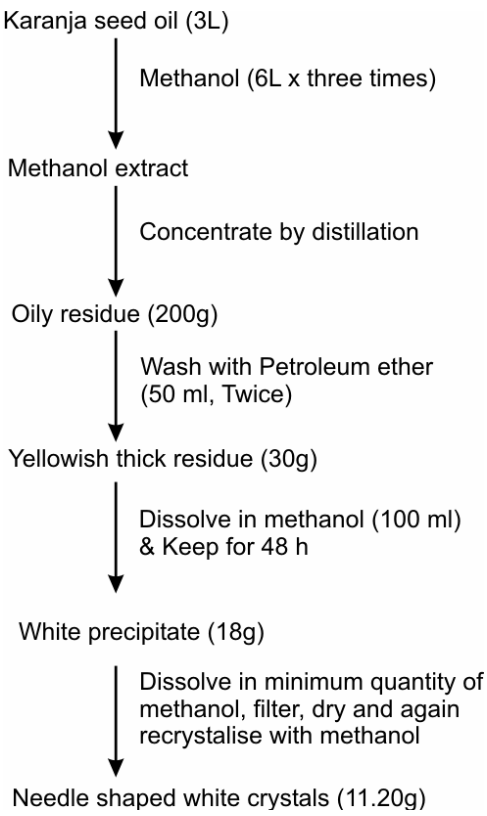

Flow chart—Isolation scheme for karanjin from *P. pinnata* seed oil

involved in isolation were simple, however the process compromises the yield[16]. The method involved the use of large number of solvents with a recovery of less than 0.5% of karanjin. In another study, losses of karanjin were minimized by using alumina for purification. Isolation of karanjin by column chromatography causes loss of karanjin[19]. All above reported studies were targeted for isolation of karanjin but in the present study focus was to develop the extraction and purification method without using any column chromatographic technique, so that good quantity of karanjin can be isolated from larger batches of *P. pinnata* seed oil.

P. pinnata seed oil (3 l) was subjected to liquid–liquid extraction with methanol in the ratio of 1:2 (*v/v*). Extraction was repeated thrice. All methanol fractions were pooled and concentrated. After keeping concentrated methanol extract for 48 h, two layers appeared. Lower oil layer was separated which was dark brown in colour. Yellowish oily precipitate (200 g) was obtained from oil layer. Precipitate was washed with 50 mL petroleum ether (Two times) to remove residual oil. Precipitate was dried (30 g) and dissolved in sufficient quantity of methanol and kept aside for some time. Product was settled after some time (18 g).

Table 1—NMR spectral data for karanjin

Atom No	^{13}C		^{1}H (*J* in Hz)	
	Isolated	Reported[17]	Isolated	Reported[17]
A	145.72	145.4	7.57 d (1.0)	7.78 d (2.0)
B	104.23	103.9	7.18 d (1.0)	7.20 d (2.0)
O-CH3	60.20	59.9	3.93 s	3.85 s
2	154.84	154.5		
3	141.81	141.5		
4	175.07	174.7		
5	121.86	121.6	8.20 d (4.00)	8.22 d (8.5)
6	109.99	109.7	7.54 d (4.00)	7.57 d (8.5)
7	158.15	157.8		
8	116.99	116.7		
9	149.93	149.6		
10	130.96	130.7		
1’	119.68	119.4		
2’	128.37	128.0	8.15 m	8.17 m
3’	128.63	128.3	7.57 m	7.60 m
4’	130.67	130.3	7.58 m	7.60 m
5’	128.63	128.3	7.56 m	7.60 m
6’	128.37	128.0	8.13 m	8.17 m

Note: δ values, ^{13}C, DMSO at 100 MHz, & ^{1}H NMR, $CDCl_3$ at 400 MHz.

Product contains amorphous and needle shaped crystals. Precipitate was dissolved by adding methanol in small fractions till whole needle shaped crystals dissolved. Amorphous powder portion was less soluble in methanol than needle shaped crystals. Solution was filtered and kept overnight which leads to precipitation of needle shaped crystals. Re-crystallization with methanol was repeated until white colored crystals appeared (11.20 g).

General experimental procedures

Melting point was determined using a Fisher Johns melting point apparatus. An IR spectrum was recorded on a Shimadzu FT-IR 8400S spectrometer Attenuated total reflector (ATR) as sample applicator. ^{1}H and ^{13}C NMR spectra were determined in the indicated solvent on a Bruker AV 300 spectrometer operating at 400 MHz (Table 1). ^{1}H and ^{13}C chemical shifts (δ, ppm) are relative to the solvent signals used as references [$CDCl_3$: δC (central line of t) 77.04; residual $CHCl_3$ in $CDCl_3$: δH 7.26]. The abbreviations s = singlet, d = doublet, t = triplet, and m = multiplet, are used throughout; coupling constants (*J*) are reported in MHz.

Positive ion mode ESIMS analysis was performed on a Thermo Scientific, LTQ-XL equipped with

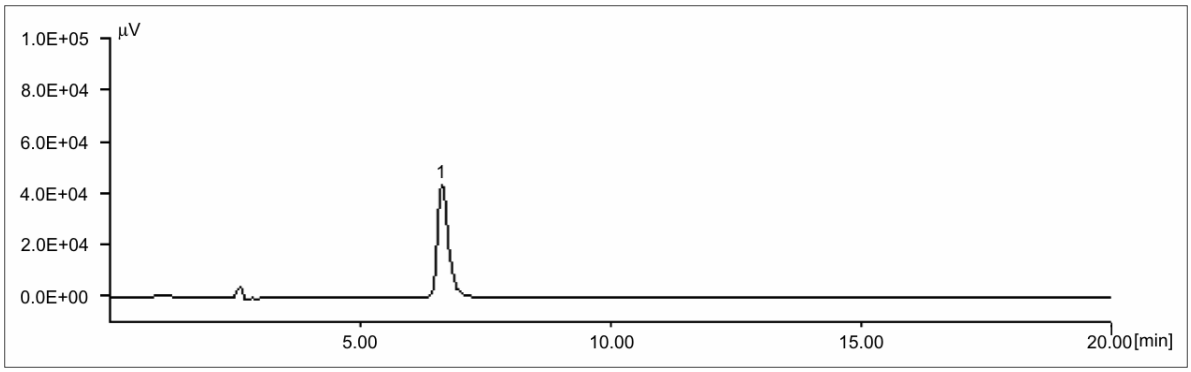

Figure 2—HPLC chromatogram of karanjin

XCalibur 1.4 software. TLC was performed on 0.20 mm silica gel 60 (E. Merck) aluminum-supported plates. Compound was visualized under UV light at 254 nm; reagent grade solvents were used for extraction; HPLC grade solvents were employed for chromatographic analysis. HPLC analysis was performed with a Jasco (Hachioji, Tokyo, Japan) system, using 250 mm × 4.6 mm i.d., RP-18 (5 μm particle size) column, an intelligent pump (PU-1580, PU-2080), a high-pressure mixer (MX-2080-31), a manual sample injection valve (Rheodyne 7725i), injection volume loop: 20 μl, monitoring by UV (UV-1575), and chromatographic data were processed with software (Borwin).

Results and Discussion

Isolated compund was analysed by using various analytical methods to confirm the structute. Product was white needle shaped crystalline solid; melting poind of 157-159° C; on TLC R_f 0.3 (Toluene: Ethyl acetate :: 7 : 3); UV/Vis λ_{max} (MeOH) nm: 260 (2.35), 304 (1.38); IR (KBr): 3132, 3053, 1621, 1451, 1406, 1369, 1284, 1222, 1162, 1034 cm^{-1}; MS (ESI, 70 eV) m/z (%) = 293.27 $[M + H^+]$(100), 293.53 (10); ^1H NMR (400 MHz, $CDCl_3$) & ^{13}C NMR (100 MHz, DMSO). NMR values are shown in Table 1 with comparison with the reported data for isolated compound. 11.20 g karanjin was isolated from 3 l of *P. pinnata* oil. Karanjin showed 98% purity by analytical HPLC which was performed with a flow rate of 1.00 mL/min, elution program: 20 min isocratic, methanol: Water: Acetic acid (85: 13.5: 1.5), monitoring by UV at 300 nm (Figure 2).

Simple isolation method for karanjin without using any chromatographic techniques provides value addition to *P. pinnata* seed oil and it would be possible to work with bulk quantity on pilot scale in industry.

Conclusion

In the present method isolation of pure karanjin has been achieved by simple extraction steps followed by re-crystallization with appropriate solvents, therefore, it was possible to isolate karanjin within short period of time. Method has shown good reproducibility of the yield and purity and this method can be used to get large quantity of karanjin, which can be employed for different applications.

Acknowledgement

Authors are thankful to Mr. Aniket Karmase (National Institute of Pharmaceutical Education and Research, Mohali) for his valuable inputs for spectroscopic study.

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