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Original Article

Antidiabetic Screening, Activity-guided Isolation and Molecular Docking Studies of Flower Extracts of *Pongamia pinnata* (L.) Pierre

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Abstract

Pongamia pinnata (L.) Pierre (Family: Fabaceae) is a famous traditional medicinal plant, the flowers of which are used for treating diabetes, however, the active constituent(s) is yet unidentified. Therefore, the present study aimed to carry out antidiabetic activity-guided isolation of extracts of flowers. Hexane extract being the most active (EC_{50}, 900 µg/mL) was fractionated by partitioning and the most active hexane fraction (EC_{50}, 570 µg/mL) was subjected to column chromatography which gave three isomers of compound 1 (4-Methoxy-7-phenyl-5H-furo [3,2-g][1] benzopyran-5-one), a furanoflavonoid. All the isomeric forms have equal antidiabetic activity (83.24%) with EC_{50} at 300 µg/mL. The activity of the isolated compound was found to be higher as compared to standard drug acarbose (43.46%). Molecular docking studies of the compound indicated higher binding energy scores with antidiabetic targets as compared to the standard drug acarbose. The results of the present study indicate that the isolated compound may be developed into an antidiabetic drug.

Keywords: *Pongamia pinnata* (L.) Pierre, diabetes, Column chromatography, Single-crystal X-ray diffraction (XRD), Molecular docking studies.

Introduction

Pongamia pinnata (L.) Pierre (Family: Fabaceae) is a versatile medicinal plant that is particularly important for its oil but also provides dyestuff, wood, fuel, insect repellent, medicines and various other supplies [1]. Traditionally, various plant parts like leaf, stem, seed, bark, flower and root are used for the treatment of bronchitis, whooping cough, rheumatic joints, piles, diarrhea and diabetes [2]. The decoction of the dried flower powder is used to treat diabetes [3,4]. The extracts of flowers are reported to possess pharmacological activities such as antioxidant, antibacterial, hepatoprotective and renal protective activities [5-9]. The literature review indicated three in vivo antidiabetic and antihyperlipidemic activities of only one extract of flowers and pods [10-12], however, the antidiabetic activity screening of different extracts of the flower was not performed and the active extract was not evaluated for the isolation of active constituents. Therefore, such extracts need to be further investigated to isolate the active compound(s) which may be developed into antidiabetic drugs.

Currently, diabetes is managed by a healthy diet, exercise and medication including insulin and oral hypoglycemic drugs [13,14]. The current medications have various adverse effects [15]. Thus, safer and cost-effective treatment therapy is direly needed for satisfactory control of hyperglycemia, with no or little side effects. Due to little toxicity and few side effects, plant-based products are being used as alternative antidiabetic medicine [16, 17]. Hence, such plants can be investigated to find antidiabetic molecules that may be developed in to new antidiabetic drugs [18].

The literature review indicated the use of various in vitro enzymatic and non-enzymatic models for assessing antidiabetic activity. In the present study, one enzymatic (alpha-amylase inhibition assay) and two non-enzymatic models such as hemoglobin glycosylation (Hb-glycosylation) inhibition and glucose uptake by yeast cell

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were used to investigate extracts of *P. pinnata.* The selection of these three models was based on the fact that they cover inhibition of glucose synthesis, utilization and glucose associated complications that are associated with diabetes.

So, the aims of the present study are: To screen different extracts of the flower of *P. pinnata* for antidiabetic activity; to evaluate active extract for the isolation of active constituents and to perform the molecular docking studies on isolated compound to support its antidiabetic activity compared to standard drug so the isolated compound may be developed into antidiabetic drug.

Material and Methods

Plant Collection

The flowers of *P. pinnata* were collected during the flowering season (April-May, 2018) from Sheikhupura, Punjab, Pakistan. The plant material was identified by Prof. Dr. Zaheer-u-Din, Department of Botany, Government College University, Lahore, Pakistan, wherein a voucher specimen bearing number GC. Herb. Bot. 3517 was deposited. The material was dried under shade and pulverized into a fine powder.

Chemicals, solvents and other supplies

All the chemicals and solvents were of analytical or HPLC grade were purchased from Merck including chloroform, n-hexane, methanol, n-butanol, ethyl acetate, sodium hydroxide, sodium chloride, anhydrous glucose (≥ 99.5%), gallic acid (≥ 98%) , aluminum TLC plates, silica gel (60-120 mesh), silica gel 60H. Sodium di-hydrogen phosphate (monobasic), disodium hydrogen phosphate, 3,5-dinitro salicylic acid (98%), potassium sodium tartarate (99%, BDH), α-Amylase (99.9%, Unichem Chemicals), potato starch (Sigma Aldrich), acarbose (Bayer), soluble haemoglobin powder (Oxoid Ltd.), gentamycin (Ray Pharmaceuticals), dimethyl sulfoxide (DMSO) (Daejung), instant baker's yeast (Rossmoor), metronidazole (Sanofi Aventis), deionized water and distilled water (In-Lab prepared) and 96-wells microtiter plates (Corning Incorporated).

Extraction

The pulverized material (4 kg) was successively extracted by maceration using n-hexane, chloroform, methanol and water. The material and solvent ratio was kept 1:3, W/V, and maceration was carried out for seven days with occasional shaking at room temperature. The extract was filtered and the solvent was evaporated at 40 ºC using a rotary evaporator (Heidolph 4002).

Antidiabetic Activity

α-amylase inhibition activity

The method of Miller [19] based on the DNS method was used with slight modification to evaluate α-amylase inhibition potential of the extracts of *P. pinnata*. Briefly, 500 µL of different plant extracts (n-hexane, chloroform, methanol and aqueous extracts) of concentration 1000 µg/ml prepared in buffer (containing 20mM sodium dihydrogen phosphate and 6.7 mM sodium chloride at pH 6.9) and DMSO were added to 500 µL of 1% (3-5 units/mg) α-amylase in ice cold buffer solution and were incubated at 37°C for 7-8 min., followed by addition of 500 µL of 0.2% starch solution in buffer. The reaction mixture was incubated again at 37°C for 10 min. The reaction was stopped with 1.0 mL of 3,5 DNSA reagent. The test tubes were then incubated in a water bath for 15 min at 85°C and cooled to room temperature and 10 mL distilled water was added and the absorbance was measured at 540 nm. The control was also prepared similarly with methanol in place of plant extracts/ standard. Acarbose (1000 µg/ml) was served as standard. The results were expressed as % inhibition calculated using the formula 1:

$$\%\ inhibition = \frac{(Absorbance\ of\ control - Absorbance\ of\ sample)}{Absorbance\ of\ control} \times 100$$

Formula 1: % inhibition by α-amylase inhibition potential using DNS method

Glucose uptake by the yeast cells

The Yeast cell suspension was prepared by the method of Cirillo [20], 1g of commercial baker's yeast was washed repeatedly by centrifugation at 4200 rpm for 5 min with ice-cold doubled distilled water until the supernatant was clear and removed. The 10% (v/v) suspension was prepared in doubled distilled water. Then, the method of Bhutkar and Bhise [21] with slight modifications was adopted. Briefly, 1ml of different plant extracts of concentration 1000 µg/ml were mixed with equal volume of glucose solution (5,10 and 25 mM/L) and incubated at 37°C for 10 min. Then the reaction was started by adding 100 µl of yeast cell suspension, vortex for 5 min and incubated at 37°C for 1hr then centrifuged at 3800 rpm for 5 min. The DNS method was used to estimate the content of glucose in the supernatant (1mL of supernatant + 1mL DNS heated at 85°C for 5 min). The absorbance was measured at 540 nm by using doubled distilled water as the blank. Metronidazole (1000 µg/mL) was used as a standard. The methanol in place of plant extracts/ standard served as control. The percent increase in glucose uptake by the yeast cells was calculated using the formula 2:

$$\%\ increase\ in\ glucose\ uptake = \frac{(Abs.of\ control - Abs.of\ sample)}{Abs.\ of\ control} \times 100$$

Formula 2: % increase in glucose uptake by Yeast cells

Non-enzymatic hemoglobin glycosylation

The method of Adisa *et al*. [22] was adopted. The mixture comprising equal volume of hemoglobin (0.6

mg/mL), gentamycin (0.2 mg/mL), glucose (20 mg/mL) and sample/standard (1000 µg/mL, plant extracts/gallic acid) prepared in phosphate buffer saline (pH 7.4) was incubated in the dark at 37 °C for 72 hours. The amount of glycosylated hemoglobin was measured from the reaction mixture at 443 nm at 24, 48 and 72 hours with buffer as a blank. The reaction mixture containing methanol was served as the control. The %age inhibition of glycosylated hemoglobin was measured by formula 3.

$$\%\ age\ inhibition = \frac{(Abs.\ of\ control - Abs.\ of\ sample)}{Abs.\ of\ control} \times 100$$

Formula 3: % age inhibition of glycosylated hemoglobin

Activity-guided isolation

Fractionation of Extracts

The most active hexane extract (HE) (10 g) was suspended in distilled water (100 mL), poured in separating funnel and fractionated by using n-hexane, chloroform, ethyl acetate and n-butanol. The fractions were evaporated on a rotary evaporator and dried at 40°C. As a result, fractions of HE (5 g n-hexane fraction (HF), 2 g chloroform fraction (CF), 200 mg ethyl acetate fraction (EF)) were obtained. These fractions were subjected to antidiabetic screening by three models described earlier.

Separation and Isolation

HF (5 g) being the most active was subjected to column chromatography as a glass column (58.42 cm long, 3.81 cm outer diameter and 3.41 cm inner diameter) was packed with 100 g silica gel of 60-120 mesh size by the wet method. Elution was carried out by gradient elution with mobile phases consisting of n-hexane, chloroform and methanol in order of increasing polarity as 10:0, 8:2, 6:4, 4:6, 2:8 and 0:10. Column chromatography gave 54 sub-fractions which were pooled on the bases TLC profiles to get 38 groups. The alpha-amylase inhibition assay was performed using 96 wells microtiter plate using the same procedure as described earlier. Group 9 (G9) containing 19-25 sub-fractions, possessing higher activity was again subjected to silica gel column chromatography. The glass column (Quick-fit small 24/29 43.18 cm long, 3.05 cm outer diameter and 2.65 cm inner diameter) was packed with 48 g silica gel of 60-120 mesh size. Isolation was carried out with an isocratic mobile phase using ethyl acetate that resulted in 7 sub-fractions as G9A to G9G. Based on TLC profiles, G9B-E were pooled and all the four subgroups were used for alpha-amylase inhibition activity using 96 wells microtiter plate with the same procedure as described earlier. The most active subgroup G9F (10 mg) was allowed to crystallize which afforded three types of colorless crystals. These crystals were recrystallized from methanol.

Characterization of crystallized compound 1

The crystals were analyzed using HPLC-DAD to check the purity of the compound. The compound was characterized using physical, spectral techniques and finally, the structure of the crystalline compound was determined by single-crystal X-ray diffraction (XRD) technique.

Structure Determination by Single-crystal X-ray Diffraction (XRD)

The crystals were examined by single-crystal X-ray diffraction (XRD) analysis at the Department of Physics, University of Sargodha, Pakistan. Single crystal data collection was performed at 296 K on a Bruker Kappa APEXII CCD diffractometer equipped with a four-circle goniometer and using MoKα graphite mono-chromated radiation. The refinement and all further calculations were carried out using SHELXL-2014/7 (Sheldrick 2015). PLATON (Farrugia 1997) was used for molecular graphics. The crystals data was submitted to the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, UK with CCDC number 1969899 (HE1PU), 1969901 (HE2NEWPU) and 1969903 (HE2NNPU). These three isomeric forms data and details of the data collection are summarized in (See Supplementary material, Table 1).

The compound 1: furanoflavonoid (4-Methoxy-7-phenyl-5H-furo[3,2-g][1] benzopyran-5-one)

Colorless, plate type (HE1PU), Needles type (HE2NEWPU, HE2NNPU) crystals, percentage yield: 0.01g, UV/Vis λmax (methanol): 305 nm, Rf: 0.75 (Chloroform-ethyl acetate, 6:4 v/v), XRD data: chemical formula; $C_{18}H_{12}O_4$, wavelength (λ); 0.71073 Å, crystal system, space group; Monoclinic, $P2_1/c$ (HE1PU, HE2NEWPU) and Triclinic, $P1$ (HE2NNPU), bonds distances *a*, *b*, *c* (Å); 8.722 (4), 15.950 (8), 9.949 (5) (HE1PU), 8.688 (2), 15.969 (4), 9.924 (3) (HE2NEWPU), 6.9714 (10), 9.7000 (13), 10.7166 (17) (HE2NNPU), α, β, γ (°); 90, 97.83(2), 90 (HE1PU), 90, 97.791(13), 90 (HE2NEWPU), 91.744 (8), 103.822 (9), 101.311 (8) (HE2NNPU), volume *V* (Å^3); 1371.1 (11) (HE1PU), 1364.0 (6) (HE2NEWPU), 687.75 (18) (HE2NNPU).

Molecular Docking Studies

The comparison of binding affinities of the isolated furanoflavonoid compound and standard antidiabetic drug acarbose for various protein targets related to hyperglycemia were performed using Mcule 1-Click Docking (Mcule, Inc. USA, https://mcule.com/apps/1-click-docking/). The binding poses conforming to the highest binding affinities were saved as “pdb” files and used later for determination of binding nature using software USCF Chimera 1.13.1 (University of California,

USA, https://www.cgl.ucsf.edu/chimera/). Various hyperglycemia related protein targets included were pancreatic α-amylase (AMYP), α-amylase 1 (AMY1), aldose reductase (ALDR), glycogen synthase kinase 3-β (GSK3B), 6-phosphofructo-2-kinase/ fructose-2,6-biphosphatase 1 (F261), hexokinase-1 (HXK1), fructose-1,6-bisphosphatase 1 (F16P1), beta-2 adrenergic receptors (ADRB2), corticosteroid 11-beta-dehydrogenase isozyme 1 (DHI1), Glucose-6-phosphate 1-dehydrogenase (G6PD) and glucokinase (HXK4).

Statistical Analysis

All the samples/standards were analyzed in triplicates and data were presented as mean ± standard deviation (SD). The EC_{50} and IC_{50} values were determined using the linear regression equation obtained from the plot of concentration versus response. The activity of all the samples/standard groups was analyzed by one-way ANOVA and post-hoc Bonferroni test using SPSS version 22.00. A $p< 0.05$ value was considered a statistically significant difference.

Result and Discussion

The results of antidiabetic activity of different extracts of flowers of *P. pinnata*, at the equal concentration (1000 µg/mL), using three in vitro models such as α-amylase inhibition, glucose uptake by yeast cells and non-enzymatic Hb-glycosylation inhibition assays (Fig.1) showed that HE had higher activity in all the three models.

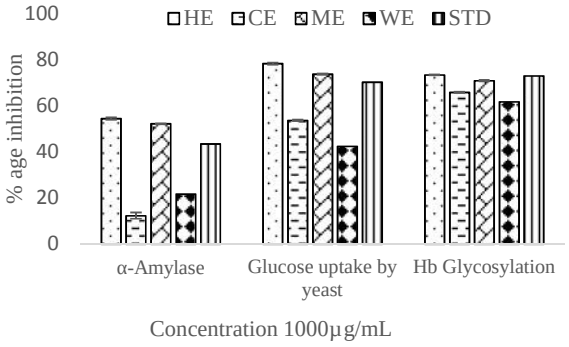


Fig. 1 Antidiabetic screening of different extracts of flowers of *Pongamia pinnata* (L.) Pierre with different standards STD (Acarbose (Acb) in alpha-amylase inhibition assay, Metronidazole (MTZ) in glucose uptake by yeast cells and Gallic acid (GA) in non-enzymatic Hb Glycosylation).

The activity of HE was found to be higher than the standard ($p<0.05$) in both α-amylase inhibition and glucose uptake by the yeast cells models, whereas the activity of HE and the standard was comparable ($p<0.05$) in Hb-glycosylation inhibition model. In glucose uptake by yeast cells, the activity of HE was higher in 5mM glucose solution as compared to 10mM and 25 mM glucose solutions (See Supplementary material, Fig. 1-4). Less activity in concentrated glucose solutions may be due to decreased activity of glucose transporters as a result of saturation of glucose in extracellular space and establishing equilibrium between intracellular and extracellular glucose.

In dose-dependent response (100-1000 µg/mL) activity in 5mM glucose concentration, the half-maximal effective concentration (EC_{50}) was found to be 580 µg/mL ($y = 0.0622x + 16.148$, $R^2 = 0.9924$). In non-enzymatic Hb-glycosylation, the activity was found to be increasing with time, maximum at 72 h (See Supplementary material, Fig. 5-8). In dose-dependent response (50-1000 µg/mL at 72 h), the half-maximal inhibitory concentration (IC_{50}) was found to be 390 µg/mL ($y = 0.043x + 32.584$, $R^2 = 0.9842$). Whereas in α-amylase inhibition activity (See Supplementary material, Fig. 9-13), the IC_{50} was found to be 900 µg/mL ($y = 0.0328x + 19.225$, $R^2 = 0.9901$). These findings were found to be contradictory to that reported in earlier three studies whereby aqueous and ethanolic extracts of flowers have shown higher antidiabetic activity [10-12]. But, in these studies in vivo model was used to assess antidiabetic activity. This difference may be due to the difference of phytochemical constituents due to various oncogenetic and exogenetic factors or in vivo experimentation. Based on these results, HE was subjected to fractionation using solvents in the order of increasing polarity.

The results of antidiabetic activity of different fractions of HE using the three in vitro models (Fig. 2). Among these fractions, HF showed the higher activity as compared to CF, whereas EF remained inactive in all the three models. The EC_{50} and IC_{50} values in glucose uptake by yeast cells, Hb-glycosylation assay and α-amylase inhibition assay were found to be 400 µg/mL ($y = 0.0439x + 26.352$, $R^2 = 0.9921$), 300 µg/mL ($y = 0.0433x + 35.48$, $R^2 = 0.9926$) and 570 µg/mL ($y = 0.0501x + 21.686$, $R^2 = 0.9915$) respectively.

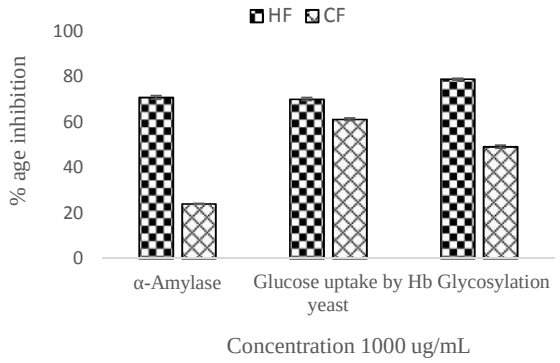
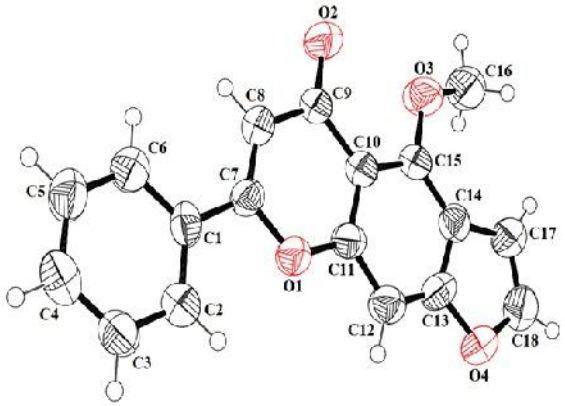
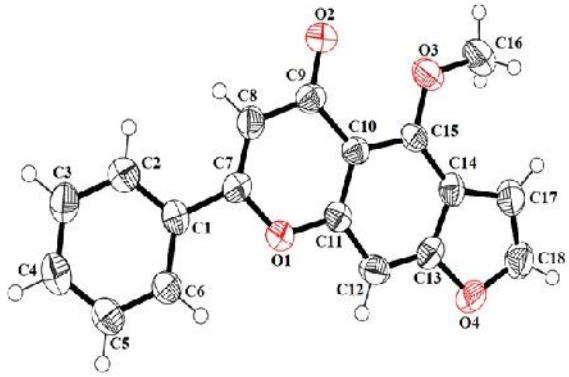


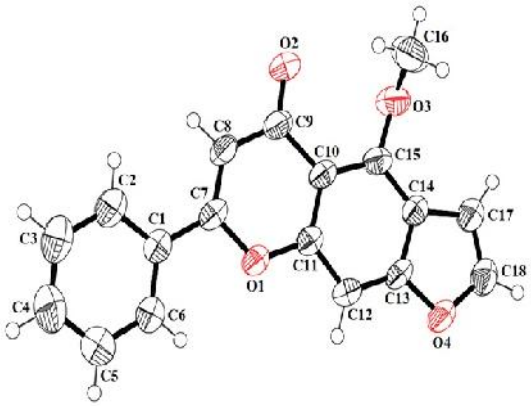
Fig. 2 Antidiabetic screening of different fractions (HF and CF) of HE of flowers of *Pongamia pinnata* (L.) Pierre



3 (a)



3 (b)



3 (c)

Fig. 3 Ortep diagrams of three isomers **3(a)** HE1PU, **3(b)** HE2NEWPU and **3(c)** HE2NNPU of 4-Methoxy-7-phenyl-5H-furo[3,2-g][1] benzopyran-5-one drawn at 50% probability level, Hydrogen atoms are shown by small circles of arbitrary radii

Then, the most active HF was subjected to activity-guided isolation using the α-amylase inhibition assay.

For activity-guided isolation, the α-amylase inhibition activity model was selected because the activity of the extract and fraction was significantly higher than the standard in this model. The inhibition of carbohydrate hydrolyzing enzymes such as α-amylase is considered an initial and primary approach to control postprandial hyperglycemia [23]. The higher activity of HF indicated that it contained active constituents which could inhibit the hydrolysis of polysaccharides having α-glycosidic linkage. The activity-guided isolation using this model gave compound 1 which at the equivalent concentration (1000 µg/mL) showed higher antidiabetic activity (82.97%) as compared to standard (ACb.) (43.46%). In dose-dependent relationship, IC_{50} values for compounds 1 was found to be 300 µg/mL ($y = 0.0484x + 33.822$, $R^2 = 0.9907$).

The compound 1 obtained from column chromatography upon crystallization produced three types of crystals. All the crystalline forms indicated a single spot on TLC having bright-yellow fluorescence at 366 nm and a black spot at 254nm after spraying with aluminium chloride spraying reagent. These crystals further gave a single peak by HPLC at different selected wavelengths (See Supplementary material, Fig. 14). Single-crystal X-Ray diffraction (XRD) revealed that the three crystalline forms were chemically 4-Methoxy-7-phenyl-5H-furo [3,2-g][1] benzopyran-5-one were denoted as HE1PU (3A), HE2NEWPU (3B) and HE2NNPU (3C) in Fig. 3.

HE1PU (4-Methoxy-7-phenyl-5H-furo[3,2-g][1] benzopyran-5-one) contains benzene ring A (C1-C6), the 4H-pyran-4-one moiety B (C7-C11/O1/O2), the anisole moiety C (C10-C16/O3) and the furan ring D (C13/C14/C17/C18/O4) which are planar with r. m .s deviation of 0.0062, 0.0220, 0.0556 and 0.0043 Å, respectively. The dihedral angles A/B, B/C and C/D are 2.68 (23)° 3.85 (18)°, 3.71 (26)°, respectively. These dihedral angles show that the moieties are almost parallel to each other. The molecules are connected through C-H…O bonding, where CH is from the methoxy group of anisole moiety and O-atom is from carbonyl oxygen of 4H-pyran-4-one moiety to form a zigzag pattern (Table 1, Fig. 4).

Table 1 Hydrogen-bond geometry (Å, º) for HE1PU

D-H···*A*	*D*-H	H···*A*	*D*···*A*	*D*-H···*A*
C16-H16*A*···O2^{i}	0.96	2.56	3.523 (7)	176
C18-H18···O2^{ii}	0.93	2.62	3.493 (7)	157

Symmetry codes: *(i) x*, −*y*+1/2, *z*+1/2; *(ii) x*, *y*, *z*+1.

HE2NEWPU (4-Methoxy-7-phenyl-5H-furo [3,2-g][1] benzopyran-5-one) has benzene ring A (C1-C6), the 4H-pyran-4-one moiety B (C7-C11/O1/O2), the anisole moiety C (C10-C16/O3) and the furan ring D (C13/C14/C17/C18/O4) are planar with r. m .s deviation of 0.0021, 0.0217, 0.0557 and 0.0067 Å, respectively. The dihedral angles A/B, B/C and C/D are 2.70 (18)° ,

3.53 (13)°, 3.62 (20)°, respectively. These dihedral angles show that the moieties are almost parallel to each other. The molecules are connected through C-H…O bonding to form R_3^2 (16) loop and forming polymeric sheets, where CH is from methoxy group of anisole moiety and also of furan ring and O-atom is from carbonyl oxygen of 4H-pyran-4-one moiety (Table 2, Fig. 5).

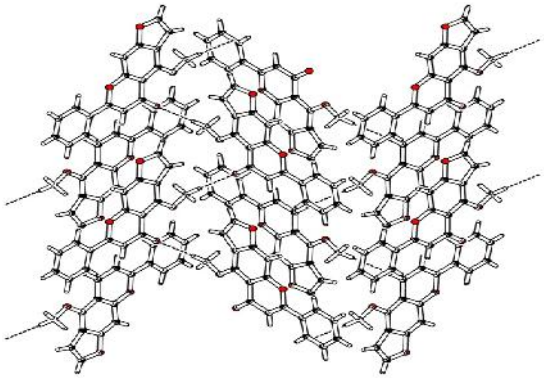


Fig. 4 Packing diagram of HE1PU (4-Methoxy-7-phenyl-5H-furo[3,2-g][1] benzopyran-5-one) showing that molecules are connected in a zigzag way.

Table 2 Hydrogen-bond geometry (Å, º) for HENEWPU

D-H···A	D-H	H···A	D···A	D-H···A
C16-H16B···O2^{i}	0.96	2.56	3.521 (5)	176
C18-H18···O2^{ii}	0.93	2.59	3.472 (4)	159

Symmetry codes: *(i) x, −y*+1/2, *z*−1/2; *(ii) x, y, z*−1.

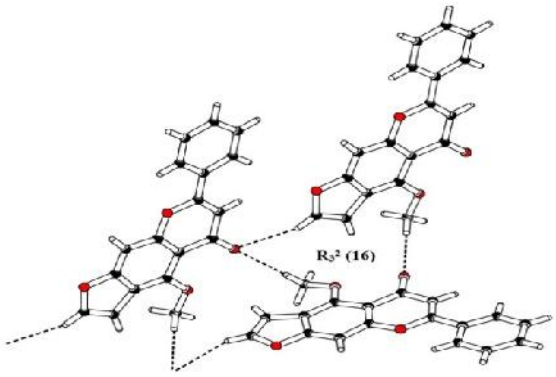


Fig. 5 Packing diagram of HE2NEWPU (4-Methoxy-7-phenyl-5H-furo[3,2-g][1] benzopyran-5-one) showing dimerization of molecules.

HE2NNPU (4-Methoxy-7-phenyl-5H-furo[3,2-g][1]benzopyran-5-one), has first benzene ring A (C1-C6), the 4H-pyran-4-one moiety B (C7-C11/O1/O2), the second benzene ring C (C10-C15) and the furan ring D (C13/C14/C17/C18/O4) in the planar form with r. m. s deviation of 0.0031, 0.0101, 0.0057 and 0.0018 Å, respectively. The dihedral angles A/B, B/C and C/D are 16.49 (3)° , 0.76 (2)°, 0.15 (3)°, respectively. The methoxy group is not in the plane of parent benzene ring C (C10-C15) but O3 and C16 are at a distance of 0.109 (2) and -1.089 (4) Å, respectively from the mean plane C. These dihedral angles show that the other moieties are almost parallel to each other. The molecules are connected through C-H…O bonding to form C (8) chains, where CH is from furan ring and O-atom is from carbonyl oxygen of 4H-pyran-4-one moiety as shown (Table 3, Fig. 6).

Table 3 Hydrogen-bond geometry (Å, º) for HE2NNPU

D-H···*A*	*D*-H	H···*A*	*D*···*A*	*D*-H···*A*
C16-H16*A*···O2	0.96	2.41	2.995 (3)	119
C18-H18···O2^{i}	0.93	2.50	3.305 (3)	145

Symmetry code: (*i*) *x*, *y*+1, *z*.

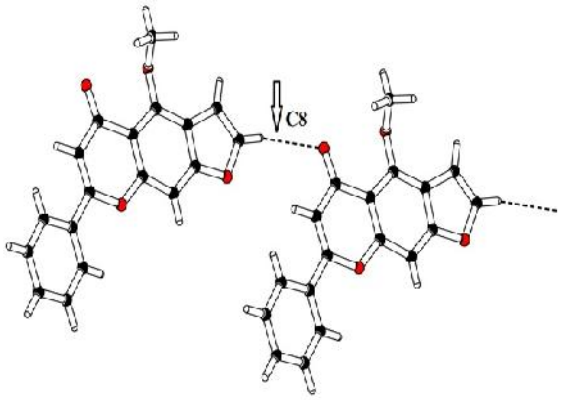


Fig. 6 Packing diagram of HE2NNPU (4-Methoxy-7-phenyl-5H-furo [3,2-g][1] benzopyran-5-one) showing C8 linkage.

These XRD results indicated that these three isomers were having different dimensions and bond angles that made them different from each other. Chemically, the isolated compound belongs to furanoflavonoid which has been isolated for the first time from flowers of the plant through antidiabetic activity-guided isolation. The literature review indicated that the stated compound was isolated from stem [24] and the roots of this plant and alpha-glucosidase inhibitory activity were performed on it [25]. This compound is also reported in the roots of *Gelonium multiflorum* A.Juss. [26] and *Fordia cauliflora* Hemsl. [27]. The compound has also been investigated for antifungal activity [28]. Therefore, this is the first report regarding the presence of this compound in flowers of the plant. The XRD parameters of the three isomeric forms were also different from the compound having the same chemical formula, isolated from the stem and root of *P. pinnata.* The difference in parameters is due to the varying arrangement of the methoxy group at benzopyran. Hence, the compound exists in three isomers which is also the first report.

The antidiabetic compound isolated from flowers of the plant is a flavonoid which is a secondary metabolite. There are several reports which indicated that flavonoids are effective substitutes for the treatment of diabetes as well as its associated diseases [29-32]. Flavonoids have strong antioxidant properties that concurrently decrease oxidative stress issues which are the main cause of diabetes-associated complications [33]. Flavonoids are also reported to protect human beings from diabetes by improving glucose metabolism, lipid profile, regulating the hormones and enzymes functions [34].

The possible mechanisms of antihyperglycemic activity of the isolated compound 1 and standard acarbose was also assessed by molecular docking. Various binding interactions with hyperglycemia-related protein targets were assessed and their scores of binding affinities were given (See Supplementary material, Table 2). These results showed that scores of binding affinities of isolated furanoflavonoid were higher than standard ACb. drugs. The binding conformations relative to the best binding poses for proteins where affinities were the highest are shown (See Supplementary material, Fig. 15-16). The composition of target binding proteins that form H-bonds with ligands are shown (See Supplementary material, Table 3). The results also confirmed that the affinity of isolated compound 1 for alpha-amylase-1 (1z32) and pancreatic alpha-amylase (1xcw) target proteins were higher i.e. (6.8-7.9) and (6.0-7.8) than standard acarbose drug (6.5-7.1) and (6.6-7.3) respectively which also supported the results of compound 1.

Conclusion

The antidiabetic screening of different extracts of flowers of *P. pinnata* and activity-guided isolation resulted in the isolated compound 1 in three isomeric forms named as 4-Methoxy-7-phenyl-5H-furo [3,2-g][1] benzopyran-5-one. The isolated compound belongs to the furanoflavonoid group and exhibits higher antidiabetic activity as compared to the standard, ACb. Molecular docking studies also indicated that the isolated compound binds to target proteins with a higher binding score as compared to the standard, ACb. The compound should be investigated further to refine this compound as a lead for antidiabetic targets.

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Conflict of Interest

The authors declare no conflict of interest.

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