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Isolation of Karanjin from Karanja Seed Oil

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Pallavi T. Jadhav*, Snehal S. Gaware, Mohini B. Mane, Trupti A. Thange

Lecturer assistant
Department of Pharmacology and Toxicology
College of Pharmacy, University of Karbala, Iraq.

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ABSTRACT

Karanjin, a furanoflavonoid has hypoglycemia, antioxidative, anti-ulcerogenic, anti-inflammatory, analgesic, uses. Considering the role of karanjin in different areas there is a need to develop simple and economic method for the isolation of karanjin

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INTRODUCTION

Pongamia pinnata belonging to family Fabaceae is medium sized glabrous tree, found throughout India up to an altitude of 1200m. The plant known as Karanj or Karanja grows in forest from which many tones of seeds are collected every year in India.

Different parts of this plant have been used as a source of traditional medicine. *P.pinnata* seeds contain 33-36% oil, 6.6% starch, 7.3% crude fibre, 17.4% proteins, 5-6% flavonoids.

Wood: Karanja is commonly used as a fuel. Its wood is susceptible to insect attack, so wood is not considered as quality timber. But it may be used in agricultural implements, tools and combs.

Oil: A thick yellow-orange to brown oil is extracted from seed. About 24% of yield is obtained by mechanical expeller. The oil has bitter test and disagreeable aroma, so it is considered as a non edible one. This oil can be used as a fuel for cooking and lamps. Oil is also used as lubricant, pesticide and in soap making industries. The oil has medicinal value in the treatment of rheumatism and in skin diseases.

Leaves: Leaves can be used for anthelmintic, digestive, and laxative, for inflammations, piles and wounds. Their juice is used for colds, coughs, diarrhea, dyspepsia, flatulence, gonorrhea, and leprosy. The fresh leaves are eaten by cattle and by goats in arid regions.

De oiled Cake: It constitutes flavonoids, furanoflavonoids, and furan derivatives and is used in treating skin diseases and in bio pesticide. The meal cake can be used as fertilizer, pesticide and used for organic farming. Seed shells can be used as combustibles.

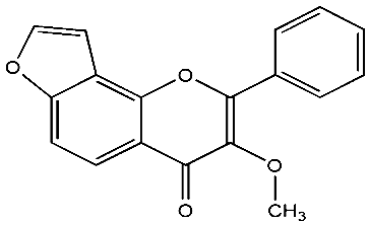
Kernel: It is used for oil extraction and the oil can be used as fuel, soap production, insecticide and medicinal use.

Fruit hull: It can be used as green manure, biogas production and combustibles. Oil cake can be used as fertilizer and combustibles.

Root and bark: (as alexipharmic, anthelmintic) used for abdominal enlargement, ascites, biliousness, diseases of the eye, skin, and vagina itch, splenomegaly, tumors, ulcers and wounds as cleaning gums, teeth and ulcers.

Seed oil is generally used in tanning industry for the dressing of leathers and to some extent in soap industry as well. Medicinally seed Oil is also employed in scabies, herpes, leucoderma. [1&2]

Structure of Karanjin



Physical Properties

M.P. – 157-159°C

Solubility – Karanjin is easily soluble in ethyl and methyl alcohol, it is also soluble in hot acetone. (10)

Crystalline nature – Karanjin is a white crystalline in nature.

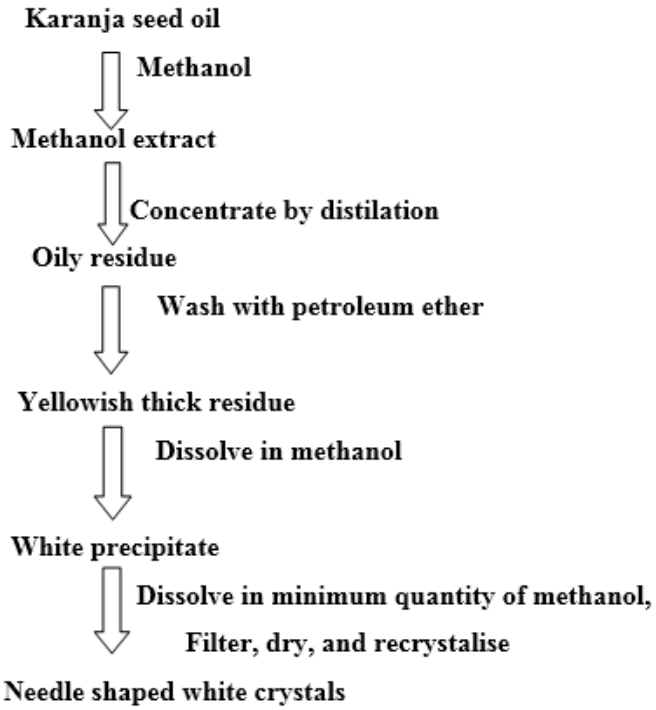
UV/V is λmax (MeOH): 260 nm, 304 nm

TLC – Rf - 0.3 Solvent system is toluene: ethyl acetate:: 7 : 3

Experimentation

In the present study, method for isolation of karanjin has been attempted (Flow chart) with a view to minimizing the processing step and to avoid tedious process. Purification of karanjin by silica gel chromatography involves greater losses of karanjin resulting in poor yield. Through the step involved in isolation were simple, however, the process compromises the yield. The method involved the use of large number of solvents with 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. 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. [4 & 5]

Liquid-Liquid Extraction



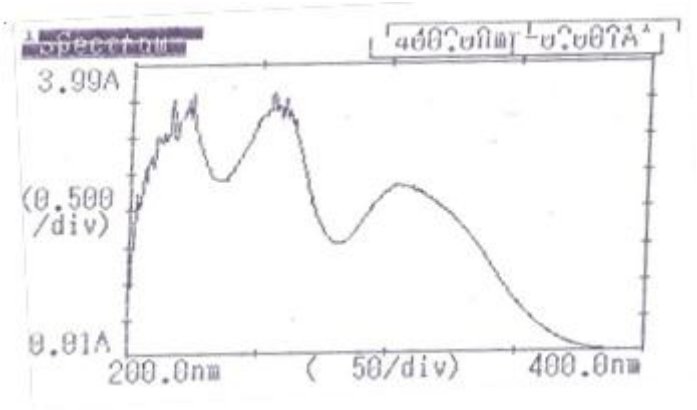
P. pinnata seed oil was subjected to liquid-liquid extraction with methanol in the ratio of 1:2(v/v). Extraction was repeated thrice. All methanol fractions were pooled and concentrated. After keeping concentrated methanol extract for 48 h. two layers appeared. Lower oil layer was separated which was dark brown in color. Yellowish oily precipitate was obtained from oil layer. Precipitate was washed with petroleum ether (Two times) to remove residual oil. Precipitate was dried and dissolved in sufficient quantity of methanol and Kept in at freezing tem. For 2-3 weeks. Product was settled after some time.

Re-crystallization process

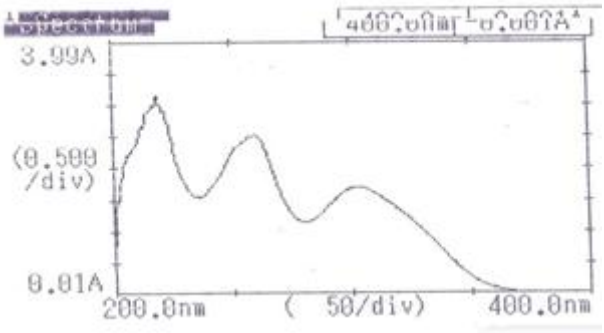
Hot acetone: - Needle shaped crystals are dissolved in hot acetone solution; it will be completely dissolved in it. Filter this solution and filtrate can be kept at a freeze temp. over a night.

Observations:

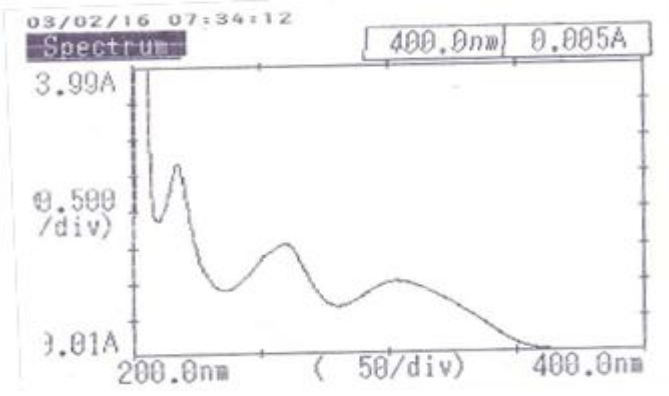
Pet ether and methanol with std. (Graph)



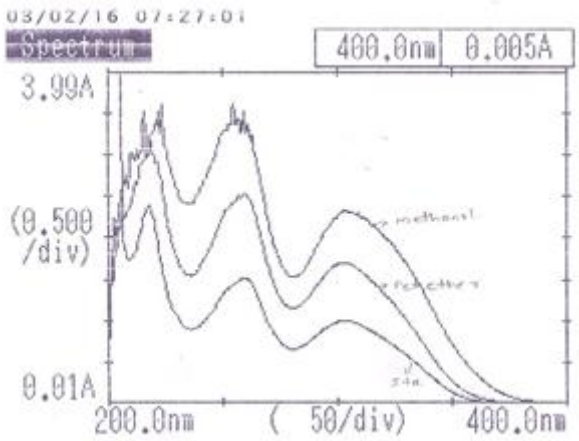
Spectra for Karanjin in Methanol



Spectra for Karanjin in Pet.Ether



Spectra for std. Karanjin in Methanol



Overlay of above 3 graphs

Interpretation:-

For the aforementioned graphs, we have concluded that the karanjin in methanol has high impurities than in Petroleum ether. Accordingly, we have chosen pet ether fraction for recovering karanjin.

Thin layer chromatography

Thin layer chromatography is similar to paper chromatography, except that a thin (0.25mm) layer of some inert material, such as Al_2O_3, MgO, or SiO_2 is used as the substrate instead of paper. A layer of any one of these oxides is made from a slurry of powder in a suitable inert solvent. The slurry is spread evenly over a flat surface (glass) and dried. It may be spread manually or mechanically. The advantage of using inert substrates instead of paper is that more reactive developing reagents, such as strong acids, can be used to detect the compounds without destroying the substrate.

Thin layer chromatography may be used with reagents such as H_2SO_4 which would react with paper and is especially useful for the separation and analysis of high molecular weight biochemical compounds. A wide variety of mixture such as amino acids, dyes, food colourings, drugs, sugars, natural products, and insecticides may be separated and identified.[3]

Preparation of TLC Plate –

The two adsorbent material most often used for TLC are alumina G and silica gel G designation stands for gypsum (calcium sulphate).

Calcium gypsum, $CaSO_4/2H_2O$, is better known as plaster of paris. When exposed to water gypsum set in a rigid $CaSO4H_2O$, which binds the adsorbent, used for TLC. About 10-13% by weight of gypsum is added as binder.

Preparing slurry of adsorbent-

The slurry is most conveniently prepared in a mortar and pestle. Foe smooth slurry without lumps, the silica gel is added to the solvent (water) and stirred. Finally stirred vigorously for through mixing.

Preparing the TLC plates-

The glass plates were cleaned with soap and water finally rinsed with 50% methanol and it was dried. Pouring method was used for coating of plates. It was activated by heating on oven for 30 min at 105.

Spotting of samples on plate -

Dipping the pulled end into the solution to be examined and empited the capillary fill the small capillary by touching it tightly to the thin layer plate at a point about 1 cm from the bottom.

Preparing a development chamber –

The glass chamber for TLC should be saturated with mobile phase. Mobile phase was poured into the chamber and capped with lid. Allowed to saturate about 30 min.

Developing TLC plates –

After the development of chamber and spotting on plate, it was kept in chamber. The solvent level in the bottom of the chamber must not be above the spot that was applied to the plate, as the spotted material will dissolve in the pool of solvent instead of undergoing chromatography. Allowed the solvent to run around 10 cm on the silica plate. Plates were removed. Plates were examined visually, under UV and after that Rf value was measured.

Isolated compound was analysed by using Thin Layer Chromatography.

The methods involves separation of components by TLC on pr-coated silica gel plates developed on Toluene: Ethyl acetate:: 7 : 3 and detection at 260 nm in absorbance mode.

RESULT AND DISCUSSION

Isolated compound was analysed by using various analytical methods to confirm the structure. Product was white needle shaped crystalline solid: melting point of 157-159˚c: on TLC Rf value is 0.3 (Toluene: Ethyl acetate::7:3). UV/V is λmax (MeOH): 260 nm, 304 nm.

CONCLUSION

In the present method isolation of pure karanjin has been achieved by simple liquid- liquid extraction followed by re-crystallization with acetone. The amount of crystalline Karinjin was found to be 2.5 gm./1 lit of karanja oil.

Method has shown good reproducibility of the yield and purity and this method can be employed for different applications.

For the aforementioned graphs, we have concluded that the karanjin in methanol has high impurities than in Petroleum ether. Accordingly, we have chosen pet ether fraction for recovering karanjin.

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