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Proceeding Paper

Effects of Delphinidin-3-Sambubiosid on Different Pathways of Human Cells According to a Bioinformatic Analysis †

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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 is the use of *Hibiscus sabdariffa* to treat conditions like hypertension or high blood glucose. Furthermore, the natural biocompounds present in this plant have been demonstrated by several authors to be hypotensive, antioxidant, anticarcinogenic, antiobesogenic, etc. One of these compounds is Delphinidin-3-Sambubiosid (DS3), the most representative anthocyanin of *Hibiscus sabdariffa*, and as such, it has been proposed to have the beneficial effects previously mentioned. However, little is known about the molecular targets of DS3. Therefore, we conducted an in silico analysis using different bioinformatic tools to determine the possible molecular targets of this molecule and the potential impact the modification of its targets could have on the proteins 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 used to identify key pathways and hub genes related to them. Also, a literature search was conducted in PubMed, where each of the hub genes was linked to DS3 so we could gather information that complemented the results of the bioinformatic tools. The results show 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 promoting further research, especially to determine the implications associated with changes in the aforementioned pathway.

Keywords: hibiscus sabdariffa; delphinidin-3-sambubiosid; bioinformatics

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1. Introduction

Hibiscus sabdariffa is a highly popular plant in Asia and America. Therefore, it has been used in a wide array of products, from flavored water to facial creams. Its popularity can also be attributed to its potential beneficial effects on health, from hypotensive to anti-cancerogenic activities, especially since this plant is rich in plenty of biocompounds. One of these biocompounds is 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 have an effect on 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 changing several pathologies' prognosis. One anthocyanin of particular interest is Delphinidn-3-Sambubiosid, which is found in particularly high quantities in *Hibiscus sabdariffa*. This anthocyanin (DS3) has shown potential therapeutic effects [3,4]; however, there is little evidence of how exactly D3S affects the cells and its targets. As such, the objective of this study is to use bioinformatic tools to determine probable targets of D3S in human cells as well as to determine possible effects 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 of 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. Regarding these last ones, the Pubmed database was used in order to find information according to what the bioinformatics suggested.

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; we achieved this with a simple search string: "Gene Name" AND "Delphinidin 3 Sambubiosid". The search included terms appearing 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 criterion was any study that included 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 were excluded.

2.4. Results

Data from the Swiss Target Prediction site. Figure 1 shows the top 15 target classes of molecules that DS3 could interact with, most of which are 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 S1 [5,6].

2.4.1. Enriched Analysis of Gene Ontology and Metabolic Pathways

The site ShinnyGo 0.77 was used to perform Gene Ontology and KEGG analysis [7]. The full results of the Gene Ontology analysis are shown in Table 1. The results of KEGG analysis are shown in Table 2, and images of each pathway with the signaling of the potential changes in the protein are displayed in Supplementary Material S2.

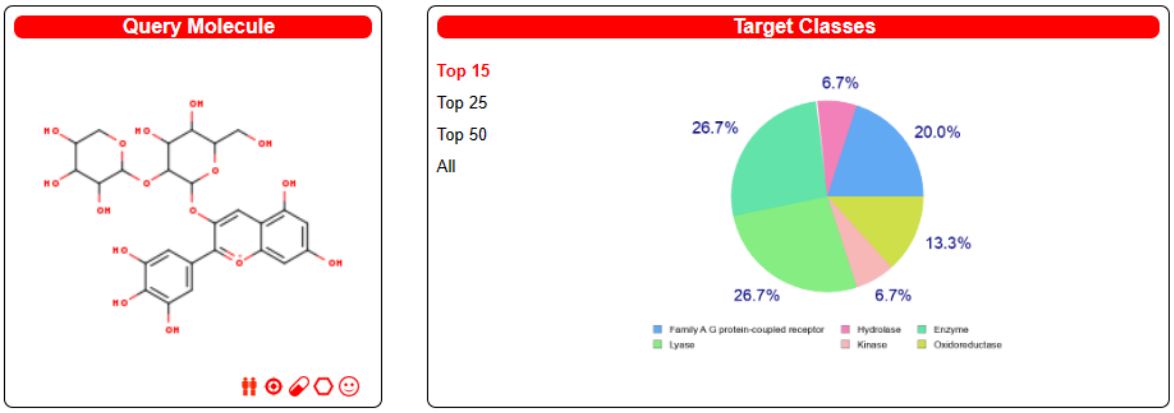

Figure 1. Top 15 molecular targets of DS3 according to the Swiss Target Prediction site.

Table 1. Functional enrichment analysis of the genes predicted to interact with DS3.

Enrichment FDR	Genes	Pathway Genes	Fold Enrichment	Pathway	Genes
4.20×10^{-18}	10	17	141.151703	Nitrogen metabolism	CA2, CA9, CA14, CA6, CA1, CA3, CA4, CA7, CA5A CA13
1.49×10^{-9}	15	354	10.1677074	PI3K-Akt signaling pathway	GSK3B, PIK3CG, MET, IL2, FLT3, PKN1, KDR, IGF1R, AKT1, MCL1, PIK3R1, EGFR, SYK, PTK2, INSR
2.21×10^{-9}	9	79	27.3369754	EGFR tyrosine kinase inhibitor resistance	GSK3B, MET, KDR, IGF1R, AKT1, PIK3R1, EGFR, AXL, SRC
2.73×10^{-9}	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
1.98×10^{-7}	8	95	20.2069806	Endocrine resistance	MMP2, MMP9, IGF1R, AKT1, PIK3R1, EGFR, PTK2, SRC
3.94×10^{-6}	7	108	15.5528265	Insulin resistance	NR1H3, GSK3B, PYGL, AKT1, PIK3R1, INSR, RPS6KA3
4.89×10^{-6}	6	70	20.5678196	Central carbon metabolism in cancer	HIF1A, MET, FLT3, AKT1, PIK3R1, EGFR
2.28×10^{-5}	8	214	8.97038859	Lipid and atherosclerosis	CAMK2B, GSK3B, MMP9, AKT1, PIK3R1, MMP3, PTK2, SRC
2.52×10^{-5}	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

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 role 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

2.4.2. Protein–Protein Interaction Network

The STRING database was used to predict the associations between protein targets of DS3 [8–20]. The network was constructed with a medium confidence of 0.400. The interactome had 257 edges, 57 nodes, an average node degree of 9.02, and a PPI enrichment p-value of $< 1.0 \times 10^{-16}$. Figure 2 shows the interactome and Table 2 shows the hub genes with at least 10 interactions.

Next, we will discuss data collection from the evidence of the hub genes regarding DS3. The results from the research in the PubMed database for articles on investigating hub genes and DS3 are shown in Table 3. These results are presented separately if direct evidence is found at the RNA, protein, or pathway level.

Table 3. Evidence found for 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]

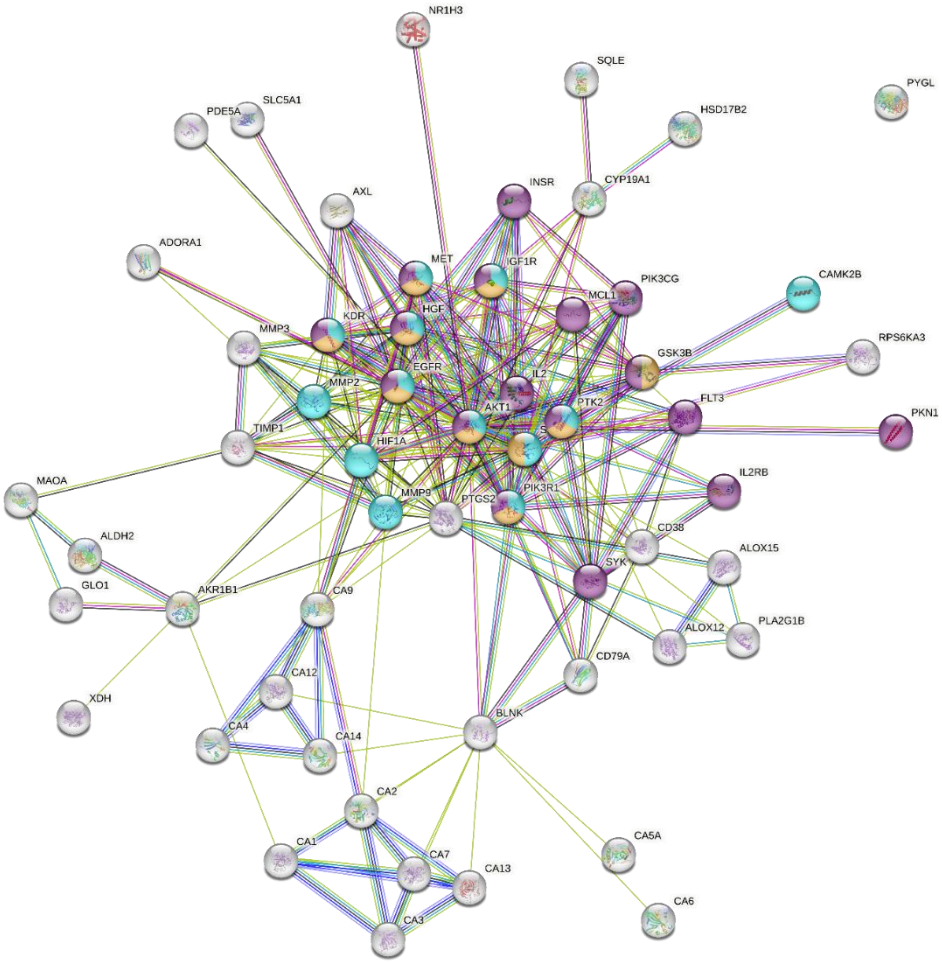

Figure 2. PPI network. Each of the edges is a specific protein with a significant protein–protein association. The blue and purple borders are known interactions recognized by several databases (previously curated and experimentally curated). The predicted interactions of each neighborhood protein, protein-gene fusion, and protein-gene concurrence are highlighted in green, red, and navy blue. Other colors, such as grass green, black, and gray, represent text mining, coexpression, and protein homology, respectively.

3. Discussion

The results obtained with the SwissTargetPrediction software (2019 version) (Figure 1) have their basis in the mathematical fundament of SwissTargetPrediction, which makes docking predictions with the software EADocks DSS (2019 version) [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) [25]; by employing this combination of tools, EADocks DSS can achieve a success rate of up to 96% in identifying ligands with fewer than 15 free dihedral angles and/or test complexes with adequately defined biding pockets. 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 2022 study, a team of researchers from China led by Lili Yan [26] looked at Erianin (a biphenyl compound) regarding its predicted molecular targets in a specific site; then, they compared the matches of these targets (along with the results of other bioinformatic tools) with then-current

information published by other authors, seeing plenty of overlap in their results. This shows how this tool (SwissTargetPrediction) has been used with good success to find information regarding molecular target information.

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

Regarding the KEGG analysis of the affected pathways (Table 1), the results indicate a dysregulation in the metabolism of nitrogen (which is key to the regulation of energy metabolism and protein metabolism) and glucose metabolism (especially in muscle and adipose tissue). Interestingly, glucose metabolism alterations seemed to mostly be attributed the PI3K-Akt pathway; this result agrees with multiple studies that have investigated the effects of DS3 on PI3K-AKT [21–24].

On the other hand, the protein-to-protein analysis also supported the idea that the PI3K-AKT pathway is a major target of DS3, with AKT being the most linked node in the whole analysis. Also, the information on the hub genes (Table 2) shows a trend of genes related to the metabolism of glucose and nitrogen, inflammation, and angiogenesis. This not only correlates 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 [28].

As for the search for evidence, using the PubMed database, we looked for any information regarding the hub genes obtained from protein-to-protein analysis and DS3. According to the results of the Gene Ontology analysis (Table 1), most of the genes of interest are related to the PI3K-Akt pathway. Out of these, it is fascinating to see the effects on EGFR, for which there is more evidence of what happens when it is exposed to DS3: reduced expression of the gene, suppressed function of the protein transcribed from it, and, finally, an association with inhibiting the whole PI3K-AKT pathway in this condition [21–24]. However, it is important to remark on 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. Conclusions

The predictive analysis indicated 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 that these changes also can occur directly in the protein, not only in mRNA. Also, quite possibly the most important result according to the bioinformatic tools is the potential modification in the function of several metabolic pathways, in particular, the effects that DS3 has on the PI3K-AKT pathway; these results are also supported by the findings presented in Tables 2 and 3. However, there are not enough published studies on DS3 and its other potential targets (suggested by the bioinformatic tools). The lack of research in this area opens up possibilities to conduct new studies with a high probability of having significant relevance, helping us to understand the mechanisms of action of DS3 in human cells.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/IECN2023-15797/s1, Supplementary Material S1: Most likely targets for DS3; Supplementary Material S2: KEGG pathways for DS3.

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draft preparation, S.R.Z.-H.; writing—review and editing, S.R.Z.-H., C.M.R.-R., T.G.-I., M.M.-C. and A.P.-L.; visualization, S.R.Z.-H.; supervision, C.M.R.-R.; project administration, C.M.R.-R.; funding acquisition, C.M.R.-R. All authors have read and agreed to the published version of the manuscript.

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