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Isolation, structural characterization, and *in silico* evaluation (ADMET, PASS, and molecular docking) of a novel flavonol-di-O-xyloside from mango honey

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The present research work incorporates isolation and structure elucidation of a new compound 3,4'-dihydroxyflavonol-5,8-di-O-β-D-Xyloside (**E**), from mango honey. The compound has been separated on silica column chromatography and further purified by preparative HPLC instrument containing column; Gemini (250×4.6 mm, 5 µ) with mobile phase (A = 20 mM ammonium bicarbonate in water, B = acetonitrile). The compound has been subjected to ADMET analysis and the results indicate that the compound can penetrate the CNS with permeability value 0.33 while Caco-2 cell permeability is favourable (–6.88 log cm/s) which demonstrates that the compound has drug-like characteristics. The docking study of compound has also been performed using the peroxisome proliferator-activated receptor gamma (PPARγ) protein as the target and the results show that compound 3,4'-dihydroxyflavonol-5,8-di-O-β-D-Xyloside (**E**) may act as a PPARγ agonist and could potentially find use in the treatment of diabetic dyslipidemia and non-alcoholic fatty liver disease (NAFLD).

Keywords: Mango Honey, 2D-NMR, Xyloside, ADMET, PASS, Anti-diabetic

In last few years, honey has drawn valuable attention of the researchers due to its medicinal and biological significance and now actively part of lifestyle as the replacement of sugar in several food items. Since ancient times, honey was traditionally used in therapeutic treatment, and has been reported to have antipyretic, antiseptic, antiviral, anti-inflammatory, antioxidant, wound healing property, anti-microbial, and also for diabetic control[1,2]. These effects have been attributed to the physiologically active secondary metabolites present in honey like polyphenols, flavonoids, saponins, alkaloids, glycosides of anthraquinone, glycosides cardiac steroids, flavonoids, and reducing compounds[3-6].

Previous research report indicates that the honey has beneficial or potential effects on the gut microbiota, in the gastrointestinal tract (GIT), in the liver, as well as on the pancreas which improve glycemic control and metabolic imbalance[7]. Previous research indicates that, various substances which may be natural/synthetic with anti-diabetic effect can modulate key glucose-regulating hormone (insulin)[8] and in comparison, to dextrose, honey samples shown lower increase in serum insulin and C-peptide level[9].

In journey of finding of novel compounds in nature, a new compound 3,4'-dihydroxyflavonol-5,8-di-O-β-D-Xyloside, isolated from honey. The honey sample was collected from a Mango tree, found in Gonda district, UP. The sample was treated with acetic anhydride and pyridine to prevent the linkage between sugar and non-sugar moieties. Resulted acetylated mixture was extracted by chloroform and water and concentrated chloroform extract was undergoes column chromatography for purification. Structure elucidation of compound was done by ^{1}H, ^{13}C NMR, HSQC, HMBC, and COSY experiments. LCMS analysis confirmed the purity and molecular weight of compound. The compound undergoes molecular docking with PDB ID: 7E0A of PPARγ and binding energy (ΔG) of compound **E** against the protein 7E0A was –7.7 kcal/mole. On basis of docking results, authors said that compound may act as PPARγ agonist and can be used as for diabetic dyslipidemia and NAFLD (non-alcoholic fatty liver disease). Results of ADMET analysis of compound indicates that Blood-brain-barrier permeability value of compound in CNS was 0.33 while Caco-2 cell permeability was –6.88 log cm/s. Hence, compound

D, complies with Pfizer Rule, has acceptable drug-like characteristics, exhibit promising pharma cokinetic profiles.

Experimental Section

Material and methods

The sugars were visualized on TLC with 50% aqueous H_2SO_4 reagent. The absorbent for TLC was silica gel G (SRL) and CC silica gel (SRL, 60 - 120) mesh. Authentic sample of xylose was purchased from Aldrich chemicals. ^1H and ^{13}C NMR spectra were recorded at 400.23, 400.32, 400.60 & 400.80 and 100.64, 100.66, 100.74 & 100.78 MHz, respectively, on a Bruker Avance III, Avance III HD &Avance Neo spectrometer. Chemical shifts (δ, ppm) are reported relative to the solvent peak ($CDCl_3$: 7.25 [^1H] or 77.00 [^{13}C]; DMSO-d_6: 2.50 [^1H] or 39.43 [^{13}C]). Proton resonances are annotated as: chemical shift (δ), multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad), coupling constant (J, Hz), and number of protons. Water Acquity H Class UPLC attached with Waters SQD 2 mass spectrometers. Ionisation method: Electro spray, Capillary (kV) 3.50, Cone (V) 25.00, Source Temperature (°C) 150, Desolvation Temperature (°C) 400, Cone Gas Flow (L/Hr) -50, Desolvation Gas Flow (L/Hr) -750, DAD Wavelength range (nm): 200 to 400, Solvent A: 0.05% Formic acid in water and Solvent B: 0.05% HCOOH in ACN: Water (90:10) and flow rate: 0.8 mL/min.

Acetylation of Honey and purification of chloroform extract

The honey sample (40g), which were collected from mango orchard from Gonda district, UP, India, was concentrated under reduced pressure and residue (20g) was further lyophilized to dryness which resulted as brown colored dry powder (10g). The powdered sample (8g) was acetylated with acetic anhydride (8 mL) and pyridine (8 mL) and was heated for one hour and stirred overnight. The reaction mixture was concentrated to dryness followed by partitioned between water and chloroform. Resulted chloroform layer was again washed with water and then was dried over anhydrous sodium sulphate which was further concentrated on reduced pressure, obtaining 8.5g acetylated compounds mixture, which showed five spots on TLC named as a, b, c, d and e. The mixture of acetylated compounds was further purified on silica column chromatography.

Silica column chromatography of acetylated mixture of compound (e)

8g of acetylated mixture was chromatographed over 400g of silica gel. The eluting solvent used was chloroform, $CHCl_3$: MeOH of varying proportion, collecting fractions of 200 mL each. The fractions containing same compounds were mixed together and an impure compound was isolated (232mg) in their acetylated form.

Deacetylation of impure compound

The impure compound was treated with methanol and sodium metal which further dried under reduced pressure. Light brown coloured compound **E** (186mg), were obtained which was further purified by preparative HPLC.

Preparative HPLC solvent system and gradient profile

Preparative HPLC was done on Waters auto purification instrument. Column name: Long Gemini (250 × 4.6 mm, 5µ) operating at ambient temperature and flow rate of 1 mL/min. Mobile phase: A=20mM Ammonium bicarbonate in water, B=Acetonitrile; Gradient Profile: Mobile phase initial composition of 95% A and 5% B, then to 95% A and 5% B in 3 min, then to 50% A and 50% B in 18 min, then to 100% B in 19 min., held this composition up to 22 min. for column washing, then returned to initial composition in 23 min. and held till 25 min.

Molecular Docking

Preparation of protein and ligand for docking

The X-ray crystallographic structure of the PPARγ ligand binding domain (PDB ID: 7E0A) was retrieved from the Protein Data Bank. The protein structure was prepared for molecular docking studies using AutoDock Tools (ADT) version 1.5.7, developed by the Molecular Graphics Laboratory[10]. During receptor preparation, heteroatoms, water molecules, and any co-crystallized ligands were removed from the PDB structure. Polar hydrogen atoms were added, and Kollman united atom charges were assigned. The prepared receptor structure was then saved in the PDBQT format for docking analysis[11]. For ligand preparation, all compounds were geometrically optimized using the B3LYP functional with the 6-31G(d,p) basis set in the Gaussian 09 program package. The optimized structures were saved in.mol2 format. Subsequently, PyMOL (version 2.4.1.0) was

used to convert the .mol2 files into PDB format. Finally, the ligand structures were imported into AutoDock Tools and saved in PDBQT format for docking studies.

Docking Study by AutoDock Vina

All molecular docking simulations were performed using AutoDock Vina, developed at The Scripps Research Institute[12]. The docking results and intermolecular interactions between the receptor and ligand molecules were analyzed using BIOVIA Discovery Studio (Dassault Systèmes BIOVIA, San Diego, USA) and PyMOL[13]. For each ligand, the binding conformation with the highest binding affinity (lowest Vina score) and the lowest root mean square deviation (RMSD) were selected for further analysis. The electrostatic grid box for docking was defined with a grid spacing of 0.375 Å. The grid box center was set at X = 49.941 Å, Y = 4.078 Å, and Z = 89.746 Å, with dimensions of 40 × 40 × 40 Å along the X, Y, and Z axes, respectively.

Results and Discussion

Structure elucidation by NMR and LC-MS

The chloroform extract of acetylated honey sample was purified by silica column chromatography in which hexane, chloroform and chloroform: methanol (increasing polarity from 0.2-8%) was used as mobile phase gave impure mixture of compounds (232mg). Further, impure mixture was followed by deacetylation to convert non polar to polar mixture which further purified by reverse phase preparative HPLC using gradient profile with initial composition of 95% of A and 5% of B in 18 min then to 50% A and 50% B in 18 min, then to 100% B in 19 min., held this composition up to 22 min. for column washing, then returned to initial composition in 23 min and held till 25 min., gave a pure novel compound (65mg), named as 3,4'-dihydroxyflavonol-5,8-di-O-β-D-Xyloside (**E**), showed positive test of flavonoid (Shinoda test)[14,15]. FT-IR analysis of compound gave peaks at 1700 and 3300 cm^{-1}, confirmed the presence of >C=O and -OH groups, respectively (16). In UV spectrum, λ_{max} were commonly referred to as Band I (usually 300-380 nm) and Band II (usually 240-285 nm). Band I was associated with absorption due to the B-ring cinnamoyl system while Band II was the result of absorption involving the A-ring benzoyl system. The λ_{max} 206, 260, 275 and 315 nm[16] confirmed the presence of chromophore and auxochromes in 3,4'-dihydroxyflavonol-5,8-di-O-β-D-Xyloside (**E**).

Table 1 — NMR data of compound 3,4'-dihydroxyflavonol -5,8-di-O-β-D-xyloside

Position of Carbon/proton	^{1}H NMR (δ)	^{13}C NMR (δ)
2		146.90
3		138.36
3-OH	9.54, s, 1H	
4		177.92
5		150.42
6	6.60, d, *J*=2.28 Hz, 1H	124.39
7	7.49, d, *J*=2.96 Hz, 1H	110.83
8		141.60
9		156.46
10		112.19
1'		121.50
2'	6.60, d, *J*=2.28 Hz, 1H	112.19
3'	7.49, d, *J*=2.96 Hz, 1H	135.48
4'		162.11
4'-OH	10.68, s, 1H	

In ^{1}H and ^{13}C NMR spectrum (Table 1), the proton and carbon signal explained with the help of HSQC, COSY and HMBC spectra in DMSO-d_6 at 400 MHz NMR instrument. The ^{1}H NMR of compound showed two proton signals at δ_H 7.49 (d, *J* = 2.96 Hz, 3H; H-3', 5', H-7) and δ_H 6.60 (d, *J* = 2.28 Hz, 3H; H-2', 6', H-6) which confirmed the presence of two benzene ring in compound.

The ^{1}H NMR spectrum of compound showed low frequency signals at δ_H 9.54 (s, 1H, 3-OH) assignable to the chelated hydroxyl groups at C-3 and δ_H 10.68 for C-4', of a flavonoid moiety. Pentose sugar moieties confirmed by anomeric proton signal δ_H 5.58 (m, 1H, H-1'') and H-2'', H-3'' and H-4'' were δ_H 4.50, 4.15 and 4.89, respectively, while H-5'' values were δ_H 3.64 (m, 1H) and 3.78 (m,1H) matched with literature values of Xylose. The ^{13}C NMR spectrum of compound showed peak of C-4 at δ_C 177.92 confirmed the presence of carbonyl group, δ_C 138.36 (C-3) and 162.11 (C-4') for aromatic ring connected with hydroxyl group and aromatic carbon. Other peaks at δ_C 150.42 (C-5) and 141.60 (C-8) in ^{13}C NMR spectrum, were for carbon connected with pentose sugar which were later confirmed as Xylose by COSY. The ^{1}H-^{1}H COSY spectrum of compound E (DMSO-d_6) at 400 MHz provided key ^{1}H-^{1}H connectivity information. The correlation between δ_H 7.49 (H-3',5', H-7) and δ_H 6.60 (H-2',6', H-6) defined vicinal coupling within the aromatic ring (Fig. 1).

The linkage between carbon and proton was further confirmed by HMBC spectrum of compound. The cross peak at δ110.83 x 7.49 showed connectivity between C-7 and H-7 while cross peak at δ124.39 x 6.60 showed connectivity between C-6 and H-6. One more cross peak at δ150.42 x 7.49 is the connectivity between C-5 and H-7. Significant cross peak at δ109.62 x 4.50 gave information about sugar moiety and showed connectivity between anomeric carbon and H-2'' of pentose sugar and it was confirmed previous research[17]. Hydroxyl group of C ring at C-3 gave cross peak at δ146.90 x 9.54. In ring B of compound E, two cross peaks were present in HMBC spectrum, δ162.11 x 7.49 and δ112.19 x 6.60, represent the connectivity between C-4' and H-3', 5' & C-6' and H-2', 6', respectively (Fig. 2).

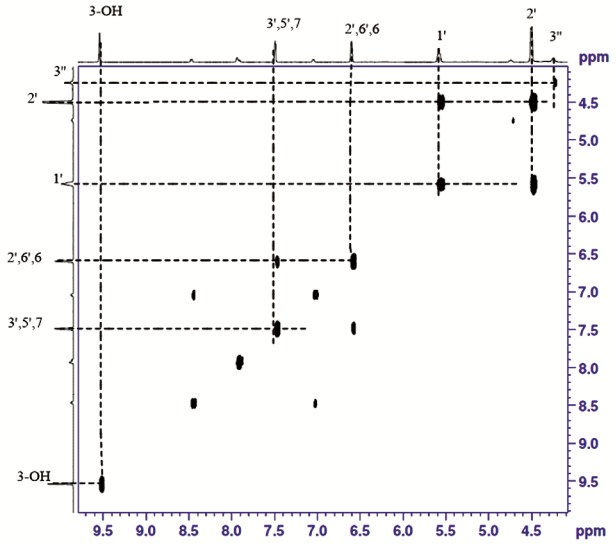

Fig. 1 — Cross peaks in COSY spectrum (^{1}H-^{1}H) connectivity

The structure of compound was further confirmed by acid hydrolysis of **E**, result of which the conversion of compound into non-sugar and sugar moiety. The sugar part of compound later confirmed as xylose (pentose sugar) by comparison with authentic sample of xylose by TLC. Structure of non-sugar moiety of **E**, was further confirmed by methylation and their data is matched with previous reported research[18]. This exercise confirmed that the structure of novel compound (**E**) and named as 3,4'-dihydroxyflavonol-5,8-di-O-β-D-Xyloside. Later, structure of 3,4'-dihydroxyflavonol-5,8-di-O-β-D-xyloside also confirmed by their LC-MS data in which molecular ion peak (*m/z*) = 550.47 Fragment ion peaks *m/z* = 551 $[M+H]^{+}$ followed by 285, 192 and 150 which were for fragments of flavonol, A & C ring and Xylose sugar confirmed the molecular formula [$C_{25}H_{26}O_{14}$] of novel compound. The fragmentation of 3,4'-dihydroxyflavonol-5,8-di-O-β-D-xyloside (**E**) suggested as given in Fig. 3.

Molecular Docking Result

The 3D molecular structure (PDB ID: 7E0A) of the PPARγ ligand binding domain was obtained from the Protein Data Bank in order to conduct molecular docking experiments on synthetic derivatives. The protein (7E0A) was used for docking studies by removing the co-crystallized inhibitor from the primary protease. The binding energy (ΔG) of compound **E** against the protein 7E0A was –7.7 kcal/mole.

Ligand **E** forms four O⋯H bonds with protein 7E0A. The first three bonds are formed by hydrogen atom of NH_2 group of the amino acid residue Glu-

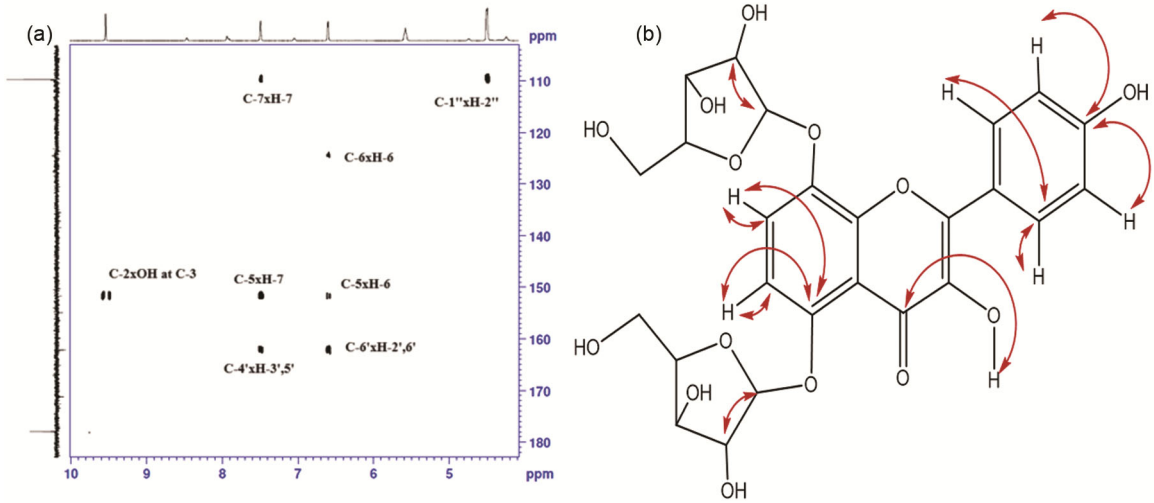

Fig. 2 — (a) HMBC spectra (b) ^{13}C-^{1}H NMR assignment with HMBC correlation of compound

A343 with O atom present at C-2″, 3″ and 5″ position of ligand **E** (Fig. 4) at a distance 2.16, 2.66 and 2.38 Å respectively. The fourth hydrogen bond formed between hydrogen atoms of NH_2 group of the amino acid residue Sar-A342 with O atom present at C-4 of ligand **E (b)** and has bond length 2.88 Å.

Based on the docking results, compound **E** may act as a PPARγ agonist and could potentially be used in the treatment of diabetic dyslipidemia and non-alcoholic fatty liver disease (NAFLD). The docking study was performed using the Peroxisome proliferator-activated receptor gamma (PPARγ) protein as the target.

ADMET Analysis of 3,4'-dihydroxyflavonol-5,8-di-O-β-D-Xyloside

ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) is a critical component of drug discovery and development, as it evaluates the pharmacokinetic and toxicological properties of chemical compounds, particularly potential drug candidates. Although a compound may exhibit strong biological activity against a target, it can still fail during development due to unfavorable ADMET characteristics. Indeed, many drug candidates are unsuccessful in clinical trials because of ADMET-related limitations rather than insufficient efficacy. In the present study, ADMET Lab 2.0 was employed to predict the ADMET properties of the investigated compounds[19]. The calculated ADMET parameter for compound **E** is summarized in Table 2.

Blood–brain barrier (BBB) permeability evaluates a compound's ability to penetrate the central nervous system (CNS) and is typically expressed as a value between 0 and 1.00, with permeability reported in cm/s. The predicted BBB permeability value for compound **E** was 0.33, indicating appreciable potential to cross the BBB. Caco-2 cell permeability is considered favorable when the predicted value exceeds −6.88 log cm/s. Based on this criterion;

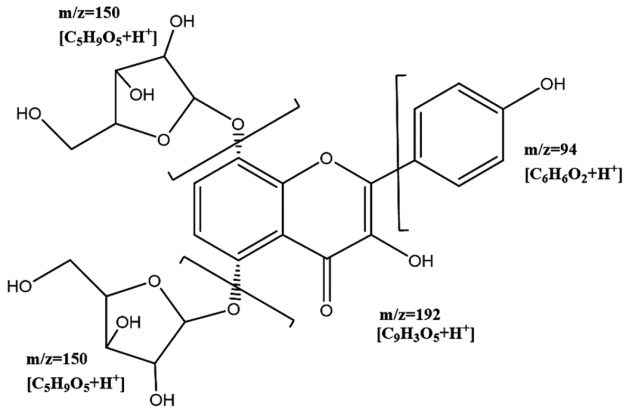

Fig. 3 — Mass fragments of compound 3,4'-dihydroxyflavonol-5,8-di-O-β-D-xyloside

Table 2 — Calculated ADMET predictions of compound 3,4'-dihydroxyflavonol-5,8-di-O-β-D-Xyloside (E)

ID	E
BBB	0.33
Caco-2	–6.88
MDCK	1×10^{-5} cm/s
HIA	0.319
CYP-2C19 inhibition	inhibitor
CYP-2C9 inhibition	inhibitor
CYP-2D6 inhibition	inhibitor
CYP-3A4 inhibtion	inhibitor
T1/2	–0.41

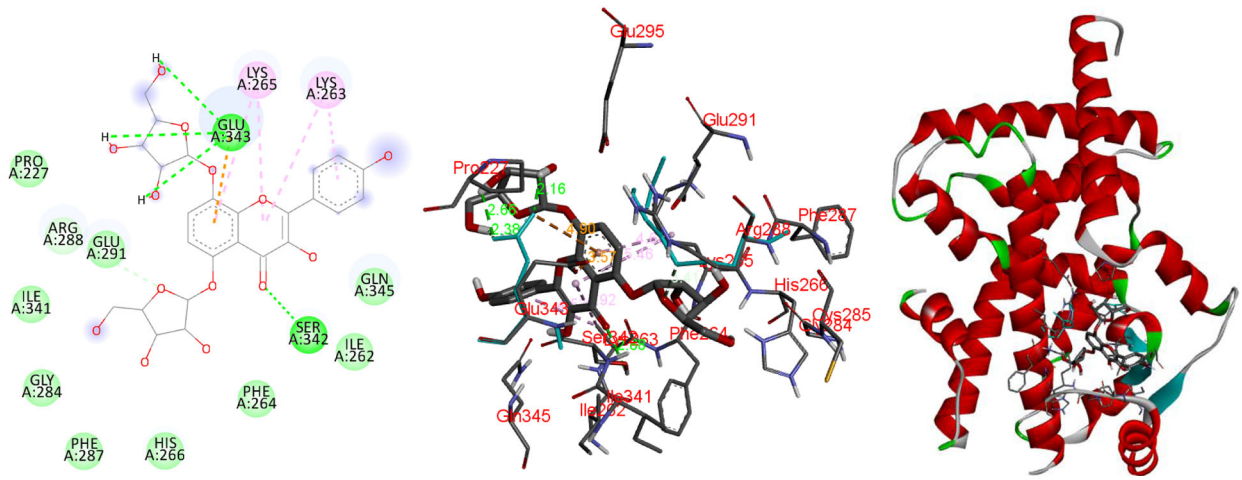

Fig. 4 — (A) Representation of 2D interaction of 1, (B) bond length with interactions and (C) Representation of docking results of E embedded into PPARγ ligand binding domain (PDB ID: 7E0A)

Table 3 — Prediction of the biological activities of 3,4'-dihydroxyflavonol-5,8-di-O-β-D-Xyloside *via* PASS

S. No.	Biological Activity	Pa Value
1	Anti-inflammatory	0.726
2	Anti-diabetic	0.675
3	Antiviral (Influenza)	0.637
4	Antifungal	0.633
5	Antibacterial	0.582
6	Antineoplastic	0.477

evaluated compound demonstrated good Caco-2 permeability.

Furthermore, ADMET analysis showed that compound **E** complies with the Pfizer Rule, suggesting acceptable drug-like characteristics. Overall, these findings indicate that isolated compound exhibit promising pharmacokinetic profiles and favorable drug-likeness, supporting their potential for further development.

Prediction of Biological Activities

The Prediction of Activity Spectra for Substances (PASS) server (https://way2drug.com/) was used to predict the potential pharmacological activities of the studied molecule based on its structural characteristics. The tool provides two key values for each predicted activity: the probability of activity (Pa) and the probability of inactivity (Pi). The value of Pa and Pi ranges from 0.00 to 1.00, allowing for an estimation of the biological effects of compound.

After completing molecular docking and ADMET analysis, the selected compound was further evaluated using the PASS server to obtain deeper insight into their possible pharmacological profiles Table 3.

As per the results from the PASS outcomes, revealed that the isolated compound may exhibited Anti-inflammatory, Anti-diabetic, Antiviral (Influenza), Antifungal and Antibacterial activities.

Conclusion

A new compound, 3,4'-dihydroxyflavonol-5,8-di-O-β-D-Xyloside, isolated from honey which was collected from mango tree, first time reported compound in nature from any source. Results of ADMET analysis indicates that reported compound showed acceptable drug like characters while molecular docking analysis indicates compound may act as a PPARγ agonist and could potentially be used in the treatment of diabetic dyslipidemia and non-alcoholic fatty liver disease (NAFLD). The docking study was performed using the Peroxisome proliferator-activated receptor gamma (PPARγ) protein as the target. The PASS analysis results revealed that the isolated compound may exhibit anti-inflammatory, anti-diabetic, antiviral (influenza), antifungal, and antibacterial activities.

Conflict of Interest

No conflict of interests.

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