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Isolation of Karanjin from Pongamia Pinnata and Its Identification by Difference Analytical Techniques

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Abstract: Karanjin is the medicinal drug that used as anti- inflammatory and anti-cancer. The object of this study is to isolate karanjin in karanja (pongamia pinnata Linn.) seed oil. The seed oil was subjected to triple petroleum with continuously constant shaking for first 48 hours, 24 hours for each second and third extraction then separated under reduced pressure at 50˚C on rotatory evaporate to get yellow viscous oil. Separate non-fatty components using ethanol solvent and reduced under 50˚C with pressure to get (43.33 g) yellow oil. The oil was kept for 3 days at 45˚C in refrigerator white deposited at bottom of oil, the residue recrystallized with methanol it should be done fast and carefully to prevent solubility of karanjin in methanol partially, the purity of isolated karanjin was found to be (99.623%). From TLC, HPL, IR, 1HNMR, 13CNMR spectra data, structure elucidation was done and the structure was confirmed as karanjin.

Keywords: Karanjin, pongamia pinnata, Karanja oil, medicinal plant**.**

1. INTRODUCTION

Pongamia pinnata Pierre belonging to the family Fabaceae (Papilionaceae). It is also called *Derris indica* and *Pongamia glabra* [1]. *Pongamia piñata* is a medium in sized and evergreen tree and a short bole. The tree is planted for shade and is grown as ornamental tree [2].

Classification of *Pongamia pinnata*,
Botanical Classification
Kingdom: Plantae,
Division: Magnoliophyta,
Class: Magnoliopsida,
Order: Fabales,
Family: Fabaceae,
Genus: Pongamia,
Species: *P. pinnata*,
Common name is Karanja [2].

Botanical Name
Pongamia pinnata (L.) Pierre

Synonyms
Derris indica (Lam.) Bennett
Millettia novo-guineensis Kane. and Hat.
Pongamia glabra Vent.
Pongamia pinnata Merr.

- **Native**

Bangladesh, India, Myanmar, Nepal, Thailand.

- **Exotic**

Australia, China, Egypt, Fiji, Indonesia, Japan, Malaysia, Mauritius, New Zeeland, Pakistan, Philippines, Seychelles, Solomon Islands, Sri Lanka, Sudan, United States of America [3] .

- **Botanical description of *P. pinnata***

Karanja is a drought tolerant, semi-deciduous and leguminous tree. A spreading crown up to 18m height or sometimes even more and (1.5m) in girth. Pod are compressed, woody , indehiscent , yellowish-gray when ripe , varying in size and shape (4.0-7.5cm) long and (1.7-3.2cm) broad, bark grayish green or brown, smooth or covered with tubercles, leaves compound, imparipinnate, leaflets opposite, 5-9 in number, ovate or elliptic; flowers white tinged with pink or violet, fragrant, in axillary racemes, , seeds usually one, rarely two, elliptical or reniform (1.7-2.0cm) long and (1.2-1.8cm) broad, wrinkled with reddish brown leathery testa. Brawn in color and poisonous in fact which should be considered in placing the tree in the landscape if many children present [4-5]. The potential of this oil as a substitute for diesel [6] and as lubricating in tuning industries is recognized [7] .

- **Distribution:**

Karanja is believed to be originated in India and Distributed throughout India in tidal and beach forest, often as a mangrove plant [8]. It is grow in the hills of south India up to elevation of about 1200 meters (4000 feet.) and in the Himalayas up to about 610 meters (2000 feet). It is widely grown from tropical dry to sub-tropical dry forest life zones. It is a shade bearer and is considered to be a good tree for planting in pastures, as grass grows well in its shade.

Figure 1A: *Pongamia pinnata* tree.

Figure1B: *Pongamia pinnata* fruit.

Figure 1C: *Pongamia pinnata* seeds.

Figure 1D: *Pongamia pinnata* flower.

The tree is suitable for afforestation especially in watershed areas and drier part of the country. *P. pinnata* is reported to be a native of India, Myanmar, Malaysia and Indonesia [6] that is distributed in tropical Asia, Australia, Polynesia, Philippine Islands [5] Unite state New Zealand, China [6,9].

- **Collection of seeds and processing**

The seed collection is prolonged due to non-synchronization of flowering & fruiting. Therefore, one time harvesting is usually not possible. The collected pods are dried for 2-3 days in the sun. The kernels are separated from the hell manually by a wooden hammer or manually operated decorticator. However, the electric decorticators of Karanja fruits have also been fabricated and being used for efficient processing. The average seed yields of Karanja is about 40-90 qtl/ha. Properties of Karanja seeds Karanja kernels are reddish brittle in colour. The air-dried kernels contain 19% moisture, 27-39% oil, 17.4 % protein 6.6% starch, 7.3% crude fibers and 2.3% ash. The kernels also contain mucilage (13.5%) and traces of essential oil. In addition, a complex amino acid glabrin is also present.

- **Physical-chemical properties and fatty acid composition of Karanja oil.**

It is known as pongam oil in trade. Fresh extracted oil is yellowish orange to brown, getting darkened during storage, having disagreeable odour and bitter quality of oil, the important properties of oil and fatty acids are given below:

Properties of oil

Colour	Dark brown
Odour	Repulsive
Refractive index at 40°C	1.434-1.4790
Specific quantity at 30°C	0.925-0.940
Iodine value	80-96
Saponification value	185-195
Non-saponification value	2.6 – 3.0

Composition of important fatty acids found in Karanja oil

Palmitic	3.7-7.9%
Stearic	2.4-8.9%
Oleic	44.5-71.3%
Linoleic	10.8-18.3%
Lignoceric	1.1-3.5%
Eicosenoic	9.5-12.4%
Arachidic	2.2-4.7%
Bethenic	4.2-5.3%

- **Use of *Pongamia pinnata***

The plant known to have a very high medicinal value [11]. The bark skin, leaves, flowers seeds and seed oil are used for medicinal purpose, karanja is used both into internally as well as externally. Bark is known to be the remedy for beriberi, Leaves are actives against microccus and their juce is used for cold cough, diarrhea [3] antibacterial, anti-giardia and anti-virial [12] antihyperammonemic. Its flowers are used for diabetes [3,13] skin disease, renal disorders [13. Externally roots are good for cleaning foul ulcers, cleaning teeth, anti-inflammatory [14], gastroprotective anti-stress activity and anti-oxidant [15]. The fruits and sprouts are use in folk remedies for abdominal remedies in India [3]. Seeds

are anthelmintic, bitter, acrid and carminative [14], inflammation, rheumatism [16], skin disease [11] , pectoral diseases , chronic fever and anemia [17] , keloid tumors in sri lanka and powder derived from plants for tumors in Vietnam, skin ailments and the oil of seeds used as an ointment for rheumatism [3] , leucodermal, scabies, herpes [16] against herpes simplex virus type-Í (HSV-1) and type 2 (HSV-2) was evaluated *in vivo* cell and antibiotics [18] leucocoderma, leprosy, lumbago [13], ulcer protective and healing effects and anti-inflammatory activity of alcoholic extract of seeds and root [13,14] high toxic to fish , alcoholic extract of oil showed activity against both Gram positive and Gram negative bacteria [16] , extract of the opulent possess significant anti- diarrhea , anti–fungal , anti–plasmodial, anti ulerogenic, anti-inflammatory and analgesic activities [5] .

- **Properties and Activity**

The flower furnishes an aliphatic waxy matter kaempferol, pongamin ($C_{15}H_{12}O_5$), γ-sitosterol glucoside, quercertin, neoglabrin (A complex amino acids) resembling glabrin and galbrosaponin ($C_{50}H_{84}O_{23}$) [19]. A furanoflavone i.e., pongone has been isolated from flowers [19] .P. pinnata contains flavonoids and other compounds including flavones, furanoflavonoids, chromenoflavone, chromenocalchones, coumarins, flavone glycosides sterol, terpenes and modified phenylalanine dipeptide [20] . The seeds contain 13.5% mucilage, traces of essential oil and complex amino acids, termed glabrin. Four furanoflavones karanjin, pongapin ($C_{19}H_{12}O_6$), kanjone ($C_{18}H_{12}O_4$) and pongaglabrone ($C_{18}H_{10}O_5$), identified as 3',4'-methylenedioxy furano [2',3',7,8] flavone, have been isolated from Indian Karanja seed [21]. Three furanoflavonoids (Pongamosides A, B and C) and a flavonol, glucoside Pongamoside D, have been reported from the n-butanol-soluble fraction of the ethanolic extract P. pinnata fruit [22]. Pongaglabol, a hydroxyfuranoflavone, and aurantiamide acetate, a rarely occurring modified phenylalanine dipeptide, have been isolated together with four furanoflavones (karanjin, lancheolatin B, kanjone and pinnatin) [23]. Two hydroxychalcones – onganones I and II – have been isolated from bark and characterized. Moreover, two phenylpropanoids – Pongapinone A and B – have been isolated from bark of Indonesian karanja plants [24] . Five flavonoids (Pongamone A, B, C, D and E) have been isolated from P. pinnata [25]. Seed is considered useful in the treatment of scabies, leprosy, piles, ulcers, bronchitis and whooping cough [26] . Seeds are mainly valued for their oil, in cosmetic industry and Ayurvedic herbal medicine [27], antihyperglycemic and antilipidperoxidative [29] antiulcer [30], analgesic [28], antimycobacterial [10] and antifilarial activity [17].

2. METHODS AND MATERIALS

- **Isolation procedure**

Powder of dried kernels (500 g) was suspended in Petroleum ether (1L). The suspension was, then, filtered. Through glutted filter paper and repeated the same procedure twice. Then all the extract was combined and was concentrated under reduced pressure at 50º on rotatory evaporator to a get yellow viscous oil. The non-fatty components were extracted from yellow viscous oil with ethanol. The ethanol extract was combined and it was concentrate under reduced pressure at 50º to get a 43.33 g of yellow oil. The oil was kept for 3 days at 4-5º in refrigerator. While residue was deposited at the bottom of oil. The oil was decanted and the obtained residue was recrystallized with methanol. TLC was taken but the T.L.C. gave 2 spots on with very small Rf value so the sample on column was loaded approximately 500 mg sample. The column was made up of silica gel having mesh size of 60-120. But still sample did not get separated. The preparative plate chromatography was used. Due to excess loading of sample it got failed. So the sample was recovered whole from preparative plate and then again recrystallization was done. Then TLC was taken. TLC gave a single spot. From this conclusion was drawn that the compound gets rearranged when it exposed to sunlight for (1 or 2) days to give another compound as an impurity.

- **TLC**

Test solution

Extract 2 g of powdered drug with 15 ml of ethanol in a Soxhlet apparatus for 18 h. Remove the solvent under reduced pressure at 50º yielding the 0.160 g crude residue. Dissolve 5 mg of the residue in 5 ml of methanol and use the solution for TLC profiling.

Standard solution

Dissolve 2 mg of karanjin in 5 ml of methanol.

Solvent system

Hexane: Ethyl acetate (80:20)

- **Procedure for TLC:**

Apply 20 µl of test solution and 5 µl of standard solution separately on a precoated silica gel 60 F 254 TLC plate (E. Merck) of uniform thickness of 0.2 mm. Develop the plate in the solvent system till the solvent rises to a distance of 8 cm.

- **HPLC Assay**

HPLC chemical and physical discretion

Chromatographic conditions:
Mobile phase: Acetonitrile (100%)
Flow rate: 0.5 ml/min
Column: Zorbax Eclipse, XDB, c8, 4.6 mm x 150 mm, reverse phase
Detector: UA Detector at 254 nm

Test Solution

Extract 2 g of powdered drug with 15 ml of ethanol in a Soxhlet apparatus for 18 h. Remove the solvent under reduced pressure 50º yielding the 160g crude residue. Dissolve 10 mg of residue in 10 ml of methanol. Filter through 0.45 µ membrane and use the solution for chromatography.

Standard Solution

Dissolve 3 mg of Karanjin in 10 ml of methanol. From this stock solution prepare a standard solutions of 0.018, 0.037, 0.037, 0.075, 0.15 and 0.3 mg/ml by transferring

aliquots (0.62, 1.25, 2.5 and 5 ml) of stock solution to 10 ml volumetric flasks and adjusting the volume of each solution to 10 ml with methanol.

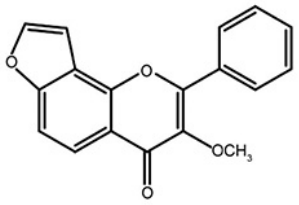
Calibration Curve
Run the HPLC of each of the standard solutions and record the respective peak area. Prepare a calibration curve by polluting peak area vs concentration of Karanjin.

- **Procedure for HPLC**

Subject 5 µ of standard and sample solutions to high performance liquid chromatographic system and record the respective peak area for the test solution and the standard solutions.

3. Results and Discussion

Different analytical techniques were used to confirm isolated structure of karanjin. White needles, melting point 163-164 º, Literature melting point1 161º, Specific Rotation: Not applicable
Solubility Soluble in benzene, ether, chloroform, acetone, alcohol. Practically insoluble in petroleum ether. Elemental analysis C 74.13%, H 4.03%, O 21.84%, expected elemental analysis: C 73.97%, H 4.14%, O 21.90 %



Karanjin Structure

- **Percentage of the marker compound**

Calculate the amount of Karanjin present in the sample from the calibration curve. The percentage of karanjin ranges from (0.06% to 0.08%) in the sample analyzed and the purity of isolated karanjin was found to be (99.623%) 400 mg; 0.08% w/w of kernels of *Pongamia pinnata*.

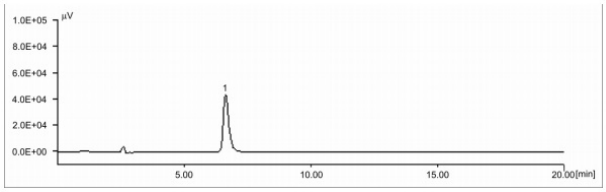


Figure 2: HPLC Chromatogram of Karanjin.

- **Characterization of the marker compound UV–VIS Spectrum**

Table 1: UV-VIS spectrum of Karanja.

UV-VIS spectrum indicates absorptions at 308 nm (ε = 9.7 x 10), 260 nm (ε = 1.61 x 10) and 208 (ε = 2.21 x 10)

Descriptions: The UV spectrum indicates the presence of strong chromophore. The three peaks obtained at 308, 260 and 208 nm suggest the presence of three chromophoric systems in the structure. This is in complete agreement with the structure

IR Spectrum:
Table 2: IR spectra of Karanjin.

2930 (C-H stretching), 2851, 1635, 1625, 1605, 1570, 1525, 1409, 1340 (C-O), 1285, 1225, 1079, 1051, 956, 795 cm^{-1}

^{1}H NMR Spectrum

Table 3: ^{1}H NMR spectra of Karanjin

δ 3.9 (3 H, s, OCH^{3}, H-11),
7.1 (1H, m, H-4"),
7.14 (1H, d, J=2 Hz, H-3'),
7.45 (1H, m, H=5"),
7.49 (1H, m H=3"),
7.50 (1H, m,H=6"),
7.70 (1H, m, H=2"),
7.75 (1 H, d, J=2Hz, H=2'),
8.10 (1Hd, J=8.5 Hz, H=6),
8.20 (1H, d, J=8.5 Hz, H=5),

^{13}C NMR Spectrum

Table 4: ^{13}C NMR spectra of Karanjin.

δ 60.9, 104.09, 109.82, 116.82, 119.47, 121.64, 128.18, 128.48, 130.52, 130.73, 141.58, 145.57, 149.64, 154.55, 157.86, 174.48

Table 5: spectra matching to each carbon in Karanjin.

Sr. No.	Dept	δ (ppm)	Assignment
1	CH3	60.9	C11
2	CH	104.09	C3'
3	CH	109.82	C2'
4	Quaternary	116.82	C8
5	Quaternary	119.47	C1"
6	CH	121.64	C4"
7	CH	128.18	C3"
8	CH	128.48	C5"
9	CH	130.52	C6"
10	CH	130.73	C2"
11	Quaternary	141.58	C10
12	CH	145.57	C5
13	Quaternary	149.64	C9
14	Quaternary	154.55	C2
15	Quaternary	157.86	C3
16	Quaternary	174.48	C4
17	CH	146.82	C6
18	CH	128.2	C7

- **Visualization of UV**

Observe the plate under UV light at 366 nm. Note the Rf and colour of the resolved bands.

- **Evaluation TLC**

A band (Rf 0.58) corresponding to karanjin is visible in both test and standard solutions as a fluroscent blue band when observed under UV light at 366 nm.

Table 6: TLC Details of Test Solution of *Pongamia pinnata L.* seeds.

Rf Value	Colour of the band
0.11	Blue
0.23	White
0.36	Fluorescent White
0.48	Fluorescent White
0.58	Fluorescent White
Karanjin	
0.77	Fluorescent White
0.92	Blue

- **Mass spectrum**

Mass spectrum could not be recorded as Karanjin does not elute under Gas-Chromatographic conditions used for mass spectrometry.

4. CONCLUSION

A simple viable method has been standardized for isolation of karanjin from *P. pinnata* seed oil where in higher yield of karanjin purity 99.623% was achieved and the product was stored in a sample vial and it was sealed with Para foil as well as aluminum foil to avoid photoreaction with sunlight, also, it would help pharmaceutical industry, for this molecule in pure form would be more useful in understanding of mechanism of its action instead of crude extract of the seed.

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