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Potential Hypoglycemic Secondary Metabolites from *Argyreia nervosa* (Burm. f.) Bojer Influencing Human Gut Health †

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Abstract: In the last few decades, natural products from plants have got immense importance in human health due to their therapeutic multi-functionality. They have also been reported to enhance human gut health, another important factor in the overall human health. Diabetes Mellitus Type 2 (DM 2) is now a global concern with 6.28% of the world's population affected by it. Many hypoglycemic drugs currently available in the market are either directly or indirectly based on a number of plant secondary metabolites. In the current study, we aimed to find out the hypoglycemic secondary metabolites from leaf methanolic extract of *Argyreia nervosa* (Burm. f.) Bojer (Family: Convolvulaceae). In the in vitro experiment, this extract showed good inhibitory activity against Porcine Pancreatic Alpha-amylase (PPA) with IC_{50} value of 1.1 mg/mL. Presence of Quercetin and Ursolic acid was identified in the leaf methanolic extract with HPTLC, HPLC and MS analysis. The calculated IC_{50} values against PPA, for standard Quercetin and Ursolic acid were 16.5 µg/mL and 13.2 µg/mL respectively. In silico studies used both of these compounds as ligands against PPA (PDB ID: 1OSE) in AutoDock 4.2.6. Significant binding energies of −9.89 kcal/mol and −8.96 kcal/mol were seen for Quercetin and Ursolic acid respectively; while Acarbose (drug used as positive control) had binding energy of −12.48 kcal/mol. Both Quercetin and Ursolic acid strongly interacted with the pivotal amino acid residues like Glu233, Asp197 and Asp300, present at the active site of PPA, which upholds our in vitro experimental results. Both the compounds have exhibited beneficial effects on human gut health in DM 2 and related complications. Docking results of them with few intestinal markers significant in gut health would also be discussed.

Keywords: *Argyreia nervosa;* Diabetes Mellitus Type 2; Quercetin; Ursolic acid; human gut health; molecular docking

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1. Introduction

Plants have impacted modern medicine by supplying a number of phytochemicals having therapeutic multi-functionality. In recent years mankind has faced various disease outbreaks, where plant-based medicines have proven their supremacy. Today, among the different life-style diseases Diabetes mellitus Type 2 (DM 2) has got the attention of scientists and researchers worldwide. It is becoming a global concern as about 6.28% world's population is affected by it [1]. DM 2 is a metabolic disorder which is characterized by hyperglycemia due to escalating defects in insulin secretion on the background of insulin

resistance. Presently, there are a number of synthetic medicines available to treat this condition. But these medicines have some side effects on human health. So, the hunt for new plant based anti-hyperglycemic molecules is still on.

One of the most common strategies to treat DM 2 is to inhibit the isoforms of α-amylase enzyme. This enzyme plays a crucial role in digestion of food and catalyses the hydrolysis of starch, increasing blood sugar levels. The association between human pancreatic α-amylase and post prandial hyperglycemia has been published previously. The delayed starch digestion caused by α-amylase inhibition reduces the rate of absorption of glucose into the blood [2]. Apart from the usual DM 2 complications, recent reports have shown that the human gut microbiota also gets negatively affected due to DM 2. Almost 90% of the adult human gut microbiota consists of either Bacteroidetes or Fermicutes. In DM 2 patients, the ratio of Bacteroidetes to Fermicutes decreased and there was a significant drop in the abundance of Bifidobacteria [3].

Plant secondary metabolites belonging to different classes such as phenols, flavonoids, terpenes, glycosides, alkaloids and saponins have been reported for their α-amylase inhibitory potential. Amongst them, flavonoids have been extensively studied for their positive effect on the gut microbiota of DM 2 patients [4]. *Argyreia nervosa* (Burm. f.) Bojer, a medicinal vine has been utilized since many years for its therapeutic properties [5]. Our own group has shown earlier that the flavonoids from *A. nervosa*, namely, Vitexin, Myricetin, Isoquercetin and Rutin have strong α-amylase inhibitory potential [6]. But none of the earlier studies have reported the effect of secondary metabolites of *A. nervosa* on human gut microbiota.

Toll-like receptors (TLRs) are the pattern recognition receptors present in the immune cells and intestinal epithelial cells of humans. They play pivotal role in initiating the inflammatory response towards broad spectrum of microbial components and control activation of both innate and adaptive immunity. Scientific reports have shown an increase in the activity and expression of TLR2 in both type-I and type-II diabetes [7]. It is reported that the microbial consortium present in the intestine is one of the important factors regulating the TLR activation. However, it is also well studied that the dietary habits modulate the gut microbiota composition [8].

In the present study, we identified two important, pancreatic α-amylase (PPA) inhibitory secondary metabolites, namely, Quercetin and Ursolic acid from *A. nervosa* that also impact the human gut microbiota. The identification of secondary metabolites was achieved by HPLC and ESI/MS analysis and the PPA inhibitory capacity was demonstrated by *in-vitro* quantitative DNSA assay. Further the results were validated by the molecular docking studies using the crucial proteins involved in the food digestion (PPA) and human gut microbiota stability (TLR2).

2. Materials and Methods

2.1. Collection and Identification

The leaves of *Argyreia nervosa* (Burm. f.) Bojer. were collected from Savitribai Phule Pune University campus. (Latitude 18.549120° N and Longitude 73.828120° E). The collected specimen has been deposited and identified at the Botanical Survey of India, Western Regional Centre, Pune-7 with voucher number (BSI/WRC/IDEN.CER./2020/94).

2.2. Preparation of Leaf Extracts

The hot extraction method was followed using Soxhlet apparatus to prepare leaf extracts. The sequential extraction using six solvents with increasing polarity viz. pet ether, chloroform, ethyl acetate, acetone and water was carried out. These extracts were concentrated using rotary evaporator and stored at 4 °C for further use.

2.3. Preliminary Phytochemical Analysis

The preliminary phytochemical tests were performed in triplicates to check the presence of different phytochemicals [9].

2.4. Qualitative Starch-Iodide assay

The preliminary check of PPA inhibition potential of leaf extracts was carried out by method proposed by Zinjarde et. al. [10] and detailed in Kulkarni & Kamble [6].

2.5. HPTLC Analysis

2.5.1. Preparation of Standard Stock Solutions

The standard solutions of Quercetin (Sigma-Aldrich, St. Louis, MO, USA, Catalogue No. Q4951) and Ursolic acid (Sigma-Aldrich, USA, Catalogue No. U6753) were prepared at a concentration of 1 mg/mL each in absolute methanol. Ultra-sonication ensured the complete dissolution.

2.5.2. Sample Preparation

The dry methanol leaf extract of *A. nervosa* was accurately weighed to 5 mg and redissolved in 1 mL methanol. This sample was sonicated and filtered through 0.45 µm membrane filter prior to application on TLC plate.

2.5.3. Instrumentation and Experimental Conditions

Chromatography was performed according to the method reported with slight modifications [11]. The HPTLC system utilized was of CAMAG (CAMAG, Muttenz, Switzerland). The mobile phases Toluene: Ethyl acetate: Formic acid (5:4:1 *v*/*v*/*v*) and Toluene: Acetone: Formic acid (7.8:2.2:0.15 *v*/*v*/*v*) were used for separation of Quercetin and Ursolic acid respectively. The data obtained was analysed by WinCATS software (Version 1.4.5) measuring peak areas.

2.6. HPLC Analysis

HPLC analysis was carried out on Waters 2998 HPLC system (MA, USA) coupled with photodiode array detector at Central Instrumentation Facility of S. P. Pune University. The eluted fractions were monitored at 254 nm and were collected at different time points. These collected fractions were checked for their PPA inhibitory potential as mentioned in Section 2.4 and the positive fractions were carried forward for ESI/MS analysis to detect the active compounds.

2.7. ESI-MS Analysis

Electrospray ionization mass spectral measurements were performed on Bruker Nano-Advance UHPLC LC-MS-MS with TOF analyzer at Central Instrumentation Facility of S. P. Pune University. The dry methanolic extract was redissolved in HPLC grade methanol and filtered through 24 µM filter before injecting into the mass spectrometer.

2.8. Quantitative 3,5-dinitrosalicyclic Acid (DNSA) Assay

To quantify the PPA inhibition, widely used DNSA assay was performed according to the method proposed by Miller in 1959 [12] and the calculations were performed using formulae detailed by Kamble et.al [13].

2.9. Statistical Data Analysis

Each experiment was performed in triplicates. The presented data are expressed as mean ± SEM (Standard Error of Mean). ANOVA was performed for statistical analysis using PAST version 4.11. The p values ≤ 0.05 were considered as significant.

2.10. In Silico Docking Studies

2.10.1. Protein Preparation

The 3D crystal structures of PPA (PDB ID: 1OSE) and TLR-2 (PDB ID: 5D3I) were retrieved from Protein Data Bank. Prior to docking, the protein was prepared by removing pre-existing water and ligand molecules using Discovery Studio Visualizer v20.1.0.19295. Polar hydrogen atoms and Kollman charges were added using AutoDock 4.2.6 software [14]. This processed protein was subsequently saved as PDBQT file and used for docking.

2.10.2. Ligand Preparation

The 3-D structures of ligand molecules were obtained from PubChem database in SDF format. Using Discovery Studio Visualizer, these ligands were converted in PDB format.

2.10.3. Docking Studies

The prepared ligands were docked on the receptor proteins PPA and TLR-2 using AutoDock 4.2.6. For PPA, a grid box with spacing 0.375A° centred at (45 A° × 40 A° × 44 A°) was generated at the centre of the co-crystallized ligand (Acarbose, positive control) [13]. For TLR-2 the grid box with spacing 0.375 A° centred at (100 A° × 90 A° × 90 A°) was generated around the binding pocket detected with the help of CASTp online server [15]. The Lamarckian Genetic Algorithm was used for searching the best conformer of ligand molecules. In the docking process, total 10 conformers were taken into consideration for each compound. From these 10 conformers, the one with the lowest binding energy and inhibition constant was regarded as having the highest affinity towards PPA and TLR2. Discovery Studio Visualizer was used to visualize docked conformations of interacting ligand molecules with selected proteins.

3. Results

3.1. Preliminary Phytochemical Analysis

Although the phytochemical analysis was performed for all the prepared extracts, here we present the data related to methanol extract as it showed good PPA inhibition. The preliminary phytochemical analysis of methanol leaf extract of *A. nervosa* showed presence of phenols, flavonoids, terpenes and alkaloids.

3.2. Qualitative Starch-Iodide Assay

The qualitative starch-iodide assay was performed and analyzed on the basis of color of the reaction which is related to the starch-iodine complex formation. The deep blue color of the reaction suggested that the PPA enzyme was inhibited by the plant secondary metabolites present in the extract keeping the starch undigested. The yellow color of the reaction indicated complete digestion of starch by fully active PPA enzyme. The brownish color of the reaction suggested the partial degradation of starch. In this assay, chloroform, acetone and methanol extracts showed good inhibitory activity. But the methanol extract was carried forward as the deep blue color of the inhibition persisted even after 24 h.

3.3. HPTLC Analysis

Through HPTLC analysis it was observed that the methanolic leaf extract of *A. nervosa* possessed two important plant secondary metabolites, namely, Quercetin and Ursolic acid. Quercetin was separated and detected on the TLC plate (Figure 1A,B) and Ursolic acid was detected and separated on TLC plate (Figure 2A,B).

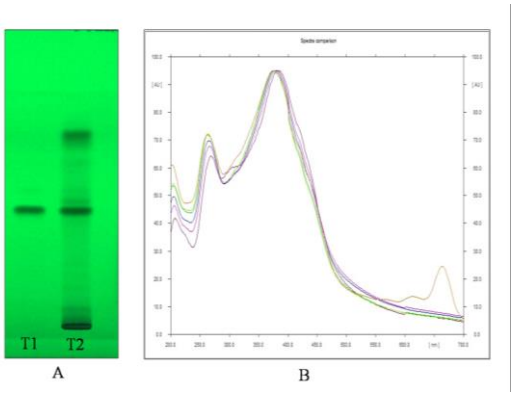

Figure 1. (**A**) Image of HPTLC separation of leaf methanolic extract of *A. nervosa*. Track T1 represents Quercetin standard and track T2 represents leaf methanolic extract of *A. nervosa* with matching band of quercetin with Rf value of 0.48. (**B**) Matching identity of Quercetin in standard and sample track by comparison of UV-VIS overlay spectra at 380 nm.

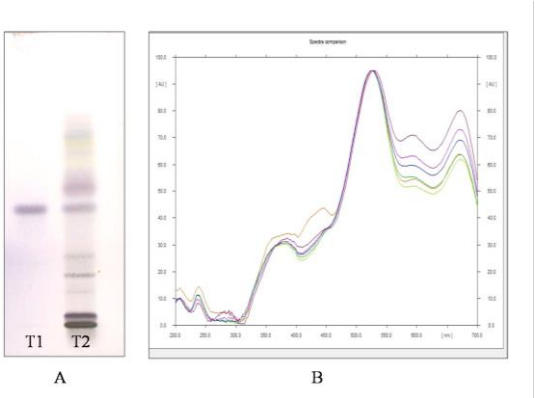

Figure 2. (**A**) Image of HPTLC separation of leaf methanolic extract of *A. nervosa* after derivatization with anisaldehyde-sulphuric acid reagent. Track T1 represents Ursolic acid standard and track T2 represents leaf methanolic extract of *A. nervosa* with matching band of Ursolic acid with Rf value 0.57. (**B**) Matching the identity of Ursolic acid in standard and sample track by comparison of UV-VIS overlay spectra at 540 nm.

3.4. HPLC Analysis

The crude methanolic leaf extract was subjected to the HPLC analysis. The peaks of this sample were collected at 3 different time points, namely, at 5 min, 6–12 min (5 components) and 14 min (Figure 3). These fractions were subjected to starch-iodide assay. Among these, fraction collected at 14 min showed strong PPA inhibitory activity. Hence, this fraction was further analysed by ESI mass spectrometry.

3.5. ESI-MS analysis

The ElectroSpray Ionization-Mass Spectrometry (ESI-MS) analysis indicated the presence of two abundant peaks of molar mass 301.1400 and 467.1931 which are assigned to Quercetin and Ursolic acid respectively (Figure 4).

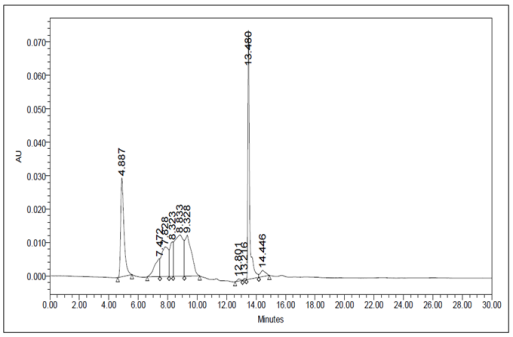

Figure 3. HPLC chromatogram of *A. nervosa* leaf methanol extract.

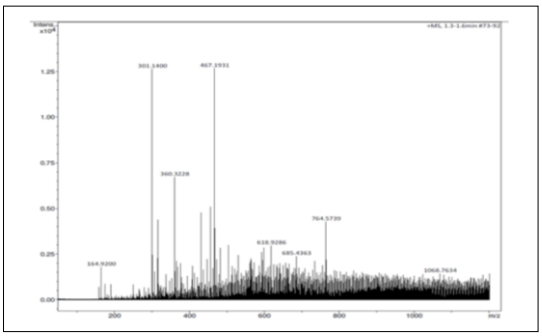

Figure 4. ESI-Mass spectrum of the methanolic extract fraction eluted at 14 min by HPLC.

3.6. Quantitative 3,5-dinitrosalicyclic Acid (DNSA) Assay

As the methanol extract showed good results for qualitative starch-iodide assay, to quantify the result we performed DNSA assay. To demonstrate that this inhibitory activity was due the presence of secondary metabolites Quercetin and Ursolic acid, separate DNSA assay was also performed for the standards alone. The percent inhibition of PPA increased linearly as the concentration of methanol extract increased. The calculated IC_{50} value of leaf methanol extract was 1.1 mg/mL. The individual IC_{50} values for Quercetin and Ursolic acid were 16.5 µg/mL and 13.2 µg/mL respectively.

3.7. In Silico Docking Studies

3.7.1. With PPA

Results of molecular docking using AutoDock 4.2.6 are shown in Table 1 indicating the docking scores and interaction details for identified compounds: Quercetin and Ursolic acid bound with PPA. The binding energy of ligand molecules is presented in kcal/mol. These studies were performed in comparison with the positive control Acarbose. The interactions between the protein-ligand complexes were analysed by Discovery Studio Visualizer. The crucial amino acids Glu233, Asp197 and Asp300 in α-amylase were found to be involved in bond formation between protein and ligand molecule underpinning the theory of α-amylase inhibition.

Table 1. Binding energies of the ligands and Acarbose with PPA.

Sr. No.	Compound Name	Binding Energy Kcal/mol	Interaction	Amino Acid Residues
1	Quercetin	−9.89	3 H-Bonds	**Asp197**, Tyr62, Gln63
2	Ursolic acid	−8.96	1 H-Bond	**Asp300**
			Van der Waal	**Glu233**, Arg195, **Asp197**, His299, Trp58, His305, Gly164, Gln 63

3	Acarbose	−12.48	9 H-Bonds	His305, His101, Lys200, Tyr151, His201, **Glu233**, **Asp300**, His299, Trp59
			Van der Waal	Val163, Ile235, Leu162, Gly306, Ala198, Arg195, Trp58, **Asp197**, Leu165, Gln63

3.7.2. With TLR-2

Quercetin interacted with TLR2 via five hydrogen bonds through amino acid residues VAL303, THR330, PHE295, GLY293 and TYR326 while Ursolic acid bound to TLR2 via a single amino acid residue, ASP327 (Figure 5). The binding energies were −11.55 kcal/mol and −11.85 kcal/mol for Quercetin and Ursolic acid respectively. From the docking analysis it can be concluded that the flavonol, Quercetin is a strong candidate for inhibition of TLR2 as compared to terpenoid Ursolic acid, as it interacted with TLR2 via a greater number of hydrogen bonds.

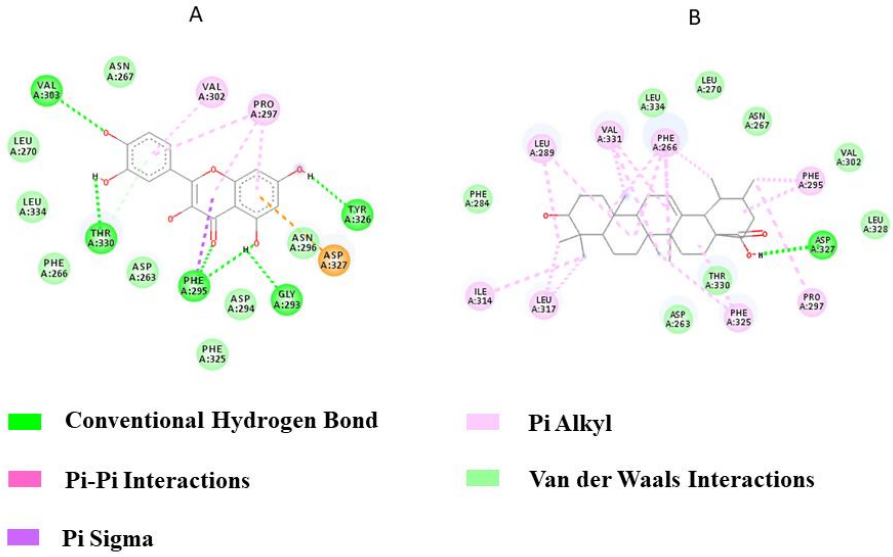

Figure 5. 2D representation of TLR2 Protein-Ligand interactions (**A**) Quercetin (**B**) Ursolic acid.

4. Discussion

Plant derived natural products have been known for their diverse and versatile therapeutic properties. These have been explored for their efficacy against many diseases such as cancer, arthritis, malaria, Alzheimer's and diabetes [16].

In the present study, the leaf methanolic extract of *A. nervosa* was explored for its α-amylase inhibitory activity. It showed presence of major classes of secondary metabolites viz. phenols, flavonoids, terpenes and alkaloids. In qualitative starch-iodide assay, methanol extract showed persistent PPA inhibition. To find out the active PPA inhibitory molecules in methanolic extract HPTLC and HPLC analysis was done. This unveiled the presence of two important secondary metabolites, namely, Quercetin and Ursolic acid. The presence of these secondary metabolites was validated by the ESI-MS analysis.

Quercetin, a well-known flavonoid is known for its α-amylase inhibitory activity. A study performed in rat pancreas, reported that Quercetin inhibited the α-amylase with IC_{50} value of 0.061 µM [17]. Ursolic acid, a terpenoid is known for its strong α-amylase inhibitory property. The in vitro study carried out by Wang et.al proved that Ursolic acid possessed α-amylase inhibitory potency with IC_{50} value of 0.482 ± 0.12 mg/mL [18]. We found IC_{50} values of Quercetin and Ursolic acid to be 16.5 µg/mL and 13.2 µg/mL (Section 3.6).

The molecular docking analysis showed that both Quercetin and Ursolic acid have binding affinity towards PPA, with binding energies of −9.89 kcal/mol and −8.96 kcal/mol

respectively. The positive control, Acarbose had binding energy of −12.48 kcal/mol. Thus, both the secondary metabolites have exceptionally good PPA inhibition potency. Molecular docking and molecular dynamics studies of Quercetin and Ursolic acid from *Morinda citrifolia* fruits against human pancreatic α-amylase demonstrated that both phytochemicals were good inhibitors with binding energies of −2.16 kcal/mol and −8.58 kcal/mol respectively [19].

It needs to be noted that although both Quercetin and Ursolic acid have shown strong PPA inhibitory potential with low IC_{50} values individually in our experiments, our methanolic extract containing these two active phytochemicals has shown a high IC_{50} value, indicating possibility for presence of certain additional molecules that are interfering with PPA inhibition. Thus, the crude extract warrants further purification.

A healthy gut microbiota is an essential aspect of overall health of the human body. Studies have shown that the gut microbiota plays a pivotal role in physiological processes in the body such as regulation of fat storage, immunity, biological barriers, nutritional absorption, growth and development [20]. Plants derived phenols, flavonoids, terpenoids and alkaloids have beneficial effects on the human gut microbiota.

A study conducted on STZ-induced diabetic peripheral neuropathy (DPN) rat models reported that these rats showed gut dysbiosis compared to control rats, as observed by fecal microbiome16S rRNA gene sequencing. Quercetin was found to be bactericidal against potential pathogenic bacteria such as *Oxalobacter*, *Klebsiella*, bacteria belonging to families Porphyromonadaceae and Oxalobacteraceae, positively correlated with DPN phenotypes and high ROS production levels. On the other hand, Quercetin enriched the abundance of *Actinobacteria,* that were negatively correlated with DPN phenotypes and ROS production levels [21].

Terpenoids also display anti-inflammatory, anti-viral, anti-cancer, anti-microbial and anti-diabetic activities. But so far, very few terpenoids have been investigated for their effects on gut microbiota. In the gut, terpenes are metabolized in liver as well as get biotransformed by intestinal microorganisms such as *Fusobacterium nucleatum*, *Bacteroides fragilis* and *Peptostreptococcus anaerobius* through the processes of hydrolysis and reduction. It is widely known that about 30% of diabetic people suffer from liver malfunction. Wan *et.al*, reported the repairing effect of Ursolic acid, a tri-terpenoid, on bacterial dysbiosis in liver fibrosis in mice. The potential beneficial bacteria, such as *Firmicutes*, *Lactobacillus* and *Bifidobacterium* were seen to be increased in the Ursolic acid group [22].

Time-dependent high glucose dose is known to induce marked increase in Toll-Like Receptors TLR2 and TLR4 mRNA and protein expression in human monocytes. TLR2 and TLR4 mediate the inflammation in activated cells via NF-ḱB signaling. Thus, targeting these inflammatory receptors by action of plant secondary metabolites may be an advantageous strategy to ameliorate the development of DM-2 and complications related to it. Especially, TLR2 is known to be responsible for production of several molecules including ROS and pro-inflammatory cytokines which contribute to the worsening of type II diabetes and related complications. Considering these facts, down-regulation of TLR2 can be used as a novel approach to treat type II diabetes [23].

5. Conclusions

The intent of present study was to find out the hypoglycemic secondary metabolites from leaf methanolic extract of *Argyreia nervosa* (Burm. f.) Bojer. The leaf methanolic extract contained Quercetin and Ursolic acid. They exhibited great α-amylase inhibitory activity in both in-vitro and in-silico experiments. For the first time, we have shown excellent in silico docking of both of these molecules on active site of TLR-2, a bridging molecule of inflammation and gut microbiota composition. Our current work proposed the multifunctional role of Quercetin and Ursolic acid as α-amylase inhibitors as well as human gut health enhancers. Further experimental studies would be carried out in this regard.

Author Contributions: Conceptualization, A.A.K. (Anjali A. Kulkarni); methodology, A.D.K. and A.A.K. (Anjali A. Kulkarni); software, A.D.K.; validation, A.D.K. and A.A.K. (Anjali A. Kulkarni); formal analysis, A.D.K.; investigation, A.D.K. and A.A.K. (Anjali A. Kulkarni); resources, A.D.K. and A.A.K. (Anjali A. Kulkarni); data curation, A.D.K., A.A.K. (Anupa A. Kumbhar) and A.A.K. (Anjali A. Kulkarni); writing—original draft preparation, A.D.K. and A.A.K. (Anjali A. Kulkarni); writing—review and editing, R.P.K., A.A.K. (Anupa A. Kumbhar) and A.A.K. (Anjali A. Kulkarni); visualization, R.P.K., A.A.K. (Anupa A. Kumbhar) and A.A.K. (Anjali A. Kulkarni); supervision, A.A.K. (Anjali A. Kulkarni); project administration, A.A.K. (Anjali A. Kulkarni); funding acquisition A.D.K. and A.A.K. (Anjali A. Kulkarni). All authors have read and agreed to the published version of the manuscript.

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References

1. Khan, M.A.B.; Hashim, M.J.; King, J.K.; Govender, R.D.; Mustafa, H.; Al Kaabi, J. Epidemiology of type 2 diabetes–global burden of disease and forecasted trends. *J. Epidemiol. Glob.* **2020**, *10*, 107. https://doi.org/10.2991/jegh.k.191028.001.
2. Tarling, C.A.; Woods, K.; Zhang, R.; Brastianos, H.C.; Brayer, G.D.; Andersen, R.J.; Withers, S.G. The search for novel human pancreatic α-amylase inhibitors: High-throughput screening of terrestrial and marine natural product extracts. *ChemBioChem* **2008**, *9*, 433–438. https://doi.org/10.1002/cbic.200700470.
3. Nie, Q.; Chen, H.; Hu, J.; Fan, S.; Nie, S. Dietary compounds and traditional Chinese medicine ameliorate type 2 diabetes by modulating gut microbiota. *Crit. Rev. Food Sci. Nutr.* **2019**, *59*, 848–863. https://doi.org/10.1080/10408398.2018.1536646.
4. Han, S.; Luo, Y.; Hu, Z.; Qin, D.; Luo, F. Targeting gut microbiota in type 2 diabetes mellitus: Potential roles of dietary flavonoids. *Food Biosci.* **2022**, *45*, 101500. https://doi.org/10.1016/j.fbio.2021.101500.
5. Galani, V.J.; Patel, B.G.; Patel, N.B. *Argyreia speciosa* (Linn. f.) sweet: A comprehensive review. *Pharmacogn. Rev.* **2010**, *4*, 172. https://doi.org/10.4103/0973-7847.70913.
6. Kulkarni, A.A.; Kamble, A.D. Flavonoids from *Argyreia nervosa* (Burm. f.) Bojer: A ready arsenal against pests as well as Diabetes. *Biol. Life Sci. Forum* **2020**, *4*, 56. MDPI. https://doi.org/10.3390/IECPS2020-08773.
7. Jialal, I.; Kaur, H. The role of toll-like receptors in diabetes-induced inflammation: Implications for vascular complications. *Curr. Diabetes Rep.* **2012**, *12*, 172–179. https://doi.org/10.1007/s11892-012-0258-7.
8. Tremaroli, V.; Bäckhed, F. Functional interactions between the gut microbiota and host metabolism.*Nature.* **2012**, *489*, 242–249. https://doi.org/10.1038/nature11552.
9. Trease, G.E.; Evans, W.C. *Pharmacognosy*, 15th ed.; Saunders Publishers: London, UK, 2002; pp. 42–44, 221–229, 246–249, 304–306, 331–332, 391–393.
10. Zinjarde, S.S.; Bhargava, S.Y. and Kumar, A.R. Potent α-amylase inhibitory activity of Indian Ayurvedic medicinal plants. *BMC Complement. Med. Ther.* **2011**, *11*, 5. https://doi.org/10.1186/1472-6882-11-5.
11. Rout, K.K., Singh, R.K., Barik, D.P.; Mishra, S.K. Thin-Layer chromatographic separation and validated HPTLC method for quantification of Ursolic acid in various *Ocimum* species. *J. Food Drug Anal.* **2012**, *20*, 22.
12. Miller, G.L. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* **1959**, *31*, 426–428. https://doi.org/10.1021/ac60147a030.
13. Kamble, R.P.; Ghosh, P.; Kulkarni, A.A. Identification of α-amylase inhibitory compounds from leaves of *Careya arborea* Roxb. and in silico docking studies. *S. Afr. J. Bot.* **2022**, *151*, 493–503. https://doi.org/10.1016/j.sajb.2022.05.018.
14. Rizvi, S.M.D.; Shakil, S.; Haneef, M. A simple click by click protocol to perform docking: AutoDock 4.2 made easy for non-bioinformaticians. *EXCLI J.* **2013**, *12*, 831.
15. Tian, W.; Chen, C.; Lei, X.; Zhao, J.; Liang, J. CASTp 3.0: Computed atlas of surface topography of proteins. *Nucleic Acids Res.* **2018**, *46*, W363–W367.
16. Ota, A.; Ulrih, N.P. An overview of herbal products and secondary metabolites used for management of type two diabetes. *Front. Pharmacol.* **2017**, *8*, 436. https://doi.org/10.3389/fphar.2017.00436.
17. Oboh, G.; Ademosun, A.O.; Ayeni, P.O.; Omojokun, O.S.; Bello, F. Comparative effect of quercetin and rutin on α-amylase, α-glucosidase, and some pro-oxidant-induced lipid peroxidation in rat pancreas. *Comp. Clin. Path.* **2015**, *24*, 1103–1110. https://doi.org/10.1007/s00580-014-2040-5.
18. Wang, J.; Zhao, J.; Yan, Y.; Liu, D.; Wang, C.; Wang, H. Inhibition of glycosidase by ursolic acid: In vitro, in vivo and in silico study. *J. Sci. Food Agric.* **2020**, *100*, 986–994. https://doi.org/10.1002/jsfa.10098.

19. Lolok, N.; Sumiwi, S.A., Muhtadi, A.; Susilawati, Y.; Hendriani, R., Ramadhan, D.S.F.; Levita, J.; Sahidin, I. Molecular docking and molecular dynamics studies of bioactive compounds contained in noni fruit (*Morinda citrifolia* L.) against human pancreatic α-amylase. *J. Biomol. Struct. Dyn.* **2022**, *40*, 7091–7098. https://doi.org/10.1080/07391102.2021.1894981.
20. Guo, P. and Wu, C.M. Gut microbiota brings a novel way to illuminate mechanisms of natural products in vivo. *Chin. Herb. Med.* **2017**, *9*, 301–306. https://doi.org/10.1016/S1674-6384(17)60109-6.
21. Xie, J.; Song, W.; Liang, X.; Zhang, Q.; Shi, Y.; Liu, W.; Shi, X. Protective effect of quercetin on streptozotocin-induced diabetic peripheral neuropathy rats through modulating gut microbiota and reactive oxygen species level. *Biomed. Pharmacother.* **2020**, *127*, 110147. https://doi.org/10.1016/j.biopha.2020.110147.
22. Wan, S.Z.; Liu, C.; Huang, C.K.; Luo, F.Y.; Zhu, X. Ursolic acid improves intestinal damage and bacterial dysbiosis in liver fibrosis mice. *Front. Pharmacol.* **2019**, *10*, 1321. https://doi.org/10.3389/fphar.2019.01321.
23. Sepehri, Z.; Kiani, Z.; Nasiri, A.A.; Kohan, F. Toll-like receptor 2 and type 2 diabetes. *Cell. Mol. Biol. Lett.* **2016**, *21*, 2. https://doi.org/10.1186/s11658-016-0002-4.

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