



Spectroscopic Analysis of Flavonoids Isolated from *Pongamia pinnata* L. Seed Oil

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Authors' contributions

Both the authors MAT and MSA A designed the project and managed the literature searches. Author MAT performed the experiment and characterization work. Author MSA A wrote the first draft of manuscript. Both authors read and approved this final manuscript.

Article Information

DOI: 10.9734/AJOCS/2020/v7i419027

Editor(s):

(1) Dr. V. Sivamurugan, Pachaiyappa's College, University of Madras, India.

Reviewers:

(1) Alexandre Ricardo Pereira Schuler, Universidade Federal de Pernambuco, Brasil.

(2) Amooru Gangaiah Damu, Yogi Vemana University, India.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/56394>

Short Research Article

Received 22 February 2020

Accepted 27 April 2020

Published 04 May 2020

ABSTRACT

Current work comprises of chemical investigation of flavonoids present in *Pongamia pinnata* (L) seed oil. Purification of flavonoids was established by using UV, FTIR, MS and NMR spectroscopic analysis. Three isolated flavonoids were identified as, namely, Kanjone, Pongapin and Lanceolatin-B. This work led to the development of easy isolation methods with improved yields and the future prospects of commercial scale processing of these flavonoids directly from plant source.

Keywords: Flavonoids; spectroscopic analysis; Pongamia pinnata L; seed oil.

1. INTRODUCTION

Flavonoids are a large group of structurally related compounds ubiquitously distributed in plant kingdom. Flavonoids have gained much attention and become a topic of interest for

researchers due to their tremendous biological importance and broader range of pharmacological activities. Flavonoids are successfully used as antioxidant, anticancer, ant tubercular, antibacterial, ant allergic, antimicrobial, anti-inflammatory, antiviral,

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antitumor, ant mutagenic, antidiabetic, hepato protective and to regulate cardiovascular activities [1-5]. Flavonoids are secondary metabolites, and play an important role in plants protection, in reproduction, pathogenesis and symbiosis [6,7]. In Plants, flavonoids help to manage stress, as caused by elevated UV-B radiation, infection by microorganisms or herbivore attack, in production of root nodules as a nitrogen fixation system [8-13]. Flavonoids help in pollination [14,15]. Flavonoids are important for human and animal's health. Their use as diet, their antioxidant properties [16,17] or their estrogenic action, and to a wide range of antimicrobial and pharmacological activities and functionalization of enzymes [18,19]. Currently we have isolated flavonoids from seed oil of *Pongamia pinnata* (L.) which is a rich source of flavonoids [20-25]. *Pongamia pinnata* (L.) is widely distributed in the coastal region and plains of South Asia, South East Asia, Australia and in all the four provinces of Pakistan, especially in north Punjab [26,27]. All parts of *Pongamia pinnata* (L.) i.e. roots, stem, bark, leaves, flowers, fruits and seeds are well known for their use in medicinal, economical and commercial values [28,29]. However different types of flavonoids have been isolated from different plant parts including leaves [30,31], Flowers [32], Fruit [33], root bark [34], stem bark [35,36] and seeds [37].

To the best of our knowledge from literature survey, no comprehensive spectroscopic analysis of Kanjone, Pongapin and Lanceolatin-B is reported.

2. MATERIALS

2.1 Plant Part

Seeds of *Pongamia pinnata* were used for study.

2.2 Chemicals

Ethyl acetate, ethanol, acetic acid, Iron (III) chloride (Merck), petroleum ether (60-95°C) (Acrosorganis), aluminum chloride (RDH), methanol (Scharlau), potassium bromide (Panreac). Silica gel 60, 0.06-0.2 mm, for CC (70-230 mesh ASTM) (Scharlau), aluminum plates precoated with silica gel for TLC 60 F254, 20x20 cm (Merck), DPPH (Fluka).

2.3 Columns

Glass Chromatographic column with reservoir, 40 x 600 mm, 750 ml (Witeg, Germany) and

glass chromatographic columns, 20 X 300 mm, (Witeg, Germany).

2.4 Instruments

The instruments used for analysis and characterization of isolated flavonoids were as follows:

- I. NMR; Bruker, spect, 300 & 400 MHz.
- II. Mass; Mat 312, maspec system (msw/A091).
- III. Melting Point App. Gallen Kamp, Cat No. MPD350.BM 3.5, Sanyo, UK.
- IV. Spectrophotometer U-2800, Model 122-6003, Hitachi, Japan.
- V. FT-IR. Spectrum One, Perkin Elmer, USA.
- VI. Fluorimeter Fluoromax-2.
- VII. Camera built in Mobile Phone Nokia 6230.

3. METHODS

3.1 Collection of Plant Material

Air dried seeds were collected from Islamabad in the month of May. The seeds were one season old. The pods were light dirty brown in color whereas seeds (endosperm) were dark brown in color.

3.2 Extraction and Isolation

The shells were separated and the seeds (endosperm) (2.73 kg) were cold pressed by a mechanical extractor. Dark brown colored oil (730.72 gm approx.) was obtained. Yellow colored residue was settled after 24 hours standing. The oil decanted.

The dark brown oil (472.22 gm) was extracted twice in 1 L MeOH for two weeks. The two methanolic extracts obtained were combined (35.66 gm). Literature survey revealed that non-polar and semi-polar solvents such as benzene, petroleum ether, diethyl ether and ethyl acetate etc. were used for the isolation of flavonoids. Therefore, to standardize the solvent system for column chromatography, TLC of the methanolic extract has been done in different combinations of petroleum ether and ethyl acetate with ratios (9:1), (8:2), (7:3), (6:4), (1:1), (4:6), (2:8), (1:9). Out of these solvent mixtures, Pet. ether and EtOAc (7:3) was found best. Fractionation of methanolic extract adsorbed on silica gel was initiated by column chromatography over silica

gel as stationary phase using petroleum ether and ethyl acetate (7:3) mixture as eluent. Total 10 pooled fractions were collected, labeled as (P1-P10). Out of these pooled fractions, flavonoids in pure or semi-pure form were isolated namely (P1-P5) by employing variety of purification procedures.

The first fraction found was flavonoid Kanjone - (PP2) (0.43 g). The rest of the two fractions were concentrated by evaporating them on rotary evaporator and keeping them on standing for a day.

The solvent was evaporated and crystalline mass appeared was recrystallized in ethanol to give small yellow needles of flavonoid Pongapin (PP4) (15 mg). The last one Lanceolatin-B (PP5) was collected in a minute amount (2 mg) as fine transparent colorless needles.

3.3 Identification of Isolated Compounds

3.3.1 Chemical analysis

Ferric chloride test for all three flavonoids was negative and Shinoda tests (positive) confirm the presence of Kanjone, Pongapin and Lanceolatin-B.

3.3.2 Physical analysis

Melting point of Kanjone, Pongapin and Lanceolatin-B are 188-190°C, 192-194°C and 137-138°C respectively and all three flavonoids best crystallized out in ethanol and ethyl acetate.

Chromatogram of isolated compounds (flavonoids) under UV light (366 nm) is shown below.

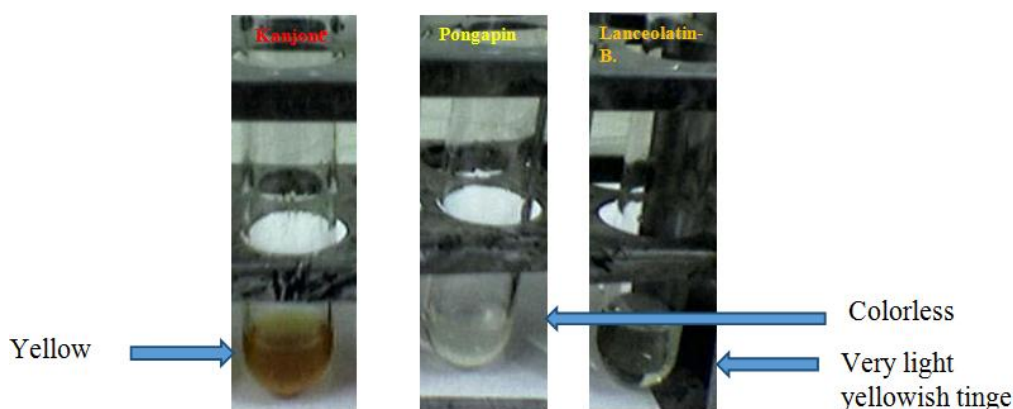


Fig. 1. Shinoda test results of isolated flavonoids

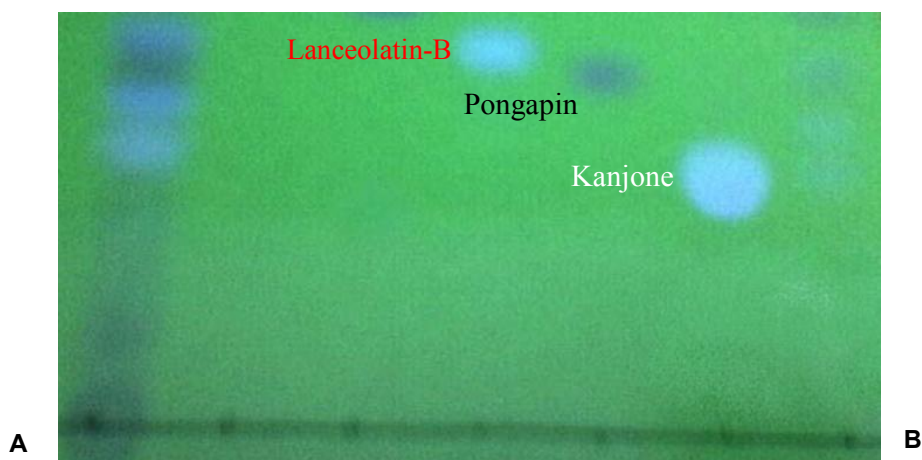


Fig. 2. Developed chromatogram of isolated compounds under UV light (366 nm)

A = Oil (before extraction), B = Oil (after extraction)

3.3.3 Spectroscopic analysis

3.3.3.1 Kanjone

UV/VIS Spectra: The isolated compound Kanjone gave characteristic UV/VIS spectra in MeOH. Its UV spectrum Fig. 3 has λ_{\max} 270 and 300 nm typical of flavone nucleus. There is a very small sharp edge at 340 as an additional feature.

FTIR Spectra: The IR spectrum of Kanjone also confirms the flavonoid structure. The stretching vibration peak at 1632 cm^{-1} is due to the $\nu(\text{C}=\text{O})$ str. Further absence of the broad bands at 1218 cm^{-1} and $3300\text{--}3500\text{ cm}^{-1}$ confirm the absence of hydroxyl group. The peaks at 1573 cm^{-1} and at 1481 cm^{-1} show aromatic (--C--H) stretching. Thus confirms the presence of aromatic nucleus. This is also supported by peak at 3094 cm^{-1} due to sp^2 (--C--H) stretch.

NMR Spectra: The ^1H and ^{13}C NMR signals indicate that the compound is a flavonoid. In ^1H

NMR the multiplets in the range of δ 7-8 indicate the presence of unsubstituted aromatic ring. The ^{13}C NMR signal at δ 178.1 points out the carbonyl function of the chromone unit. The two protons of furan ring gave their signals at δ 7.78 (d, $J=1.9$, H-2'') and at δ 7.21 (d, $J=1.9$, H-3''). The furan ring is attached to position C-7 and C-8 in an angular way as supported by the presence of methoxy group at position C-6 evident from its signal at δ 4.09 (s, 3H)/ δ 56.4 (C-6). A proton δ 7.23 (s, 1H) is present at C-5. The NMR data show that the furano flavonoid could be like Kanjone an isomer of karanjin.

Mass Spectra: The molecular formula of Kanjone was determined as $\text{C}_{18}\text{H}_{12}\text{O}_4$ by ESIMS of its $[\text{M}+\text{H}]^+$ ion at m/z 292. The major fragments observed are at m/z 292 (100%), 262 (37%), 189.9 (63.75%), 159.9 (54.97%), 118.9 (50.28%), 101.9 (13.51%), 76.9 (7.75%), 75.8 (24.07%). Thus NMR, IR and UV spectra confirms the compound is flavone, trivial name is Kanjone [36].

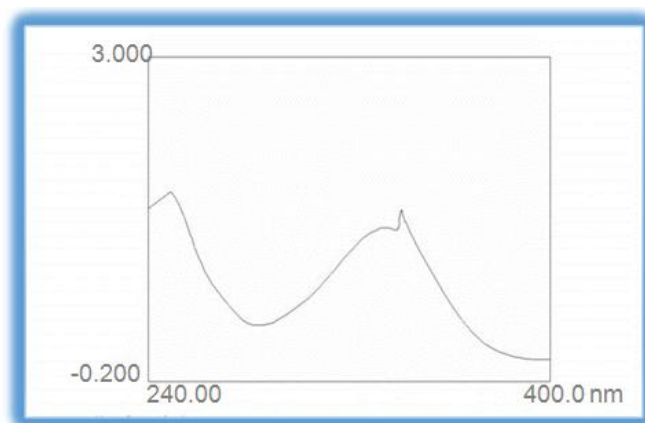


Fig. 3. UV/VIS spectra of compound Kanjone measured in ethanol

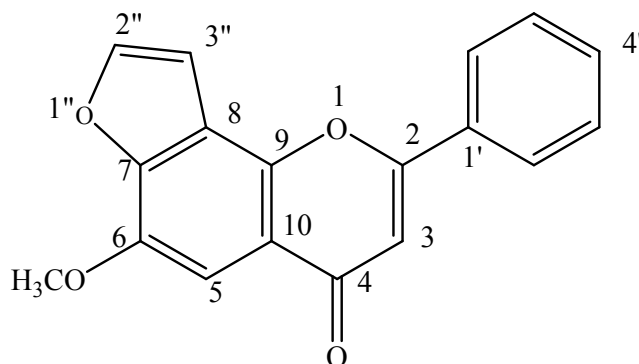


Fig 4. Structure of Kanjone

3.3.3.2 Pongapin

UV/VIS Spectra: The isolated compound Pongapin gave characteristic UV/VIS spectra in MeOH. Its UV spectrum has λ_{\max} 250 and 330 with a big sharp peak edge at 340. It looks different to flavonoid's spectra already taken.

FTIR Spectra: The figures appeared from IR spectra of Pongapin did not match exactly with that of flavonoid skeleton as already reported. There is one strong and broad peak at 1445 cm^{-1} and one small but broad peak at 1625 cm^{-1} . It is difficult to gain information about any key structural feature. There is need of advance spectroscopic technique to study the structure.

NMR Spectra: The ^1H and ^{13}C NMR spectra of compound reveal the presence of flavonoid skeleton. The ^{13}C NMR signal at δ 174.8 point out the carbonyl function of the chromone unit. The methoxy group is present at position C-3 as indicated by Proton signal at δ 3.90 (s, 3H) and ^{13}C - signal at δ 60.0. The ^1H and ^{13}C signals for a

furan ring at δ 7.16 (1H, d, $J= 1.6$, H-3'')/ δ 104.2 (C-3'') and δ 7.75 (1H, d, $j= 1.6$, H-2'')/ δ 145.6 (C-2'') were observed. An aromatic proton is present at C-2'' as indicated by proton signal at δ 7.24 (s, 1H) and ^{13}C - signal at δ 123.4. Similarly, ^1H -NMR signals at δ 7.52 and δ 7.65 correspond to the protons substituted aromatic ring attached to the benzochromone unit of flavonoids. In ^1H -NMR spectrum, the signal for 3', 4'-methylenedioxy substitution on ring B appeared at δ 6.07 (s, 2H). Finally, on the basis of spectral results the compound was identified as Pongapin.

Mass Spectra: The molecular formula was determined as $\text{C}_{19}\text{H}_{12}\text{O}_6$ by ESIMS of its $[\text{M}+\text{H}]^+$ ion at m/z 335.7. The major fragments observed are at m/z 334.7 (89%), 292.7 (15%), 189.9 (63.75%), 159.8 (38.2%), 145.8 (27.1%), 132.8 (29.71%), 115.9 (11.96%), 82.8 (20.10%), 75.9 (30.83%), 74.9 (46.08%), 62.9 (23.15%). Thus NMR, IR and UV spectra confirms with the structure of flavonoid commonly called Pongapin [38].

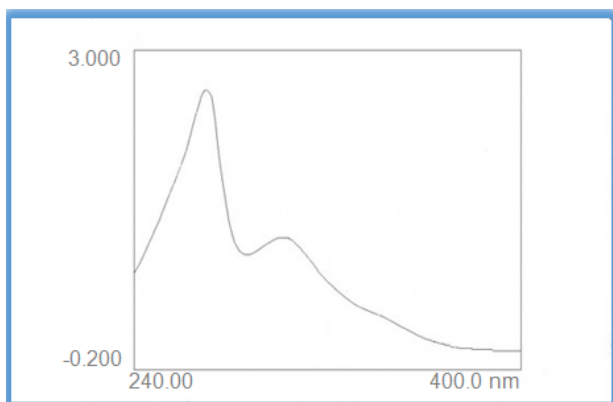


Fig. 5. UV/VIS spectra of compound Pongapin measured in ethanol

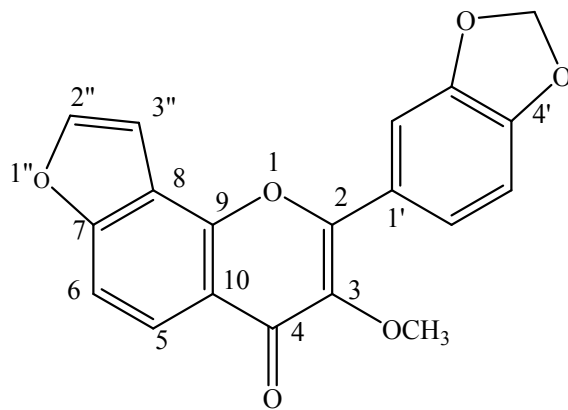


Fig. 6. Structure of Pongapin

3.3.3.3 Lanceolatin-B

UV/VIS Spectra: The isolated compound Lanceolatin-B gave characteristic UV/VIS spectra in MeOH. Its UV spectrum has λ_{\max} 260 and 300 with very small shoulder at 250. It also looks different to flavonoid's spectra already taken.

FT/IR Spectra: The figures appeared from IR spectra of Lanceolatin-B partially matched with that of flavonoid skeleton shown in Fig. 8. There is one strong and broad peak at 1649.71 cm^{-1} indicate the presence of C=C aromatic bending vibration of aromatic structure. Aromatic C-H stretching signals over 3000 cm^{-1} are weak and condensed which may be due to moisture and noise. Aromatic C-H bending signals in the region 860 cm^{-1} to 880 cm^{-1} also support the compound is Lanceolatin-B. Two small sharp peaks at 1407 cm^{-1} and 1362 cm^{-1} are also supportive for Lanceolatin-B. So confirmation of Lanceolatin-B on the basis of FTIR absorption data was also confirmed by following advance spectroscopic techniques.

NMR Spectra: The study of $^1\text{H-NMR}$ signals favors flavonoid structure of molecule under study. The signal at δ 6.87 (s, 1H) indicated H-3, whereas signal at δ 8.15 (d, $J = 8.0$, 1H) and at δ 7.55 (d, $J = 8.0$, 1H) point out H-5 and H-6 respectively. The multiplet signal at 7.9 (m) corresponds to the aromatic protons of unsubstituted phenyl ring attached to Benzochromone unit of a flavonoid. The $^1\text{H-NMR}$ signals for protons of annulated furan ring at δ 7.20 (d, $J = 1.8$, 1H) for H-3'' and at δ 7.76 (d, $J = 1.8$, 1H) for H-2'' were observed respectively. On the basis of $^1\text{H-NMR}$ data the structure is identified as Lanceolatin-B.

Mass Spectra: The molecular formula was determined as $\text{C}_{17}\text{H}_{10}\text{O}_3$ by ESIMS of its $[\text{M}+\text{H}]^+$ ion at m/z 261.8. The major fragments observed are at m/z 159.9 (100%), 131.9 (14.8%), 77 (3.68%), 75.9 (43.71%), 69.0 (9.55%). Thus NMR, IR and UV spectra confirms with the structure of flavonoid commonly called Lanceolatin-B [39].

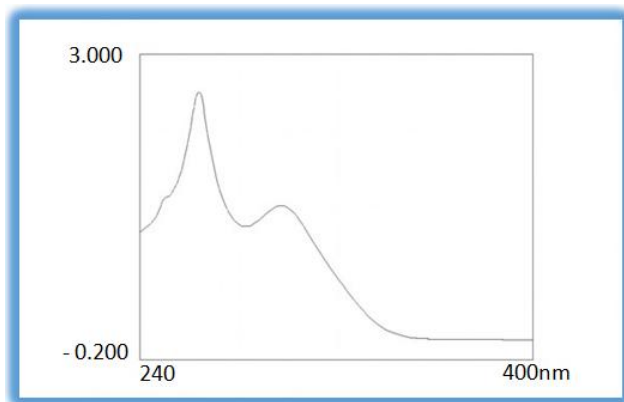


Fig. 7. UV/VIS spectra of compound Lanceolatin-B measured in ethanol

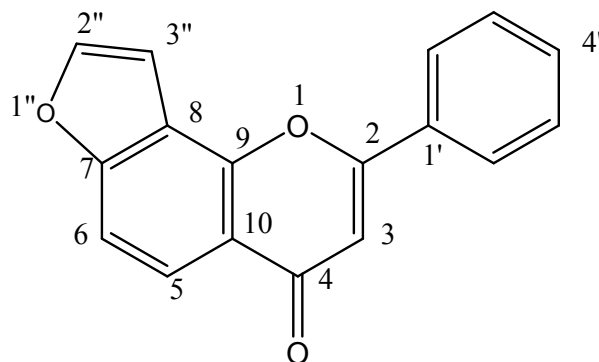


Fig. 8. Structure of Lanceolatin-B

4. CONCLUSIONS

Phytochemical investigation of *Pongamia pinnata* seed oil led to the isolation of flavonoids. The compounds were confirmed by extensive spectroscopic studies including UV, IR, NMR and MS. These include Kanjone, Pongapin and Lanceolatin-B. It was also concluded that local specie does not contain pongamol, a commercially important flavonoid known to occur as one of the major crystalline components from Indian species. So presence or absence of particular flavonoid, large differences in amount of a same flavonoid among same species depends upon habitat and region. These findings not only support the evidences regarding the distribution of *Pongamia* species in perplexingly variable forms but also to the effect of change in habitat on phytochemical composition. It invites the expansion of phytochemical research further to the species of other habitat such as Pakistan.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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