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Effects of Delphinidn-3-sambubiosid in different pathways of human cells according to a bioinformatic assay.

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Abstract: The use of food and its nutrients as a remedy for diseases is historically, and culturally well-rooted in plenty of societies. An example of this it's the use of *Hibiscus sabdariffa* to treat conditions like hypertension or high blood glucose. Furthermore, the natural biocompounds present in this plant have been associated by several authors as hypotensive, antioxidant, anticarcinogenic, antiobesogenic, etc. One of those compounds it's Delphinidin-3-Sambubiosid (DS3) the most representative anthocyanin of *Hibiscus sabdariffa*, and as such, it has by large been attributed with the beneficial effects previously mentioned. However, little is known about the molecular targets that DS3 has. Therefore, we made an *in-silico* analysis using different bioinformatic tools to see the possible molecular targets of this molecule and the potential impact the modification of its targets could have on the protein and/or pathways of humans. We used the Swiss Target Prediction site to identify all the molecular targets of DS3, and then, ShinnyGo 0.77, KEGG, and Stringdb were helpful in identifying key pathways and hub genes related to them. Also, a literature search was made in PubMed where each of the hub genes were linked to DS3 so we could gather information that complemented the results of the bioinformatic tools. The results showed that DS3 can modify the behavior of genes related to nitrogen and glucose metabolism, inflammation, angiogenesis, and cell proliferation. Additionally, DS3 has direct effects on the PI3K-AKT pathway, which could be a key finding to promote further research, especially in the implications associated with changes in the aforementioned pathway.

Keywords: Hibiscus sabdariffa; Delphinidin-3-sambubiosid; Bioinformatics

1. Introduction

Hibiscus sabdariffa is a highly popular plant in Asia and America. Therefore, has been used in a wide array of products going from flavored water to facial creams. Its popularity can also be attached to its potential beneficial effects on health, going from hypotensive to anti-cancerogenic, especially since this plant is rich in plenty of biocompounds. One of these biocompounds are anthocyanins, which are a group of phenol-derived compounds quite common in many fruits, vegetables, plants, and especially in *Hibiscus sabdariffa*. They are generally responsible for such foods' blue, red, or purple colors (1). Structurally they are aliphatic or aromatic compounds with three rings and one or more sugar molecules. As for the potential therapeutic effect of anthocyanins, there is substantial evidence that most if not all anthocyanins can have an effect in different cells of mammals (2) with some

of them directly linked to alterations in biological pathways, multiple biological models have shown that anthocyanins are capable of change the way plenty of pathologies prognosis. One anthocyanin of particular interest is Delphinidn-3-Sambubiosid, found particularly in high quantities in *Hibiscus sabdariffa*. This anthocyanin (DS3) has shown potential therapeutic effects (4), however, there is little evidence of how exactly D3S affects the cells and what targets it has in them. As such, the objective of this study is to use bioinformatic tools to determine which probable targets D3S has on human cells as well as to determine the effects that changes in such pathways.

2. Methods

2.1. Bioinformatic analysis

The site SwissTargetPrediction was used to determine possible molecular targets of the interaction of D3S. Once the list of possible targets for D3S was obtained, the ShinnyGo 0.77 site was used to obtain the Fold Enrichment (FE) of each one by FDR (cut-off of 0.05). Out of those, the ones with an FE higher than 5 were used in KEGG to identify the pathways where there could be a key interaction caused by D3S. Also, the website Stringdb site was used to obtain a hub of genes gathered from the FE data. From these last ones, evidence was searched using the Pubmed database.

2.2. Literature search and data selection

A search was conducted using PubMed to identify relevant articles that have information about the genes obtained from the bioinformatic analysis against D3S, these were made with a simple search string "Gene Name" AND "Delphinidin 3 Sambubiosid". The search used terms in titles, abstracts, or a combination of both. Finally, inclusion and exclusion criteria were used to determine which articles could be considered for the final discussion.

2.3. Inclusion and Exclusion criteria

The inclusion criteria were: Any study that checks for any of the genes (or protein derived from them) obtained from the bioinformatic analysis with DS3. As for the exclusion criteria: studies with duplicated or overlapping data, papers that only presented abstracts, conferences, editorials, or author responses, articles without full text available, and systematic reviews.

2.4. Results.

Data from the Swiss Target Prediction. Figure 1 shows the top 15 target classes of molecules that DS3 could interact with, most of them being enzymes and lyases followed closely by a family of G protein-coupled receptors. Also, the full information on all the possible targets is shown in supplementary material 1 (5,6).

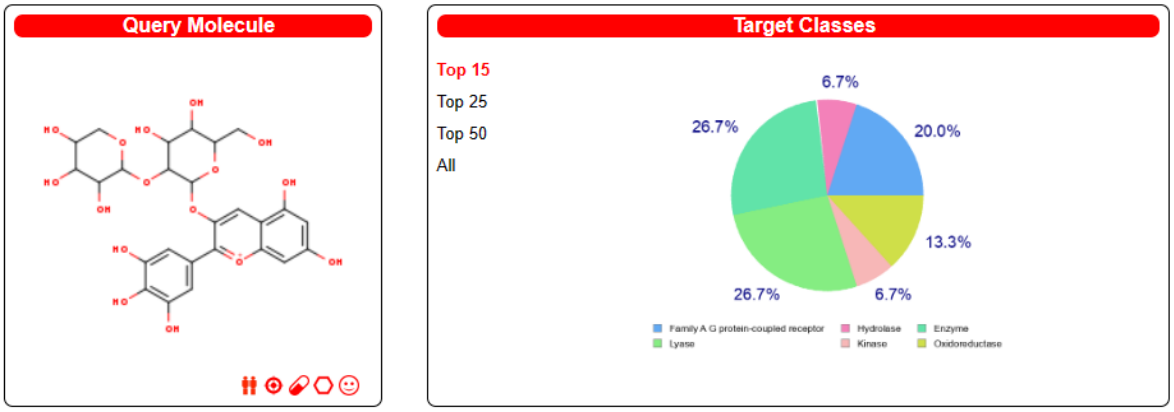

Figure 1. Top 15 molecular targets of DS3 according to the Swiss Target Prediction site.

Enriched Analysis of Gene Ontology and Metabolic Pathways. The site ShinnyGo 0.77 was used to perform the Gene Ontology and KEGG (7). The full results from the Gene Ontology are shown in Table 1. The results of KEGG are shown in Table 2 and the images of each pathway with the signaling of the potential changes in the protein are displayed in supplementary material 2.

Table 1. Functional enrichment analysis of the genes that have a prediction of interaction with DS3.

Enrichment FDR	genes	Pathway Genes	Fold Enrichment	Pathway	Genes
4.20E-18	10	17	141.151703	Nitrogen metabolism	CA2 CA9 CA14 CA6 CA1 CA3 CA4 CA7 CA5A CA13
1.49E-09	15	354	10.1677074	PI3K-Akt signaling pathway	GSK3B PIK3CG MET IL2 FLT3 PKN1 KDR IGF1R AKT1 MCL1 PIK3R1 EGFR SYK PTK2 INSR
2.21E-09	9	79	27.3369754	EGFR tyrosine kinase inhibitor resistance	GSK3B MET KDR IGF1R AKT1 PIK3R1 EGFR AXL SRC
2.73E-09	27	1527	4.24287044	Metabolic pathways	CD38 PTGS2 CA12 AKR1B1 HSD17B2 PYGL CA2 SQLE PIK3CG CA9 ALOX12 ALDH2 CA14 GLO1 CA6 CA1 CYP19A1 PDE5A XDH ALOX15 CA3 CA4 CA7 PLA2G1B CA5A CA13 MAOA
1.98E-07	8	95	20.2069806	Endocrine resistance	MMP2 MMP9 IGF1R AKT1 PIK3R1 EGFR PTK2 SRC
3.94E-06	7	108	15.5528265	Insulin resistance	NR1H3 GSK3B PYGL AKT1 PIK3R1 INSR RPS6KA3
4.89E-06	6	70	20.5678196	Central carbon metabolism in cancer	HIF1A MET FLT3 AKT1 PIK3R1 EGFR
2.28E-05	8	214	8.97038859	Lipid and atherosclerosis	CAMK2B GSK3B MMP9 AKT1 PIK3R1 MMP3 PTK2 SRC
2.52E-05	5	56	21.424812	Regulation of lipolysis in adipocytes	PTGS2 AKT1 PIK3R1 ADORA1 INSR
0.0001554	8	294	6.52946652	MAPK signaling pathway	MET FLT3 KDR IGF1R AKT1 EGFR INSR RPS6KA3
0.00042661	5	112	10.712406	TNF signaling pathway	PTGS2 MMP9 AKT1 PIK3R1 MMP3
0.0008788	5	137	8.7575874	Insulin signaling pathway	GSK3B PYGL AKT1 PIK3R1 INSR
0.00134156	5	155	7.74057725	Non-alcoholic fatty liver disease	NR1H3 GSK3B AKT1 PIK3R1 INSR
0.0024404	3	47	15.3164614	Carbohydrate digestion and absorption	SLC5A1 AKT1 PIK3R1
0.00373995	4	120	7.99859649	AMPK signaling pathway	IGF1R AKT1 PIK3R1 INSR
0.01950816	3	107	6.72779144	Glucagon signaling pathway	CAMK2B PYGL AKT1
0.02834824	2	46	10.432952	Type II diabetes mellitus	PIK3R1 INSR
0.02929805	2	47	10.2109742	Pyruvate metabolism	ALDH2 GLO1

Protein-Protein Interaction Network. The STRING database was used to predict the associations of protein targets of DS3 (8-20). The network was constructed with a medium confidence of 0.400. The interactome has 257 edges, 57 nodes, an average node degree of 9.02, and a PPI enrichment p-value of <1.0e-16. Figure 3 shows the interactome and Table 2 shows the hub genes with at least 10 interactions.

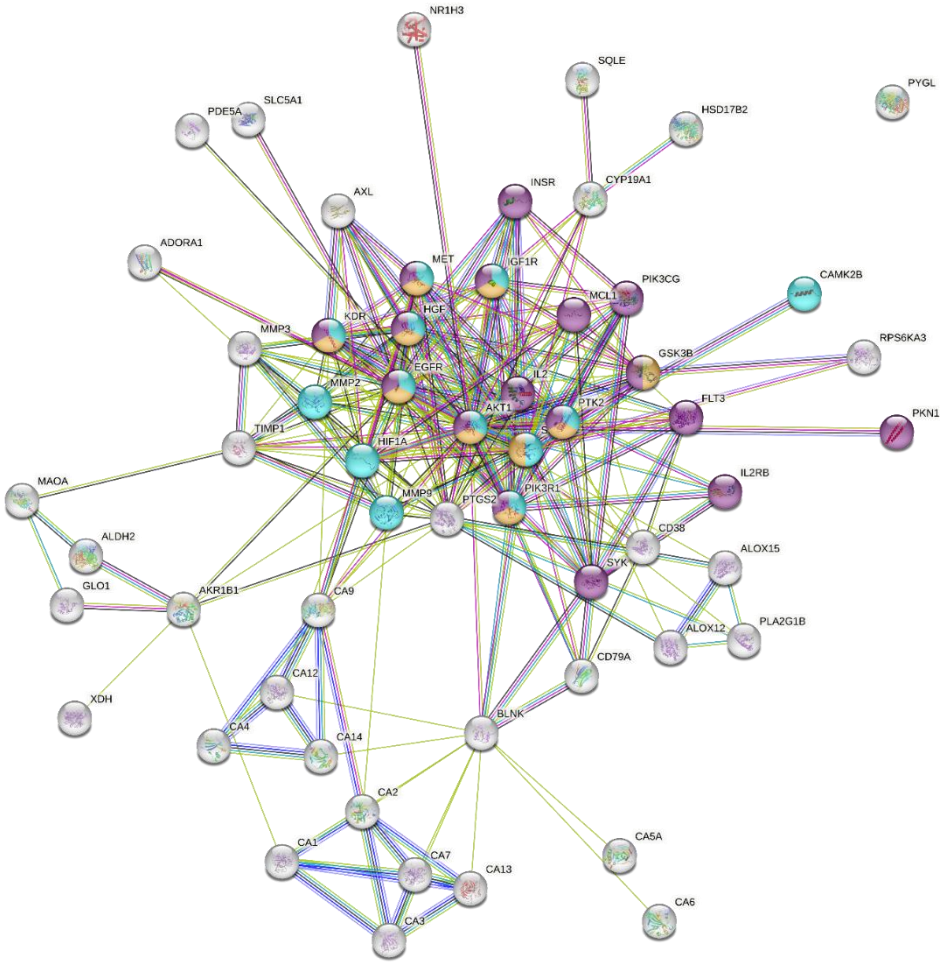

Figure 3. PPI network. Each of the edges is a specific protein with a significant protein-protein association. The blue and purple borders are known interactions from plentiful databases (previously curated and experimentally curated). As for the predicted interactions of each neighborhood gene, gene fusion, and gene concurrence are identified as green, red, and navy blue. Other borders such as grass green, black, and grey are text mining, coexpression, and protein homology respectively. .

Table 2. Hub genes with at least 10 interactions in humans were obtained from the predictions of interactions with DS3.

Gene symbol	Protein name	Protein-Function
AKT1	RAC-alpha serine/threonine-protein kinase	Regulates many processes including metabolism, proliferation, cell survival, growth, and angiogenesis.
PTK2	Focal adhesion Kinase 1	Related to the increase in glucose uptake and glycogen synthesis in insulin-sensitive tissues.
IL2	Interleukin-2	Required for T-cell proliferation and other cells of the immune system
PIK3R1	Phosphoinositide-3-kinase regulatory subunit alpha/beta/delta	Necessary for the insulin-stimulated increase in glucose uptake and glycogen synthesis
SYK	Spleen-associated tyrosine kinase	Regulates biological processes including immunity, cell adhesion, vascular development, and others.

PTGS2	Prostaglandin G/H synthase 2	Plays a role in the production of inflammatory prostaglandins
MMP9	Matrix metalloproteinase-9	Key in local proteolysis of the extracellular matrix and leukocyte migration
HIF1A	Hypoxia-inducible factor 1-alpha	Master transcriptional regulator in response to hypoxia
MMP2	Matrix metalloproteinase-2 (gelatinase a)	Involved in angiogenesis, tissue repair, tumor invasion, inflammation, and atherosclerotic plaque rupture
KDR	Vascular endothelial growth factor receptor 2	Essential in the regulation of angiogenesis, promotes the proliferation, survival, and migration of endothelial cells
MET	Hepatocyte growth factor receptor	Regulates processes like proliferation, scattering, morphogenesis, and survival
HGF	Hepatocyte growth factor	Growth factor for a broad spectrum of tissues and cell types
EGFR	Epidermal growth factor receptor	Converts extracellular cues into appropriate cellular responses
IGF1R	Insulin-like growth factor 1 receptor	Involved in cell growth and survival control
CA9	Carbonic anhydrase 9	Involved in pH regulation
BLNK	B-cell linker protein	Important for the activation of NF-kappa-B and NFAT

Data recollection from the evidence of the hub genes about DS3. The results from the search in the database PubMed results from the articles of the hub genes when tested in a study with DS3 are shown in Table 4. These results are presented separately if the evidence is found at RNA, protein, or pathways level directly.

Table 3. Evidence found of the Hub genes when tested against DS3.

Genes	Results at the gene expression level	Results at the protein level	Results of pathway impact
MET		Syed, D. N. 2008: Suppress the phosphorylation of the protein (21)	
IGF1R		Teller et al, 2009: Inhibition of its kinase activity (22)	
EGFR	Harish Chandra Pal, et al, 2013: Reduction in the expression of the gen (23)	Fredrich D, Et all, 2008: Suppress phosphorylation of the protein (24)	Harish Chandra Pal, et al, 2013: Inhibition of the PI3K-Akt pathway (23)

*The hub genes that are not shown had no relevant information (if any) for this study in the database PubMed at the date of recollection of the data.

3. Discussion

The results of the SwissTargetPrediction software (Figure 1) have their bases in the mathematical fundament of SwissTargetPrediction which makes docking predictions with the software EADocks DSS (5). EADocks DSS primarily uses an algorithm that determines targets of molecules on proteins by using a binding model within all possible 3D cavities. According to Grosdidier A., Zoete V., and Michielin O., in this task, this software has a success rate of close to 70% in correctly predicting binding models. Moreover, it also discriminates and filters its results thanks to other tools such as the Chemistry at HARvard Macromolecular Mechanics (CHARMM) and Fast analytical continuum treatment of solvation (FACTS) (28), with this conjunction, the ligands with less than 15 free dihedral angles and/or test complexes with adequately defined biding pockets have a success rate of up to 96% in EADocks DSS. It is important to remark on how SwissTargetPrediction has been used in the determination of molecular targets of small molecules that come from plants or foods (not dissimilar to the one in this study). For example, in a study of 2022, a team of researchers from China led by Lili Yan (26) looked at Erianin (a biphenyl compound)

information regarding its predicted molecular targets in this site, then, they compared the matches of those targets (along with the results of other bio-informatic tools) with then-current information published by other authors getting plenty overlap in their results. This shows how this tool (SwissTargetPrediction) has been used to good success in order to find information regarding molecular target information.

The results from the Gene Ontology assay (Table 1) show that DS3 affected several processes involving metabolism and inflammation, in particular nitrogen metabolism, insulin resistance signaling, PI3k-Akt pathway, metabolic pathways, insulin signaling pathway, regulation of lipolysis, TNF signaling pathway, lipid and atherosclerosis, and endocrine resistance. These results are supported by the fundament of the database, the use of the FE of each one by FDR (cut-off of 0.05) has been widely accepted as a tool in bioinformatics to delimit the possibility of false positives and have (27). These processes are related to a significant number of the effects described for DS3 or plants with high quantities of this anthocyanin (2,3).

Regarding the KEGG analysis on the pathways affected (table 1), the results indicate a dysregulation in the metabolism of nitrogen (key in the regulation of energy metabolism and protein metabolism) and glucose metabolism (especially in muscle and adipose tissue). Interestingly, as for the glucose metabolism alterations seem to be attached mostly to alterations in the PI3K-Akt pathway, this result agrees with multiple studies that have studied the effects of DS3 in PI3K-AKT (21-24)

On the other hand, the protein-to-protein analysis also supports the idea that the PI3K-AKT pathway is a major target of DS3, by having AKT being the most linked node of the whole analysis. Also, the information on the hub genes (table 2) shows the trend of the genes to be related to the metabolism of glucose and nitrogen, inflammation, and angiogenesis. This not only has concordance with the previous results shown but also includes angiogenesis, a process related to the production of nitric oxide and therefore to blood pressure. This is of interest since another of the most reported effects of DS3 is its potential as a hypotensive (25).

As for the evidence research, using the PubMed database we looked for any information regarding the hub genes obtained from the protein-to-protein analysis and DS3. According to the results of the gene ontology (table 1) most of the genes of interest are related to the PI3K-Akt pathway. Out of those, it is fascinating to see the effects in EGFR, being the one that has more evidence of what happens when exposed to DS3: reducing the expression of the gen, suppressing the function of the protein transcribed from it and, finally, being associated with inhibit the whole PI3K-AKT pathway in this condition (21-24). However, it is important to remark how most of the other genes do have some predicted alterations but, there was not much information to be found about their relationship with DS3 (if any), therefore the importance of studying them *a posteriori* is suggested.

4. Conclusion

The predictive analysis indicates that DS3 has the potential to trigger changes in genes related to nitrogen and glucose metabolism, inflammation, angiogenesis, and cell proliferation. The information currently available suggests those changes also can occur directly in the protein and not only in mRNA. Also, quite possibly the most important result according to the bioinformatic tools is the potential modification in the function of plenty of metabolic pathways, in particular the effects that DS3 has in the PI3K-AKT, these results are also supported by the findings that were discussed in table 2 and table 3. However, there are not enough published studies about DS3 and its other potential targets (suggested by the bioinformatic tools), the lack of research in that area opens a wide field to conduct new studies with a high probability of showing significant relevance to understand the mechanisms that DS3 has in human cells.

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