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Isolation and Hypoglycemic Activity of a Novel Pongamiaflavonylflavonol from *Pongamia pinnata* Pods

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Abstract: *Pongamia pinnata* (family Papilionaceae) has been used for bronchitis, whooping cough, rheumatic joints and quench dipsia in diabetes. This study deals with the isolation of a new hypoglycemic phytoconstituent from *P. pinnata* pods. The hypoglycemic activity of the isolated phytoconstituent was evaluated in comparison of the methanolic extract of the pods. Methanolic extract of *P. pinnata* pods was fractionated by column chromatography and the isolated compounds were identified by spectral analysis. A new compound named Pongamiaflavonylflavonol was isolated from chloroform: methanol (97:3) eluant. This new isolated compound was studied for hypoglycemic activity in streptozotocin induced diabetic rats. Methanolic extract of *P. pinnata* pods and pongamiaflavonylflavonol showed significant hypoglycemic activity in streptozotocin-induced hyperglycemic rats after oral administration. At the end of 6 h the new compound showed 12.15% reduction in blood glucose level in comparison of extract (11.36%) against the standard (16.93%). It can be concluded that the novel Pongamiaflavonylflavonol isolated from *P. pinnata* pods may be useful as oral hypoglycemic therapeutic agent. This may serve as a lead compound for development of more potent drugs for clinical use in diabetes.

Key words: *Pongamia pinnata*, hypoglycemic activity, streptozotocin, novel flavonol, antidiabetic phytoconstituent, isolation

INTRODUCTION

Diabetes is a life threatening metabolic disorder. Recently WHO has calculated that worldwide almost 3 million deaths per year are attributable to diabetes. The global diabetic population is expected to increase alarmingly in the coming decades, rising to 380 million people in 2025 (WHO, 2011). Developed countries have higher prevalence rates than developing countries. It is estimated that about 5.4% of the world population would be suffering by the year 2025. India followed by China and the U.S. shall be the capital of in the year 2025. Presently, India, China, the United States, Russia and Germany are the five countries with the largest numbers of people with diabetes (Facts and Figures, WHO 2011). According to data from National Diabetes Fact Sheet of U.S., 25.8 million children and adults in the United States (8.3% of the population) have diabetes. It was also stated that diabetes is the leading cause of new cases of blindness and kidney failure among adults aged 20-74 years in the US. Moreover, the risk for stroke is 2 to 4 times higher among people with diabetes. Diabetes is the seventh leading cause of death in the United States (American Diabetic Association, 2011).

Diabetes is a disease to which only symptomatic relief can be given. The glucose levels can be controlled either by a variety of oral hypoglycemic agents (like sulphonylurea and biguanides etc.) or by the hormone replacement therapy (Insulin). But, the complete cure is still to be (and being) explored. Moreover, the presently prescribed antidiabetic drugs show various side effects and compulsion of being dependent on the drugs (Inzucchi, 2002; Semalty and Semalty, 2008). Nowadays, phytomedicines are gaining popularity and widespread acceptance in the treatment of diabetes also. A lot of investigations are being focused to explore the herbal drug and its chief hypoglycemic constituents (Karim *et al.*, 2011). The study focuses on the hypoglycemic activity of *Pongamia pinnata* which belong to family Papilionaceae commonly known as Karanja. The plant is distributed throughout India as roadside avenue tree in tidal and beach forest. It is used medicinally in India, China, Australia and Philippine Island (Wealth of India, 2003). In Indian traditional system of medicine-Ayurveda, different parts of *P. pinnata* have been used for bronchitis, whooping cough, rheumatic joints and quench dipsia in diabetes

(Meera *et al.*, 2003). Its oil is externally applied to cure herpes and eczema (Qureshi and Khan, 2001). The plant extract has also been reported as phytopesticide against *Okra mosaic virus*. The yield of crop was found to be highest with maximum plant height, flower production and fruits formation (Bhyan *et al.*, 2007). *P. pinnata* is distributed up to the altitude of 1200 m and is the native of western ghat, chiefly found along the bank of rivers, streams or near sea coast at beach and tidal forest. *P. pinnata* is a medium-sized glabrous tree with short bole and spreading crown up to 18 m high or sometime even more and 1.5 m in girth. Bark is grayish green or brown, smooth or covered with tubercles, leaves are imparipinnate, leaflets 5-7, ovate or elliptic. Pods are compressed, woody, indehiscent, yellowish gray when ripe, varying in size and shape, elliptic to obliquely oblong, 4.0-7.5 cm long and 1.7-3.2 cm broad with short curved beak. Seed usually 1 rarely 2, elliptical or reniform 1.7-2.0 cm long and 1.2-1.8 cm broad, wrinkled with reddish brown leathery testa (Khare, 2004).

The plant flowers for a short period, so the pods may be used alternatively. Therefore, the pods were screened for the potential antidiabetic activity. A significant antihyperglycemic and antilipidperoxidative activity of *P. pinnata* flowers have been already reported in streptozotocin induced diabetic rats but the activity has not been reported in pods (Punitha *et al.*, 2006; Shirwaikar *et al.*, 2003). Therefore, in the present study hypoglycemic activity of *P. pinnata* pods was investigated in streptozotocin induced diabetic rats against the standard (glibenclamide). A new phytoconstituent was isolated from pods and its hypoglycemic activity was studied in comparison of the methanolic extract of the pods.

MATERIALS AND METHODS

Plant material: The pods and flowers of *P. pinnata* were collected from Jamia Hamdard campus in October 2006 and were identified by Dr. Javed Ahmad, Department of Botany, Jamia Hamdard. (Voucher No. PRL-001-06). The voucher specimens are kept for the record in Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi. Flowers pods were dried for 7 days in air and latter at temperature below 45°C in an oven (2.2 kg).

Extraction and isolation of novel pongamiaflavonylflavonol: 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. Elution of the column with chloroform: methanol (97:3) yielded a compound (PP1) of green amorphous powder nature and recrystallized from methanol (140 mg).

The melting point was obtained on a Perfit apparatus. Both ¹H and ¹³C-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-d₆) using trimethylsilane (TMS) as internal standard with chemical shift δ expressed in ppm and coupling constant (J) in Hertz. The IR Spectra were obtained in KBr pellet on Win IR FTS-135 instrument (Biorad, USA). ESI MS scanned at 70 eV on a Jeol D-300 instrument (Jeol, USA).

In vivo hypoglycemic activity

Animal: Colony bred, healthy Wistar Albino rats were obtained from the animal house of Jamia Hamdard, New Delhi, after obtaining approval from institutional ethical committee. All the animals were weighted (200-250 g) and marked separately. Animals having similar weight and sex were kept in same group. The animals were housed in standard cages (48×35×22 cm) at room temperature (25±2°C), with artificial light from 7.00 am to 7.00 pm and provided with pelleted food and water *ad libitum*.

Acute toxicity study: Acute toxicity was performed for aqueous extract according to the acute toxic classic method as per guidelines of Organization for Economic Cooperation and Development (OECD, 1996). Albino Wistar rats were used and animals were kept fasting for overnight providing only water, after which the extract was administered orally at the dose of 200 mg kg⁻¹ and observed for 24 h. If the mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If the mortality was observed in one animal, then the same dose was repeated to confirm the toxic dose. If mortality was not observed, the procedure repeated for further higher dose i.e., 2000 mg kg⁻¹. One tenth of maximum dose of the extract tested for acute toxicity was selected as dose i.e., 200 mg kg⁻¹.

Hypoglycemic activity: Albino Wistar rats (200-250 g) were randomly divided into six groups with six animals in each group. Except the group I (normal control), in animals of all other groups, diabetes was induced by injecting

Streptozotocin (50 mg kg⁻¹ i.p. for 4 consecutive days) which was freshly prepared in citrate buffer (pH 4.5). After 4 days these hyperglycemic rats were used for the study:

- **Group I:** (normal control) rats received only buffer (orally)
- **Group II:** (diabetic control) these rats were kept without any treatment to study the diabetic nature of rat
- **Group III:** (standard) received the reference standard drug glibenclamide (3 mg kg⁻¹) administered orally
- **Groups IV:** (*P. pinnata* pods methanolic extracts): *P. pinnata* pods methanolic extract (200 mg kg⁻¹) in 1% CMC through oral route
- **Groups V:** (pure compound PP-1) received new isolated difurano flavonoids (50 mg kg⁻¹) in 1% CMC through oral route

After administration of standard, extract or new compound the blood samples were taken from the orbital sinus of each rat at 0, 2nd, 4th and 6th h with the help of a capillary tube for the estimation of blood sugar (Semalty and Semalty, 2008).

Statistical analysis: The data are represented as mean±SEM and statistical significance between treated, untreated and control group was analyzed by ANOVA followed Dunnett's multiple comparison *t*-tests. Student's *t*-test. The *p*<0.05 implies significance.

RESULTS AND DISCUSSION

Extractive yield of methanolic extract of pods was 7.2% of dry plant. Methanolic extract of *P. pinnata* was fractionated by column chromatography and isolate named Pongamiaflavonylflavonol (PP1), as a green amorphous powder from chloroform: methanol (97:3) eluant. It 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.

Its UV spectrum showed absorption maxima at 221, 264 and 322 nm typical to flavones. The IR spectrum of Pongamiaflavonylflavonol displayed characteristic absorption bands for hydroxyl (3257, 3160 cm⁻¹) and carbonyl (1667 cm⁻¹) groups.

Its mass spectrum showed molecular ion peak at *m/z* 642 corresponding to a biflavone moiety, C₃₄H₂₆O₁₃. The prominent ion fragments generated *m/z* 311 and 331 indicated the attachment of tetrahydroxy-methoxyflavone with a dihydroxy-methoxy ethyl flavonone. The prominent ion peak at *m/z* 300 was formed due to removal of methoxy group from the mass unit at *m/z* 331.

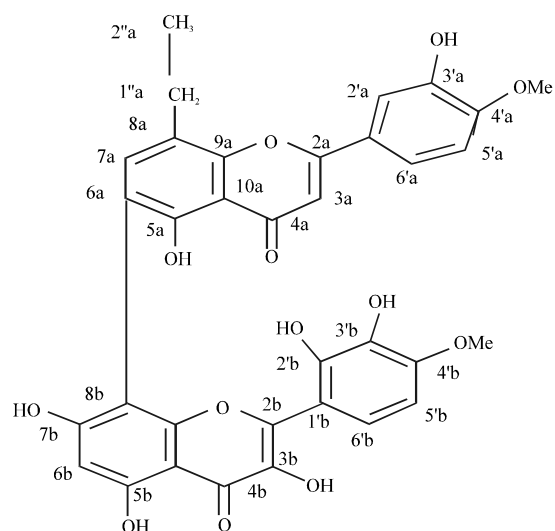


Fig. 1: Chemical structure of novel isolated antihyperglycemic compound PP1

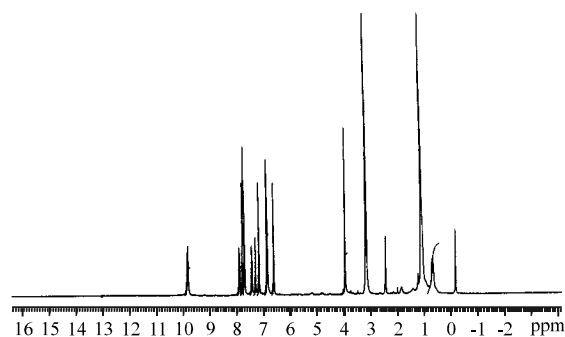


Fig. 2: ¹H NMR spectrum of compound PP1 (DMSO-d₆)

Pongamiaflavonylflavonol (5a, 3'a, dihydroxy-4'a-methoxy 8-ethylflavonyl (6a→8b)-5b, 7b, 2'b, 3'b-tetramethoxy-4'b-methoxyflavonol) (Fig. 1).

m.p 240-242°C, UV λ_{max}(MeOH): 221, 264, 322 nm (log ε 5.6, 3.2, 4.8); IR ν_{max}(KBr): 3257, 3160, 2921, 2852, 1667, 1579, 1503, 1448, 1367, 1288, 1252, 1170, 1069, 1027, 832 cm⁻¹; ¹H NMR (DMSO-d₆): δ 7.94 (1H, d, J = 8.8 Hz, H-5'a), 7.81 (1H, brs, H-6b), 7.77 (1H, d, J = 8.4 Hz, H-5'b), 7.73 (1H, d, J = 8.4 Hz, H-6'b), 7.46 (1H, d, J = 8.8 Hz, H-7'a), 7.18 (1H, brs, H-3a), 6.88 (1H, d, J = 2.5 Hz, H-2'a), 6.63 (1H, brs, H-7a), 3.95 (6H, brs, 2×OMe), 2.46 (2H, brs, H₂-1'a), 0.84 (3H t, J = 6.3 Hz, Me-2'a); ¹³C NMR (DMSO-d₆) EIMS *m/z* (rel. int.) 642[M]⁺ (C₃₄H₂₆O₁₃) (11.3), 331 (100), 311 (22.1), 300 (80.3) (Fig. 2 and 3).

The ¹H NMR spectrum (Fig. 2) of compound isolated from the pod extract showed four one – proton doublets at δ 7.94 (J = 8.8 Hz), 7.77 (J = 8.4 Hz), 7.73 (J = 8.4 Hz) and

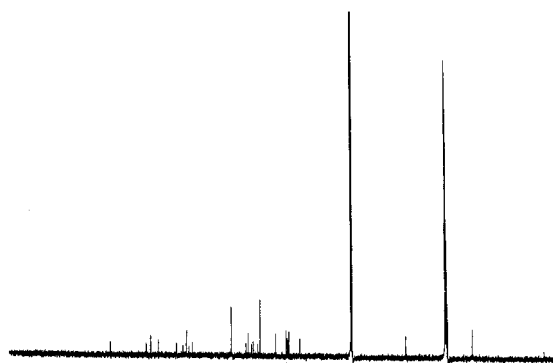


Fig. 3: ^{13}C NMR spectrum of Compound PP1

7.46 ($J = 8.8$ Hz) assigned to ortho-coupled H-5'a, H-5'b, H-6'a and H-6'b, respectively. A one-proton doublet at δ 6.88 with coupling interaction of 2.5 Hz was ascribed to meta-coupled H-2'a. Three one proton signals at δ 7.81, 7.18 and 6.63 were attributed to aromatic H-6b, H-3a and H-7a, respectively. A six-proton broad signal at δ 3.95 was accounted to two methoxy protons. A two-proton broad signal at δ 2.46 was associated with the methylene H₂-1''a protons. A three-proton triplet at δ 0.84 ($J = 6.3$ Hz) was assigned to primary methyl H₃-2'a protons. The ^{13}C NMR spectrum (Fig. 3) exhibited signals for carbonyl carbon at δ 177.61 (C-4a) and 177.46 (C-4b), aromatic carbon between δ 163.11- 104.33, methoxy carbons at δ 56.34, methylene carbon at δ 29.55 and methyl carbon at δ 14.53. The absence of carbon signals near δ 95.0 supported flavones moiety attachment at C-8b, with C-6 of a flavanol part. The ^1H NMR and ^{13}C NMR signals of Pongamiaflavonylflavonol were compared with the related flavonoids molecules. Ahmad *et al.* (2004) isolated and reported 3'-O-b-d-glucopyranosyl[2'',3'':7,8] furanoflavone, 6-methoxy-3'-O-b-d-glucopyranosyl [2'',3'':7,8] furanoflavone, 3-methoxy-6-O-b-d-glucopyranosyl [2'',3'':7,8] furanoflavone and 3-methoxy-3',4'-methylenedioxy-7-O-b-d-glucopyranosyl flavone and named those as pongamoside A, B, C and D, respectively from fruits of *P. pinnata*. This supports the present study in terms of the presence of flavonoids in the fruits. Therefore, the abundance of flavonoids was most likely in pod extract also. On the basis of spectral data analysis and chemical reaction, the structure of Pongamiaflavonylflavonol has been established as 5a, 3'a, dihydroxy-4'a-methoxy 8-ethylflavonyl (6a-8b)-5b, 7b, 2'b, 3'b-tetramethoxy-4'b-methoxyflavonol (Fig. 1). This is a new molecule isolated from a natural or synthetic source for the first time.

In vivo hypoglycemic activity: In the present study, a comparative chronic antidiabetic study was carried out between methanolic extract and new compound (PP1) of

P. pinnata pods. The dose of 200 mg kg⁻¹ body weight per oral did not produce any toxic effect. Administration of Streptozotocin (50 mg kg⁻¹) led to elevation of blood glucose level. A comparative antidiabetic study was carried out between methanolic extract and new compound (PP1) of *P. pinnata* pods. Treatment with oral methanolic extract of *P. pinnata* pods and Pongamiaflavonylflavonol (PP1) elicited hypoglycemic activity on blood glucose level significantly $p < 0.01$ in normal rats (Table 1). In normal rats, the initial blood glucose level of 84.18 \pm 3.32 mg/100 mL was reduced to 68.13 \pm 4.11, 63.44 \pm 3.55 and 55.12 \pm 3.12 mg/100 mL at the end of 2, 4 and 6 h, respectively with the extract. On the other hand, with the new compound PP1, the initial blood glucose level of 83.55 \pm 3.45 mg/100 mL was reduced to 53.54 \pm 4.12, 56.58 \pm 3.76 and 61.87 \pm 4.16 mg/100 mL at the end of 2, 4 and 6 h, respectively (Table 1). It was observed that in STZ induced diabetic rats after 6 h, blood glucose level was reduced by 11.36% (from 288.25 to 255.51 mg/100 mL), 16.93% (from 281.85 to 234.12 mg/100 mL) and 12.15% (from 283.13 to 247.56 mg/100 mL) with *P. pinnata* pods methanolic extract (200 mg kg⁻¹), standard (Glibenclamide 3 mg kg⁻¹) and new compound PP1 (50 mg kg⁻¹), respectively (Table 2). Therefore, after 6 h of treatment, antidiabetic activity was found to be in the decreasing order of Std. (Glibenclamide 3 mg kg⁻¹) > compound PP1 (50 mg kg⁻¹) > *P. pinnata* pods' extract (200 mg kg⁻¹) in the STZ induced diabetic rats. The compound PP1 showed the hypoglycemic activity comparable to that of standard.

In previous investigations on *P. pinnata*, the hypoglycemic activities have been reported in flower and bark also (Punitha *et al.*, 2006; Badole and Bodhankar, 2009a, b). All these studies support the presence of hypoglycemic activity in various parts of the plant and also flavonoid compounds responsible for hypoglycemic activities. *P. pinnata* flower extract was reported to have good antihyperglycemic hypolipidemic activity which was found comparable to the standard drug glibenclamide (Punitha *et al.*, 2006). Badole isolated a new antiheperglycaemic compound - Cycloart-23-ene-3 β , 25-diol, from stem bark of *P. pinnata* (Badole and Bodhankar, 2009a). It was also reported that the concomitant administration of petroleum ether extract of the stem bark with glyburide, pioglitazone or metformin showed a synergistic antihyperglycaemic effect (Badole and Bodhankar, 2009b). This study is well supported by a previous study by authors, in which a new difuranoflavonone compound PP (named Pongamiaflavonol) was isolated from methanolic extract

Table 1: Effect of methanolic extract of *P. pinnata* pods and Pongamiaflavonylflavonol (PP1) on blood glucose level in normal rats

Drug	Dose (mg kg ⁻¹ p.o.)	Blood glucose level mg/100 mL			
		Initial	2 h	4 h	6 h
Control	---	83.16±5.10	82.83±3.11	83.01±3.12	83.11±3.14
Extract	200	84.18±3.32	68.13±4.11*	63.44±3.55*	55.12±3.12*
Glibenclamide	3	84.13±2.15	45.83±4.27*	44.54±3.45*	52.21±5.55*
PP1	50	83.55±3.45	53.54±4.12*	56.58±3.76*	61.87±4.16*

All values are Mean±SEM, n = 6, *Significant at p<0.01

Table 2: Effect of methanolic extract of *P. pinnata* pods and Pongamiaflavonylflavonol (PP1) on blood glucose level in streptozotocin induced hyperglycemic rats

Drug	Dose (mg kg ⁻¹ p.o.)	Blood glucose level mg/100 mL			
		Initial	2 h	4 h	6 h
Control	---	282.11±8.45	279.22±7.11	228.12±11.64	285.77±9.23
Extract	200	288.25±9.88	258.56±7.11	228±1.12**	255.51±7.45**
Glibenclamide	3	281.85±9.77	245.38±9.58*	224.15±9.55*	234.12±9.96*
PP1	50	283.13±8.12	243.43±8.61*	234.22±10.13*	247.56±11.07*

All values are Mean±SEM, n = 6, *,**Significant at p<0.01 and p<0.05, respectively

of *P. pinnata* pods (Kumar *et al.*, 2010). The compound PP showed the significant hypoglycemic and hypolipidemic activity like that of aqueous pods and flower extract of *Pongamia*. The present study supported the presence of antihyperglycemic activity of the plant with a focus on activity of flower pod extract. The activity of the novel compound (PP1) from the flower pod extract of *P. pinnata* has been explored and reported very first time.

CONCLUSIONS

Therefore, it can be concluded that methanolic extract of *P. pinnata* pods and a novel isolated molecule Pongamiaflavonylflavonol (PP1) significantly decreased blood glucose level in normal and STZ-induced diabetic rats. It can be concluded that the novel Pongamiaflavonylflavonol may be useful as oral hypoglycemic therapeutic agent. This may serve as a lead compound for development of more potent drugs for clinical use in Diabetes.

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