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# Isolation and Characterization of Novel Flavonoid from Methanolic Extract of *Pongamia pinnata* Pods

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## ABSTRACT

*Pongamia pinnata* (Linn) Pierre (family Papiolanaceae) is an important medicinal plant found in tidal forests of India and has been largely used in the traditional Indian system of medicine for bronchitis, whooping cough, rheumatic arthritis and diabetes. Significant hypoglycemic activity has already been reported in flowers of the plant. But due to the short flowering period of the plant, the pods of the plants were studied as a potential alternative of flowers for getting some novel compounds with potential of the similar activity. In the present study, methanolic extract of *P. pinnata* pods were fractionated with column chromatography from its methanolic extract. Elution of the column with petroleum ether: chloroform (1:3) in fractions 51-79 yielded a novel flavonoid as yellow crystalline powder that was recrystallized from methanol. It showed $R_f$ value: 0.90 (Petroleum ether: chloroform:: 1:3) and mp as 131°C The isolated novel compound was characterized with UV-Vis., Infrared (IR), Mass, $^1$H and $^{13}$C-NMR (Nuclear Magnetic Resonance) spectroscopy. The isolated novel compound was found to be 13'-(3'-hydroxy-4'-methoxychalconyl-13-3, 4-dimethoxychalcone and hence named as Pongamiachalcone). Being a novel flavonoid isolated from the plant there are very good chances that this can also be another novel hypoglycemic agent from *P. pinnata*. Therefore, *in vivo* hypoglycemic activity of the novel compound may be carried out for its probable hypoglycemic activity like that of other flavonoids obtained from *P. pinnata* pods.

**Key words:** *Pongamia pinnata*, flavonoid, chalcone, novel compound, *P. pinnata* pods

## INTRODUCTION

*Pongamia pinnata* (family Papiolanaceae) is a common plant which is distributed throughout India and known as Karanja. The medicinal importance of the plant is well known in south east Asia, China and Australia (Wealth of India, 2003). In particular the Indian traditional system of medicine-"Ayurveda" recognizes the plant and the parts thereof for the treatment of bronchitis, whooping cough, rheumatic joints and diabetes (Meera *et al*., 2003). Various part of the plant including the flowers and the pods have been investigated and were found to be showing significant antibacterial/antifungal and hypoglycemic/hypolipidemic activity (Kumar *et al*., 2010a, b; Semalty *et al*., 2012). *P. pinnata* is found mainly along the river banks or the coastal area up to the altitude of 1200 m. *P. pinnata* is a medium-sized glabrous tree with grayish green or brown bark (smooth or covered with tubercles). The plant bears the imparipinnate ovate or elliptic leaves; compressed, woody, yellowish gray (when ripe) pods with different size and shape (Semalty *et al*., 2012).

As mentioned earlier, the flowers of *P. pinnata* have been found to show significant antidiabetic activity. But the problem is short flowering period of the plant. Various studies have reported that the pods have the similar activity profile as that of the flower (Punitha *et al*., 2006; Shirwaikar *et al*., 2003). So, the problem can be overcome by using pods alternatively for the same potential. Therefore, the present study deals with the isolation some new phytoconstituents from methanolic extract of *P. pinnata* pods.

## MATERIALS AND METHODS

**Plant material:** The pods of *P. pinnata* were collected from Modinagar (Uttar Pradesh) August 2009 and were identified in Department of Botany, H.N.B. Garhwal University Srinagar Garhwal Uttarakhand. A specimen for further reference was retained. The pods of were dried in an oven at a temperature below 45°C for 2 days and coarsely powdered (3 kg). The ground pods were extracted exhaustively first with hexane and then with methanol. The methanolic extract was concentrated under reduced pressure to yield dark brown, viscous syrupy mass.

**Extraction and isolation of novel flavonoid:** The dried flower pods were coarsely powdered and extracted with water and methanol at room temperature. The extracts were vacuum dried in rotator vacuum film evaporator (Perfit Model No. 5600 Buchi type). The methanolic extract yielded as a viscous residue (160 g). The fractionation of methanolic residue was carried out in column with solvents in increasing polarity viz. pet. ether, chloroform and methanol.

**Characterization of isolated compounds:** The melting point was obtained on a Perfit apparatus. Both $^{1}H$ and $^{13}C$-NMR spectra were recorded with a Bruker Advance 003 version, Germany NMR instrument operating at 400 and 100 MHZ, respectively. The spectra were recorded in deuterated dimethyl sulfoxide (DMSO-$d_6$) using trimethyl silane (TMS) as internal standard with chemical shift $\delta$ expressed in ppm and coupling constant (J) in Hertz. The Infra Red (IR) Spectra were obtained in KBr pellet on Win IR FTS-135 instrument (Biored, USA). Electrospray Ionization Mass Spectroscopy (ESI MS) was done at 70eV on a Jeol D-300 instrument (Jeol, USA).

## RESULTS AND DISCUSSION

**Pongamiachalcone:** Elution of the column with petroleum ether: chloroform (1:3) in fractions 51-79 yielded a yellow crystalline powder (500 mg) that was recrystallized from methanol.

It showed $R_f$ value:0.90 (Petroleum ether: chloroform:: 1:3) mp:130-131°C; UV $\lambda_{max}$ (MeOH) 216, 238, 348 (log $\epsilon$ 2.8, 5.3,4.7) IR$\nu_{max}$ (KBr): 3492, 2923, 1690, 1596, 1550, 1466, 1260, 1216, 1132, 971, 853 $cm^{-1}$. $^{1}$HNMR ($CDCl_3$): 7.99 (1H, d, J = 7.2 Hz, H-5), 7.97 (1H, d, J = 7.2 Hz, H-5'), 7.88 (1H, d, J = 8.8 Hz, H-8), 7.86 (1H,d, J = 8.8 Hz, H-8'), 7.61 (1H, d, J = 1.6 Hz H-2), 7.57 (1H, d, J = 2.4 Hz, H-2'), 7.55 (1H, m, H-6), 7.53 (1H, m, H-6'), 7.49 (2H, m, H-11, H-11'), 7.47 (2H, m, H-12, H-12'), 7.31 (1H, d, J = 8.8 Hz, H-7), 7.29 (1H, d, J = 8.8 Hz, H-7'), 7.17 (2H, brs, H-14, H-14'), 6.99 (2H, m, H-15, H-15') 4.13 (6H, brs, 2×OMe), 3.94 (3H, brs, OMe), ESIMS m/z (rel. int.): 520 $[M]^+$ ($C_{33}H_{28}O_6$).

Pongamiachalcone was obtained as a yellowish crystalline powder from petroleum ether: $CHCl_3$ (1:3) eluants. Its UV spectrum showed absorption maxima at 238 and 348 nm typical to chalcones. The IR spectrum of compound displayed characteristic absorption bands for hydroxyl group (3492 $cm^{-1}$), carbonyl group (1690 $cm^{-1}$) and aromatic rings (1596, 1550, 971 $cm^{-1}$).

Its mass spectrum showed a molecular ion peak at m/z 520 corresponding to a Molecular formula $C_{33}H_{28}O_6$. Its $^1$HNMR spectrum exhibited two one-proton ortho-coupled doublets as δ 7.99 (J = 7.2 Hz) and 7.97 (J = 7.2 Hz) assigned correspondingly to H-5 and H-5'. Two-one proton meta-coupled doublets at δ 7.61 (J = 1.6 Hz) and 7.57 (J = 2.4 Hz) were ascribed to H-2 and H-2', respectively. Two one-proton multiplets at δ 7.55 and 7.53, two multiplets at δ 7.49 and 7.47 integrating for two protons each and two broad signal at δ 7.17 and one multiple at δ 4.13 integrating for two protons each were accounted to the remaining aromatic protons. Four one-proton doublet at δ 7.88 (J = 8.8 Hz), 7.86 (J = 8.8 Hz), 7.31 (8.8 Hz) and 7.29 (J = 8.8) were attributed to cis-proton H-8, H-8', H-7 and H-7', respectively. A six-proton broad signal at δ 6.99 and three-proton broad signal at δ 3.94 were associated with the three methoxy protons.

The $^{13}$C NMR spectrum of the isolated compounds showed signals for carbonyl carbons at δ 186.14 (C-9) and 184.32 (C-9'), aromatic carbons between δ 154.69-97.94, vinylic carbons at δ 106.07 (C-7), 128.78 (C-8), 105.33 (C-7') and 128.35 (C-8') and methoxy carbons at δ 61.16, 60.05 and 54.27. The absence of any signal between δ 0.5-3.94 in $^1$H NMR spectrum between δ 10.0-54.27 ruled out the existence of an aliphatic chain in the molecules.

On the basis of foregoing discussion the structure of was established as 13'-(3'-hydroxy-4'-methoxychalconyl-13-3, 4-dimethoxychalcone. The absence of any carbon signal near δ 163.0 in the $^{13}$C NMR spectrum suggested the attachment of chalconyl moiety between δC-7 and C-7'.

Extractive yield of methanolic extract of pods was 8.5% of dry plant. Methanolic extract of *P. pinnata* was fractionated by column chromatography. One new compound was isolated and named as Pongamiachalcone (13'-(3'-hydroxy-4'-methoxychalconyl-13-3, 4-dimethoxychalcone). The compound responded positively to Shinoda test (Danmalam *et al.*, 2009) indicating flavonoid nature of the molecule. The compound was characterized for melting point and various spectral analyses. On the basis of spectral data analysis and chemical reaction, the structure of Pongamiachalcone was established as 13'-(3'-hydroxy-4'-methoxychalconyl-13-3, 4-dimethoxychalcone (Fig. 1). It is a novel molecule isolated from the plant for the first time by the research group.

This study is well supported by previous studies by authors in which two novel flavonoids were isolated from *P. pinnata* pods (Kumar *et al.*, 2010b; Semalty *et al.*, 2012). In the first study, a new difuranoflavonone named Pongamiaflavonol $C_{20}H_{12}O_6$ (5,7-difurano-4'-methoxyflavanone) was isolated from methanolic extract of *P. pinnata* pods and was found to be showing significant hypoglycemic and hypolipidemic activity in streptozotocin-induced diabetic rats (Kumar *et al.*, 2010b). In another study a novel flavonoid named Pongamiaflavonylflavonol (5a, 3'a, dihydroxy-4'a-methoxy 8-ethylflavonyl (6a→8b)-5b, 7a, 2'b, 3'b-tetramethoxy-4'b-methoxyflavonol) was isolated from *P. pinnata* pods and showed significant hypoglycemic activity in streptozotocin-induced diabetic rats (Semalty *et al.*, 2012). Various previous studies have also reported the presence of flavonoids in flower and bark of *P. pinnata*. The present study is well

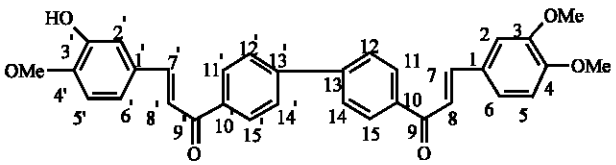


Fig. 1: Chemical structure of isolated novel compound (Pongamiachalcone)

supported by these previous studies which have also found the isolated flavonoids from the plant for the hypoglycemic and hypolipidemic activity (Ahmad *et al*., 2004; Punitha *et al*., 2006; Badole and Bodhankar, 2009).

## CONCLUSION

*Pongamia pinnata* is rich in abundance of flavonoids in various parts of the plant. The flavonoids obtained from the plant chiefly show hypoglycemic activity. The present study reported a novel compound named Pongamiachalcone (13'-(3'-hydroxy-4'-methoxychalconyl-13-3, 4-dimethoxychalcone) from *P. pinnata* pods for the first time. The in vivo hypoglycemic activity of the novel compound may be carried out for its probable hypoglycemic activity like that of other flavonoids obtained from *P. pinnata* pods.

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