Fishing the targets of bioactive compounds from *Psidium guajava* L. leaves in the context of diabetes

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Abstract:

Over the last decade, *Psidium guajava* L. (guava) leaves have demonstrated their *in vitro* and *in vivo* effect against Diabetes mellitus (DM). However, there is a lack of literature concerning the effect of the individual phenolic compounds present in the leaves in DM disease. For this reason, the aim of the present work was the *in silico* evaluation of each phenolic compound. The identification of an 80% ethanol extract of guava leaves revealed the presence of 73 compounds. The *in silico* study carried out with the DIA-DB web server revealed that aldose reductase was the target protein with heterogeneous affinity. Naringenin exhibited the highest number of interactions with target proteins, and compounds like catechin, quercetin, naringenin and morin displayed similarities with known antidiabetic drugs. In conclusion, the computational workflow showed that guava leaves contain several compounds that can play a therapeutic role in the treatment of DM.

Keywords: *Psidium guajava* L., guava, leaves, in silico, diabetes mellitus, phenolic compounds.

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1. Introduction

Diabetes mellitus (DM) is one of the most serious and increasing health disorders in the world. Nowadays, this disease affects 382 million people, and it is expected that the number of affected could rise to 592 million people by 2035 [1]. The cause of clinical diabetes is due to a deficiency of the effect of insulin at the tissue level and it is usually accompanied by an increase in oxidative stress. This deficiency is caused by an autoimmune destruction or by the dysregulation of insulin release from the pancreatic B-cells (type 1 and 2, respectively) [2]. Therefore, treatment of DM is based on the use of clinical drugs which reduce blood glucose levels.

Furthermore, there is evidence that herbal medicines possess diabetic inhibitory properties through different mechanisms such as α-glucosidase, α-amylase, dipeptidyl peptidase IV (DPP-4), and protein tyrosine phosphatase 1B (PTP-1B) inhibition, as well as the activation of peroxisome proliferator-activated receptor γ (PPARG) [3]. In many plants, these effects have been associated with the presence of bioactive compounds which could be effective as adjuvant in diabetes therapy [4]. Regarding the plant drugs used in traditional medicine, the leaves of *Psidium guajava* L. have been widely employed as hypoglycaemic agents [2].

Guava tree (*P. guajava* L.) is originally from Mexico although it can grow in tropical and subtropical conditions. Apart from the anti-diabetic effect, different parts of this crop have exhibited *in vitro* and *in vivo*, properties against several diseases like diarrhoea and dysentery, and these activities have been related mainly to its phenolic composition, which is greater in the leaves than in the other parts of the tree [5]. Recent studies have revealed the phenolic profile of Spanish guava leaves and the relation of some of the compounds tentatively identified with their anti-diabetic properties [6,7]. Despite the comprehensive study, and the *in vitro* and *in vivo* assays conducted, it is still not clear which compounds

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from the extract are responsible for its anti-diabetic effect. Therefore, we propose in this work to carry out *in silico* anti-diabetic activity studies to identify the responsible bioactive compounds. We will use two approaches, a) prediction of the interaction of potentially bioactive molecules with relevant DM targets, providing the characterization of binding modes [8], and b) prediction of similarity of extract compounds against already known anti-diabetic agents, following the principle *"similar compounds bind to similar targets"* [9]. Based on these premises, the purpose of this work was to evaluate *in silico* the potential of every phenolic compound present in Spanish guava leaves against the principal targets related to DM.

2. Results and Discussion

2.1 Identification of the phenolic compositions

Tentative identification of phenolic compounds present in guava leaves via high performance liquid chromatography coupled to electrospray ionization and quadrupole time-of-flight mass spectrometry (HPLC-ESI-QTOF-MS) was accomplished due to a previous work performed by our research group [7] and data are summed up in Table 1. Due to the nature of the phenolic compounds present in the leaf extracts, both negative and positive ionization modes are employed in Table 1 for identification [10]. Despite being detectable by both ionization modes, most of phenolic subclasses are detected in negative mode because the sensitivity is better and they mainly produce the ion $[M-H]^-$ [10,11]. In contrast, positive mode is used for anthocyanins subclass since $[M]^+$ is the predominant ion specie generated due to its structure [11].

Furthermore, the identification of the compounds was achieved according to its retention time, mass spectra and literature. Accordingly, with Table 1, the retention time, calculated and experimental *m/z*, molecular formula, the score, and the error (ppm) are data obtained

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from HPLC-ESI-QTOF-MS for each compound. Briefly, the MassHunter Workstation Software (version B.06.00 Qualitative Analysis, Agilent Technologies) reports the Score, which means the feasibility between de mass spectra of the measured compound and the molecular formula that is reporting (in terms of accurate mass, isotope abundance pattern and spacing), and by the error (ppm) term, which reveals the difference amongst experimental and calculated mass/charge (*m/z*). It is noteworthy that the exact mass of the parent ion is characteristic of each compound as well as its fragmentation pattern.

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Table 1. Identification of phenolic compounds in *Psidium Guajava* L. leaves by HPLC-DAD-ESI-QTOF-MS

No.	Compound	rt (min)	*m/z* exp	*m/z* calc	Molecular Formula	Score	Error (ppm)
	Negative mode						
1	HHDP glucose isomer 1	1.929	481.0640	481.3406	C20H18O14	96.51	-2.55
2	HHDP glucose isomer 2	2.139	481.0638	481.3406	C20H18O14	99.09	-0.19
3	HHDP glucose isomer 3	2.516	481.0639	481.3406	C20H18O14	97.21	-2.24
4	Prodelphinidin B isomer	3.85	609.1276	609.5111	C30H26O14	97.84	-1.7
5	Gallic acid	4.022	169.0142	169.1116	C7H6O5	99.27	0.37
6	Pedunculagin/ Casuariin isomer 1	5.865	783.0699	783.5332	C34H24O22	98.57	-1.29
7	Prodelphinidin Dimer isomer 1	7.272	593.1311	593.5117	C30H26O13	96.51	-2.35
8	(epi)-gallocatechin isomer 1	7.814	305.0698	305.2595	C15H14O7	95.55	-3.32
9	Vescalagin/castalagin isomer	7.953	933.0649	933.6216	C41H26O26	99.19	-0.79

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10	Prodelphinidin Dimer isomer 2	8.119	593.1316	593.5117	C30H26O13	96.51	-2.35
11	Uralenneoside	9.387	285.0624	285.2268	C12H14O8	97.8	-2.69
12	Geraniin isomer 1	9.497	951.0749	951.6369	C41H28O27	99.56	-0.2
13	Pedunculagin/ Casuariin isomer 2	9.536	783.0699	783.5332	C34H24O22	98.39	-1.36
14	Geraniin isomer 2	9.652	951.0752	951.6369	C41H28O27	99.56	-0.2
15	Procyanidin B isomer 1	10.018	577.1367	577.5123	C30H26O12	95.68	-2.55
16	Galloyl(epi)catechin-(epi)gallocatechin	10.345	745.1420	745.6160	C37H30O17	96.9	-0.62
17	Procyanidin B isomer 2	10.356	577.1367	577.5123	C30H26O13	99.41	-0.61
18	Tellimagrandin I isomer	10.738	785.0851	785.5491	C34H26O22	99.13	-0.96
19	Pterocarinin A isomer 1	10.998	1067.122	1067.7521	C46H36O30	99.82	-0.11
20	Pterocarinin A isomer 2	11.208	1067.122	1067.7521	C46H36O30	98.39	-1.26
21	Stenophyllanin A	11.247	1207.1495	1207.8903	C56H40O31	98.64	-1.08
22	Procyanidin trimer isomer 1	11.247	865.1998	865.7645	C45H38O18	97.53	-1.59

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23	(epi)-catechin	11.258	289.0727	289.2601	C15H14O6	96.76	-3.18
24	Procyanidin tetramer	11.336	1153.2612	1153.0246	C60H50O24	99.6	-0.5
25	Procyanidin trimer isomer 2	11.413	865.1998	865.7645	C45H38O18	97.53	-1.59
26	Guavin A	11.496	1223.1423	1223.8897	C56H40O32	99.05	0.85
27	Casuarinin/ Casuarictin isomer	11.895	935.081	935.6375	C41H28O26	97.67	-1.43
28	Galloyl(epi)catechin-(epi)gallocatechin	12.1	745.142	745.6160	C37H30O17	96.9	-0.62
29	Procyanidin pentamer	12.144	1441.3234	1441.2688	C75H62O30	95.66	1.97
30	Galloyl-(epi)catechin trimer isomer 1	12.166	1017.2097	1017.8687	C52H42O22	99.72	-0.01
31	(epi)-gallocatechin isomer 2	12.327	305.0702	305.2595	C15H14O7	95.55	-3.32
32	Tellimagrandin I isomer	12.504	785.0855	785.5491	C34H26O22	98.44	-1.38
33	Vescalagin	12.758	933.0649	933.6216	C41H26O26	96.33	-0.8
34	Stenophyllanin A isomer	12.925	1207.1472	1207.8903	C56H40O31	98.37	0.89
35	Galloyl-(epi)catechin trimer isomer 2	12.985	1017.2097	1017.8687	C52H42O22	98.17	-1.35

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36	Myricetin hexoside isomer 1	13.284	479.0836	479.3678	C21H20O13	98.36	-0.92
37	Stachyuranin A	13.412	1225.1587	1225.9055	C56H42O32	95.54	1.35
38	Procyanidin gallate isomer	13.517	729.1476	729.6166	C37H30O16	96.89	-1.91
39	Myricetin hexoside isomer 2	13.677	479.0835	479.3678	C21H20O13	97.89	-0.08
40	Vescalagin/castalagin isomer	13.844	933.0645	933.6216	C41H26O26	88.32	-1.57
41	Myricetin -arabinoside/ xylopyranoside isomer 1	13.988	449.0728	449.3418	C20H18O12	98.39	-1.65
42	Myricetin -arabinoside/ xylopyranoside isomer 2	14.214	449.0726	449.3418	C20H18O12	98.02	-1.65
43	Procyanidin gallate isomer	14.563	729.6356	577.5123	C30H26O12	98.17	-1.73
44	Myricetin -arabinoside/ xylopyranoside isomer 3	14.99	449.0726	449.3418	C20H18O12	98.66	-1.65
45	Myricetin hexoside isomer 3	15.034	479.0839	479.3678	C21H20O13	97.08	-1.92
46	Myricetin hexoside isomer 4	15.217	479.0841	479.3678	C21H20O13	97.08	-1.92

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	Myricetin -arabinoside/ xylopyranoside						
47	Isomer 4	15.604	449.0743	449.3418	C20H18O12	98.39	-1.65
48	Quercetin -galloylhexoside isomer	15.626	615.1008	615.4726	C28H24O16	99.16	-0.98
49	Ellagic acid deoxyhexoside	15.837	447.0578	447.3259	C20H16O12	91.25	-3.19
50	Quercetin -galloylhexoside isomer	16.036	615.0999	615.4726	C28H24O16	99.16	-0.98
	Myricetin -arabinoside/ xylopyranoside						
51	isomer 5	16.191	449.0736	449.3418	C20H18O12	98.39	-1.65
52	Morin	16.28	301.0362	301.2278	C15H10O7	97.46	-2.5
	Myricetin -arabinoside/ xylopyranoside						
53	isomer 6	16.462	449.0735	449.3418	C20H18O12	98.39	-1.65
54	Ellagic acid	16.507	300.9996	301.1847	C14H6O8	98.88	-1.71
55	Hyperin	16.616	463.0895	463.3684	C21H20O12	96.41	-2.65
56	Quercetin glucoronide	16.723	477.0659	477.3519	C21H18O13	98.1	-1.83
57	Isoquercitrin	16.95	463.0893	463.3684	C21H20O12	97.04	-2.33

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58	Procyanidin gallate isomer	17.038	729.1476	729.6166	C37H30O16	96.89	-1.91
59	Reynoutrin	17.498	433.0792	433.3424	C20H18O11	95.94	-2.9
60	Guajaverin	17.802	433.0795	433.3424	C20H18O11	97.99	-1.91
61	Guavinoside A isomer 1	17.985	543.1159	544.4610	C26H24O13	98.1	-1.77
62	Avicularin	18.206	433.0803	433.3424	C20H18O11	96.7	-2.2
63	Quercitrin	19.194	447.0947	447.3690	C21H20O11	95.23	-3.02
64	Myrciaphenone B	19.208	481.0999	481.3836	C21H22O13	97.2	-2.23
65	Guavinoside C	19.768	585.0898	585.4466	C27H22O15	97.19	-1.92
66	Guavinoside B isomer 1	20.77	571.1470	571.5062	C28H28O13	97.26	-2.05
67	Guavinoside A isomer 2	20.702	543.1159	543.4530	C26H24O13	98.1	-1.77
68	Guavinoside B isomer 2	21.667	571.147	571.5062	C28H28O13	97.26	-2.05
69	2,6-dihydroxy-3-methyl-4-O-(6″-O-galloyl-β-D-glucopyranosyl)-benzophenone	21.971	557.1318	557.4796	C27H26O13	96.93	-2.12

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70	Guavin B	22.237	693.1110	693.5414	C33H26O17	97.82	-1.67
71	Quercetin	22.314	301.0358	301.2278	C15H10O7	98.9	-1.34
72	Naringenin isomer	26.738	271.0622	271.2448	C15H12O5	96.09	-3.67
	Positive mode						
73	Cyanidin-3-o-glucoside	3.661	449.1089	449.3911	C21H21O11	96.97	-2.34

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2.2 In silico results and bibliography searches

Compounds from Table 1 were processed with the DIA-DB server using Ligand similarity based virtual screening (LBVS) and Structure based virtual screening (SBVS) approaches. The results obtained after docking and molecular shape similarity analyses are shown in Figures 1 and 2, respectively. Based on these results and, to confirm or refute them, a bibliographic review of existing experimental studies for the different compounds present in *P. guajava* leaves was carried out, considering the targets involved in the regulation of glycemia.

The results of the docking analysis showed that aldose reductase (AKR1B1) (Protein data bank (PDB) [12] :3G5E) was the target that presented a more heterogeneous affinity, interacting with: avicularin, (epi)-catechin, ellagic acid, (epi)-gallocatechin isomers 1 and 2, guaijaverin, isoquercitrin, morin, naringenin isomer, quercetin and quercitrin (Figure 3). Compounds morin, naringenin, catechin and quercetin were also observed to have high similarity scores with tolrestat, a known AKR1B1 inhibitor. AKR1B1 is an enzyme of the polyol pathway that has been implicated in diabetic complications. In a study by Anand et al (2016) [13], a *P. guajava* leaf extract was found to inhibit rat lens aldose reductase *in vitro.*

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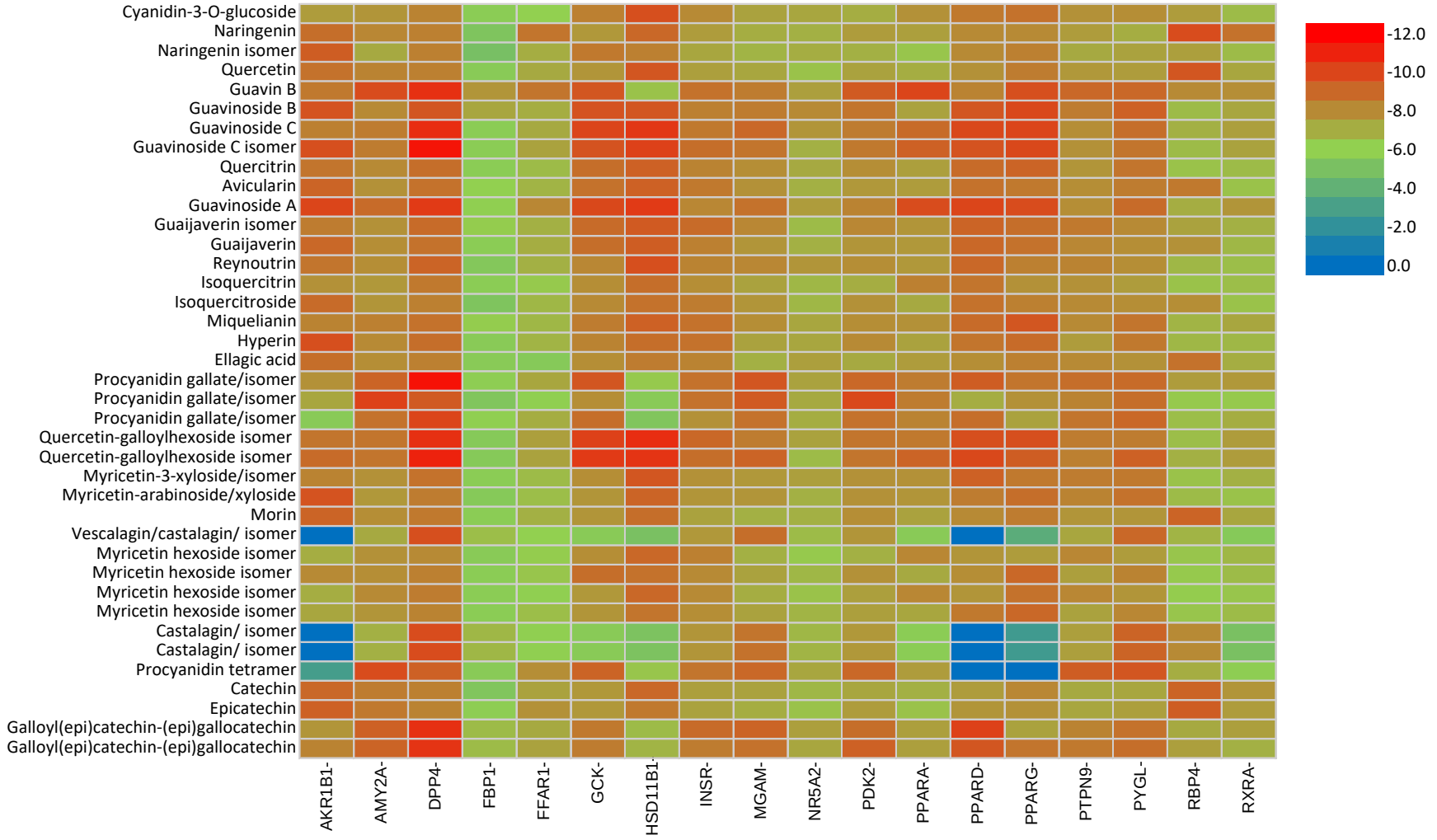

Figure 1 Heat map with the docking results of compounds from guava leaves extract against DM targets. Colour scale denotes docking score from blue (no interaction) to red (highest interaction). Each column represents the DM protein target, and each row is assigned to each compound from extract.

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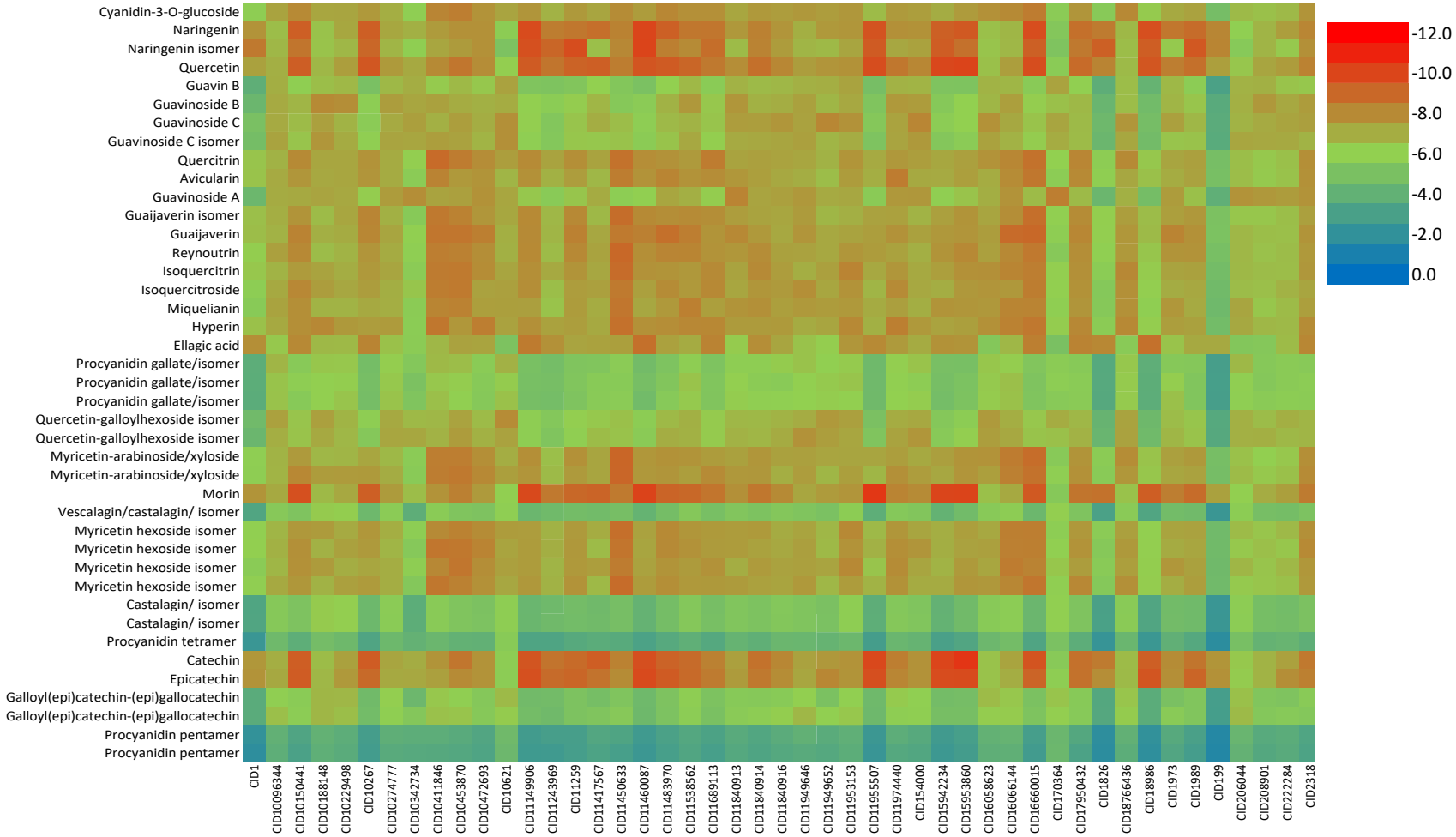

Figure 2. Heat map with the molecular shape similarity results of compounds from *P. guajava* extract against already known anti-diabetic compounds from DIA-DB database. Color scale denotes normalized similarity score from blue (no similarity) to red (highest similarity value). Each column represents each anti-diabetic compound from DIA-DB database (Pubchem ID) while each row is related to each compound from extract.

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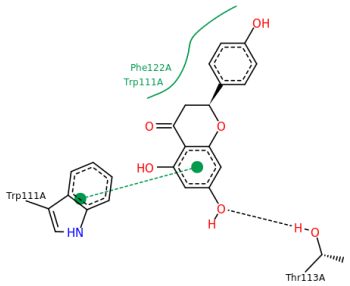
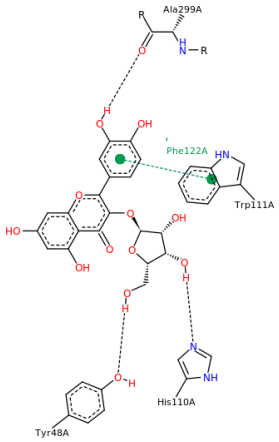
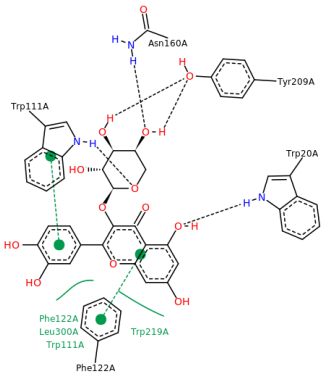
Naringenin
(Score -8.6 kcal/mol)



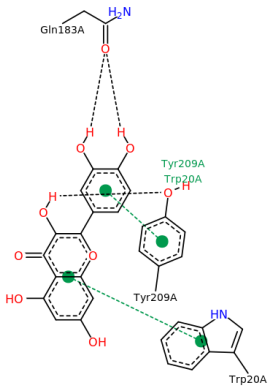
Avicularin
(Score -8.9 kcal/mol)



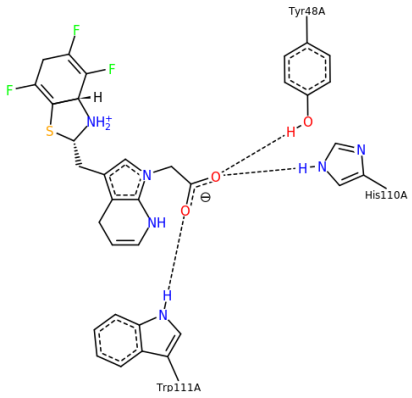
Guaijaverin
(Score -9.5 kcal/mol)



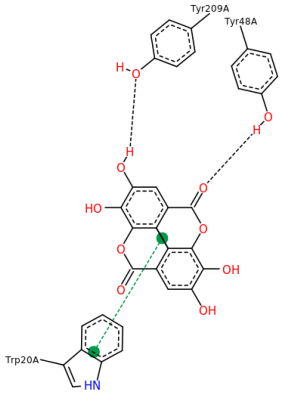
Quercetin
(Score -8.5 kcal/mol)



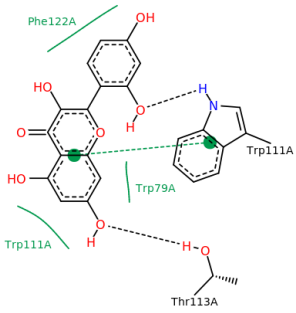
IDD 740 (PDB 3G5E ligand)
(Score -11.3 kcal/mol)



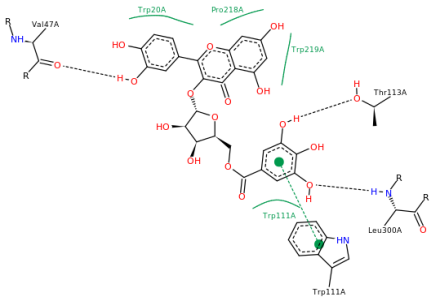
Ellagic acid
(Score -8.8 kcal/mol)



Morin
(Score -10.9 kcal/mol)



Guavinoside C
(Score -9.2 kcal/mol)



Catechin
(Score -10.4 kcal/mol)

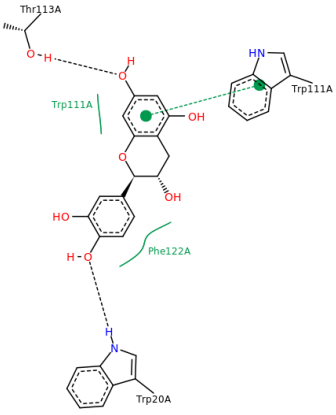


Figure 3 2D representations of docking results for the extract compounds with aldose reductase (PDB 3G5E). Black dashed lines represent hydrogen bonds, green dashed lines aromatic interactions and solid green lines hydrophobic interactions.

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Likewise, of all the compounds evaluated, naringenin was the one that presented interaction on the highest number of targets: dipeptidyl peptidase-4 (DPP-4) (PDB:4A5S), hydroxysteroid 11-beta dehydrogenase 1 (HSD11B1) (PDB:4K1L), AKR1B1 (PDB:3G5E), and PPARG (PDB:2FVJ) and peroxisome proliferator-activated receptor delta (PPARD) (PDB:3PEQ). There is some evidence in literature supporting the interactions of naringenin with some of these targets identified here. The binding of naringenin to HSD11B1 (PDB:4K1L) stands out with a score value of -8.5 kcal/mol. This result is in line with those obtained in the trials of Ortiz-Andrade et al. (2008) [14] in which an IC_{50} of 1000 nM was obtained for this molecule.

In a recent study by Khan et al. (2020) [15], naringenin was found to inhibit aldose reductase in an uncompetitive manner with an IC_{50} of 2.6 µM. Fan et al. (2013) [16] demonstrated the ability of naringenin to inhibit in porcine kidney DPP4 enzyme (88% sequence homology with human counterpart) with an IC_{50} of 0.24 µM. Goldwasser et al. (2010) [17] showed that naringenin could bind to the ligand-binding domain of PPARG in Hela reporter cell line HG5LN GAL4-PPARG and activate PPARG up to 57% at 80 µM.

The heterogeneity of interaction shown by naringenin in the docking analysis agrees with the heterogeneity of the analysis of similarity. This indicates that naringenin is similar to other already known anti-diabetic flavonoid compounds such as luteolin or myricetin, as well as to different antidiabetics such as carbutamide and chlorpropamide (sulfonylureas), compound MB07803 (fructose 1,6-bisphosphatase inhibitor), tolrestat (AKR1B1 inhibitor) or picolinate of chromium (III), a drug capable of improving the fluidity of the cell membrane and increasing the rate of internalization of insulin (Figure 4).

The docking analysis showed that quercetin binds to AKR1B1 (PDB:3G5E) with a score value of -8.6 kcal/mol and to HSD11B1 (PDB:4K1L) with a score value of -9.5 kcal/mol

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respectively. These results agree with the inhibition tests on AKR1B1 (PDB:3G5E) carried out by Chethan et al. (2008) [18]; de la Fuente et al. (2003) [19] and Ueda et al. (2001) [20] in which quercetin showed IC_{50} values ranging from 14 nM to 248 nM. With regards to HSD11B1 (PDB:4K1L), Torres-Piedra et al. (2010) [21] showed that quercetin was able to produce a decrease in the activity of HSD11B1 (PDB:4K1L) of up to 27%.

Several studies indicate that quercetin could also influence protein tyrosine phosphatase (PTP) (PDB: 4GE6) and PPARG (PDB:2FVJ) [22-24].

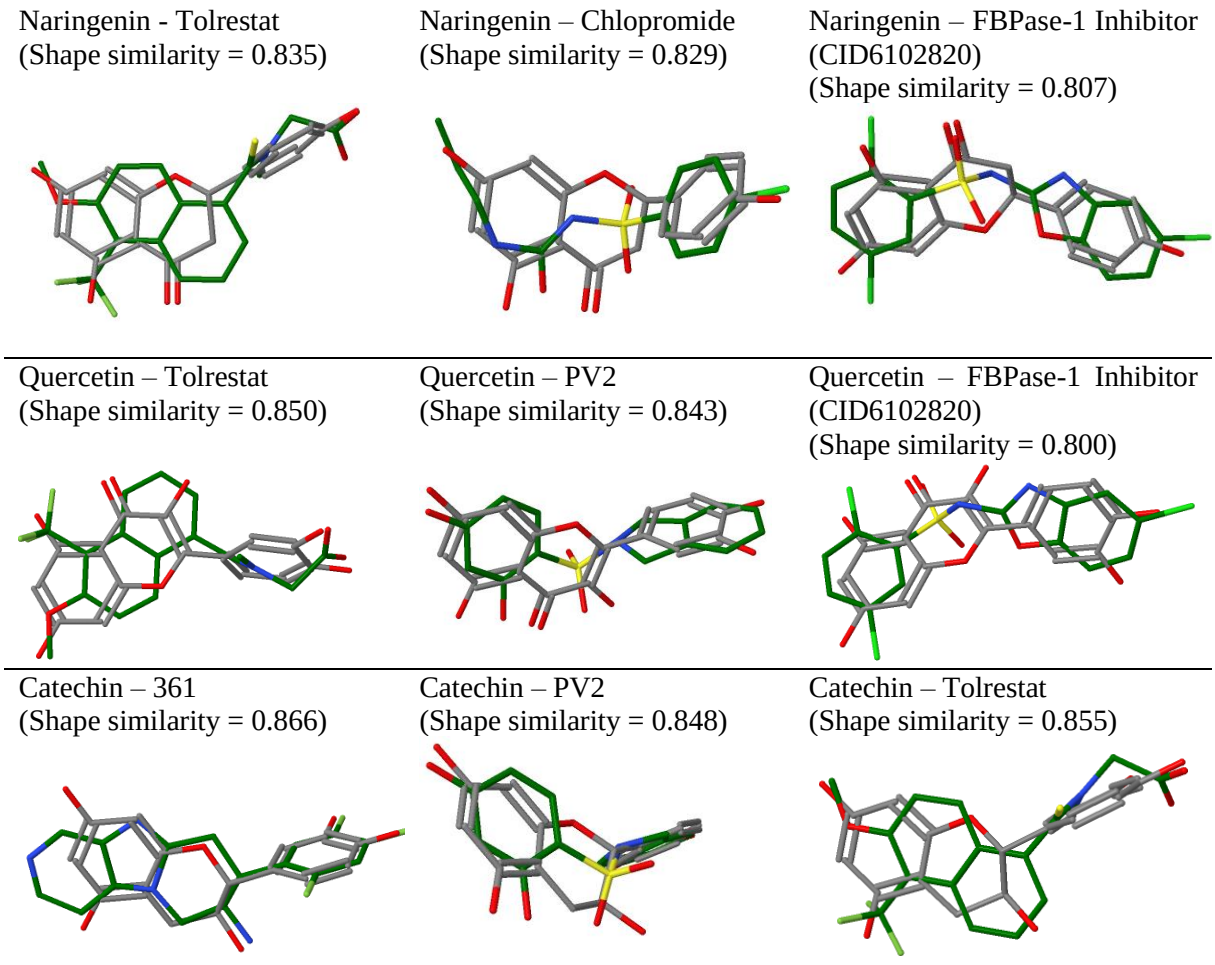

Figure 4. Similarity analyses for the extract compounds naringenin, quercetin and catechin with known/ experimental anti-diabetic drugs.

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This diversity of targets shown by quercetin agrees with the results of similarity analysis in which this compound found high similarity values against other flavonoids (luteolin and myricetin) and with antidiabetics such as the compound PV2 (inhibitor of pyruvate dehydrogenase kinase mitochondrial), the compound MB07803, tolrestat (AKR1B1 inhibitor) or the chromium picolinate (III) (Figure 4).

Quercitrin also showed a good interaction with AKR1B1 (PDB:3G5E) with a score of -8.7 kcal/mol. This interaction was corroborated by the trials of Dhagat et al. (2008) [25], Kim et al. (2015) [26]; Jung et al. (2011) [27] and Yoshikawa et al. (1998) [28] in which quercitrin gave IC_{50} values between 150 and 340 nM. In addition, the studies carried out by Choi et al. (2010) [29] indicate that quercitrin could influence the receptor activated by PPARG (PDB:2FVJ).

Isoquercitrin presented a score value with AKR1B1 (PDB:3G5E) of -8.9 kcal/mol; This value is consistent with the tests developed by Kim et al. (2015) [26] in which an IC_{50} value of 320 nM was obtained for this molecule. Also, in their studies with laboratory rats, Brindis et al. (2013) [30] showed that isoquercitrin caused a decrease in postprandial glucose peaks similar to that obtained with a dose of acarbose of 5 mg/kg.

Guaijaverin showed a score value of -8.9 kcal/mol in its binding with AKR1B1 (PDB:3G5E). These results agree with laboratory rat tests carried out by Yoshikawa et al. (1998) [28] in which they obtained an IC_{50} of 180 nM.

Ellagic acid, on the other hand, was predicted to bind AKR1B1 (PDB:3G5E) and insulin receptor (INSR) (PDB:3EKN) with a score value of -8.8 kcal/mol and -8.2 kcal/mol respectively. In the case of AKR1B1 (PDB:3G5E), trials such as those of Akileshwari et al. (2014) [31]; Hundsdörfer et al. (2012) [32]; Naeem et al. (2012) [33]; Sawant et al. (2015) [34] or Kawanishi et al. (2004) [35], in which the IC_{50} values of ellagic acid were found

 doi:10.20944/preprints202211.0420.v1

between 48 nm and 397 nM again confirmed the good predictions obtained by the DIA-DB application. Similarly, the INSR inhibition analyses carried out by Sawant et al. (2015) [34] where an IC_{50} of 340 nM was obtained, further support the predictions made by DIA-DB.

Several studies indicate that ellagic acid could also influence hepatic glycogen phosphorylase (PYGL) (PDB:3DDS) as well as PPARG (PDB:2FVJ) [36,37].

The docking analysis revealed interaction of (epi)-catechin with DPP4, retinol 4 transport protein (RBP4) (PDB:2WR6), AKR1B1 (PDB:3G5E), pancreatic α-amylase (AMY2A) (PDB:4GQR) and HSD11B1 (PDB:4K1L). Of note here is the binding of catechin 1 and 2 to AMY2A (PDB:4GQR) with a score of -8.4 kcal/mol; these results agree with the *in vitro* studies carried out by Adisakwattana et al. (2011) [38] and Toma et al. (2014) [39] in which they found that catechin reduced the activity of this enzyme by between 5 and 6%. Similarly, catechin and epi-catechin were observed to inhibit the rat lens aldose reductase enzyme *in vitro* by 38% and 41% at 30 µM, respectively.

The wide diversity of interactions shown by catechin agrees with the results of similarity analyses, in which this compound showed the highest degree of similarity with other compounds, being very similar to: myricetin, luteolin, chromium picolinate (III) and the compounds: 361 (DPP4 antagonist), PFT and PV1 (inhibitors of the HSP90 thermal shock protein), PV0, PV2 and PV8 (inhibitors of mitochondrial pyruvate dehydrogenase kinase) and MB07803 (Figure 4).

Geraniin 1 and 2 bind to AMY2A (PDB:4GQR) with score values of -8.9 and -8.2 kcal/mol respectively. These results agree with the tests carried out by Palanisamy et al. (2011) [40] in which they found an IC_{50} value of 970 nM for this compound.

Finally, it is worth mentioning compounds guavinoside C and stachyuranin A. The docking analysis showed that guavinoside C binds with good score values to: DPP4 (PDB:4A5S),

 doi:10.20944/preprints202211.0420.v1

intestinal maltase-glucoamylase (MGAM) (PDB:3L4Y), pyruvate dehydrogenase kinase (PDK2) (PDB:4MPC), PTP (PDB: 4GE6), AMY2A (PDB:4GQR), glucokinase (GCK) (PDB:3IMX), HSD11B1 (PDB:4K1L), AKR1B1 (PDB:3G5E) and INSR. In the case of stachyuranin A, the range of unions is smaller but not negligible, and potential targets identified were DPP4 (PDB:4A5S), PYGL (PDB:3DDS), (AMY2A) (PDB:4GQR) and the insulin receptor.

The analysis of similarity indicated that neither of the two molecules had high similarities with any of the known antidiabetic compounds. In addition, no bibliographic references regarding the antidiabetic activity of these compounds were found.

3. Discussion

Treatment of streptozotocin-/alloxan-induced diabetic rats with *P. guajava* extracts *in vivo* is associated with a reduction in hyperglycaemia. Several protein targets identified in this study could assist in reducing hyperglycaemia through insulin sensitization and regulation of glucose homeostasis. Compounds naringenin, (epi)-catechin, guavinoside C and stachyuranin A were identified as DPP4 inhibitors. Inhibition of DPP4 would increase the half-life of the incretin hormones and thereby increase insulin secretion and thus allowing time to normalize blood glucose levels [41]. Compounds naringenin, quercetin, (epi)-catechin) and guavinoside C also through their inhibition of 11-beta-HSD1 could inhibit glucose production by the liver and improve glucose-dependent insulin sensitivity [42]. Similarly, in a study by Shen et al. (2008) [43], a *P. guajava* extract was found to decrease fructose-1,6-bisphosphatase (FBP1) activity, an enzyme also responsible for glucose production by the liver, and in this study naringenin, quercetin as well as (epi)-catechin were observed to share high similarity to MB07803, an FBP1 inhibitor.

 doi:10.20944/preprints202211.0420.v1

P. guajava extracts have been observed to stimulate glucose uptake by hepatocytes, adipocytes, myotubes and intestinal cells possibly through regulation of the insulin signalling pathway [13,43-46]. PTP1B disrupts the insulin signalling pathway and thus treatment with inhibitors would result in insulin sensitization and improve glucose homeostasis [47]. On the other hand, activation of INSR by agonists will stimulate the insulin signalling pathway, thereby improving insulin sensitivity, and promoting glucose uptake by the tissues [48]. Quercetin and guavinoside C were identified as inhibitors for PTP1B while ellagic acid and stachyuranin A were found to interact with INSR. Postprandial blood glucose levels may also be decreased through the inhibition of AMY2A and MGAM, two enzymes responsible for carbohydrate digestion. (Epi)-catechin and stachyuranin A were identified as AMY2A inhibitors while guavinoside C was identified as an inhibitor of both AMY2A and MGAM. *In vitro* studies by Liu et al. (2014) [49], Oghogho and Nimenibo-Udia (2019) [50] and Wang et al. (2010) [51] with porcine pancreatic alpha-amylase and yeast/ rat intestinal alpha-glucosidase showed good inhibitory activity by Psidium guajava extracts, comparable to positive control acarbose.

Besides reducing hyperglycaemia, *P. guajava* extracts have also been shown to improve the associated hyperlipidaemia. Treatment with *P. guajava* extracts is associated with a reduction in total cholesterol, triglycerides, low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) while increasing high-density lipoprotein (HDL) levels in the blood of diabetic rats [52-54]. The PPARs play various roles in lipid metabolism by regulating the genes involved in lipogenesis, triglyceride synthesis, reverse cholesterol transport, lipolysis, and fatty acid oxidation. Quercetin, quercitrin and ellagic acid were found to bind PPARG while naringenin was found to bind both PPARD and PPARG.

 doi:10.20944/preprints202211.0420.v1

In conclusion, DIA-DB web server was used to process information about 73 phenolic compounds present in the extract of guava leaves and to predict their potential bioactivity in the context of DM. After detailed analyses, catechin, quercetin and naringenin showed the highest molecular shape similarity values against already available antidiabetic drugs. In addition, we reported several compounds that act in the DM mechanism by the interaction with specific DM protein targets. Some of them are well known phenolic compounds in guava leaves, such as catechin, ellagic acid, naringenin, guavinoside C, and quercetin and its derivatives guaijaverin and isoquercitrin. However, guaijaverin and isoquercitrin are specific of guava leaves extract although were not previously reported in the DM context. Besides, compound like stachyuranin A that has not been previously identified in guava leaves, has demonstrated to contribute to the anti-diabetic properties of the leaves. In addition, the bibliographic analysis confirms the validation of the DIA-DB predictions. Finally, this work paves the way for the isolation or selective extraction of some of the specific compounds reported here, and their application as nutraceuticals and/or food additives and for further in vivo studies with target compounds.

4. Materials and Methods

4.1 Plant Material and Sample Preparation

Middle age intense green leaves were collected in Motril, Spain (36°44′43″N 3°31′14″W), in February 2015. The samples were air-dried at room temperature, ground and extracted with ethanol/water 80/20 (v/v) by ultrasound assisted extraction as was previously reported Díaz-de-Cerio et al. (2016) [6].

4.2 HPLC-ESI-QTOF-MS analyses

Chromatographic analyses were performed using an HPLC Agilent 1260 series (Agilent Technologies, Santa Clara, CA, USA) equipped with a binary pump, an online degasser, an

 doi:10.20944/preprints202211.0420.v1

autosampler, and a thermostatically controlled column compartment. Moreover, MS analyses were carried out using a 6540 Agilent Ultra-High-Definition Accurate-Mass Q-TOF-MS coupled to the HPLC, equipped with an Agilent Dual Jet Stream electrospray ionization (Dual AJS ESI) interface.

Phenolic compounds from *P. guajava* L. leaves, in negative mode, were analysed by the chromatographic and the detection method described by Díaz-de-Cerio et al. (2016) [6]. Phenolic compounds were also separated in the positive mode using the method that Gómez-Caravaca et al. (2013) [55] reported to determine the anthocyanins.

4.3 In silico approaches

We used the DIA-DB [56] web server (http://bio-hpc.ucam.edu/dia-db) to predict the antidiabetic activity of compounds. DIA-DB uses two different approaches, namely LBVS and SBVS.

LBVS methods exploit all existing available information (structure, physicochemical parameters, binding affinities, etc) about known active and inactive compounds. DIA-DB exploits shape information for checking existence in the database of compounds similar to the ones used in the input query. For that purpose, DIA-DB uses internally the shape complementary tool WEGA [57].

SBVS identifies compounds which can bind to a target protein with high affinity. This is achieved by determining the optimal binding position by docking each query molecule to a database of protein targets involved in diabetes and available in DIA-DB and then ranking the compound-targets interactions according to their estimated binding affinity values, namely docking scores. The SBVS protocol implemented in DIA-DB employs the Autodock Vina docking program [58]. Vina finds well-binding ligands for a protein receptor of known structure in an input database that contains the three-dimensional

 doi:10.20944/preprints202211.0420.v1

structures of many ligands. Each ligand of the database is docked into the whole surface of the protein using an all-atom representation of the protein and ligand.

4.4 Bibliography searches

Based on the obtained SBVS and LBVS DIA-DB predictions, we checked posteriori the existence of bibliographical references confirming our predictions.

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