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Malacca Pharmaceutics

Vol. 2, No. 2, 2024

A Comprehensive Network Pharmacology Study on the Diabetes-Fighting Capabilities of Yacon Leaf Extract

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Article History

Received 15 April 2024
Revised 2 June 2024
Accepted 13 June 2024
Available Online 19 June 2024

Keywords:
Smallanthus sonchifolius
Diabetes Mellitus Type 2
Protein target
Network pharmacology

Abstract

Indonesia ranks fourth in the world for the number of diabetes mellitus (DM) sufferers. DM is a group of metabolic disorders characterized by hyperglycemia due to insulin abnormalities. This research employs Network Pharmacology analysis to examine the target proteins and pharmacological network profiles predicted to be influenced by compounds in the leaves of *Smallanthus sonchifolius* (yacon) for their anti-diabetic effects. Gas chromatography-mass spectrometry (GC-MS) identified 41 secondary metabolite compounds in yacon leaves, seven of which have a Pa value > 0.5. Compound C28 has the highest Pa value as an insulin promoter, at 0.662. A total of 129 target proteins were found for the secondary metabolite compounds in yacon leaves, and 5,112 target proteins were identified for Type 2 Diabetes Mellitus (T2DM). The intersection analysis between yacon leaves and T2DM revealed 32 common proteins. Network analysis highlighted 10 top proteins: ESR1, PPAR-α, HMGCR, CYP19A1, PPARD, PTP1N, GRIN2B, FYN, AR, and SHBG. Among these, PPAR-α shows great potential and promising prospects as a target for further exploration. Considering several parameters, it can be concluded that PPAR-α is a promising protein and a potential target for new drug candidates for T2DM.

1. Introduction

Smallanthus sonchifolius, commonly known as the insulin plant or yacon, belongs to the *Smallanthus* genus within the Asteraceae family. Various parts of the insulin plant include its tubers, leaves, and flowers. Each part of the insulin plant offers numerous benefits, particularly its leaves. The insulin plant leaves contain phenolic compounds belonging to the group of free organic acids, such as chlorogenic acid (caffeic acid and its derivatives), flavonoids, diterpenes, and sesquiterpene lactones [1].

Several investigations have documented significant phenolic compounds in Yacon leaf and tuber extracts, including chlorogenic, protocatechuic, ferulic, rosmarinic, gallic, gentisic, and caffeic acids and their variations. Furthermore, research has highlighted yacon leaf extracts' antioxidant properties and protective effects against oxidative stress. These extracts also positively impact glucose metabolism in rat liver cells and exhibit insulin-mimicking effects. Additionally, caffeic acid has been shown to lower plasma glucose levels in diabetes,

while chlorogenic acid increases glucose tolerance and reduces insulin resistance [2].

The effects of yacon leaf extract are known to induce improvements in dysmetabolism and DM-related cardiomyopathy, manifested as a 63.9% decrease in glycemia, a 49.3% increase in insulin concentration, reduced concentrations of insulin, triacylglycerol, and free fatty acids, pancreatic cell improvements, increased antioxidant enzyme activity, and cardiac tissue repair [3], 96% ethanol extract of yacon leaves has α-glucosidase enzyme inhibitory activity with an inhibition percentage of 75.53%. Therefore, this compound has the potential to control blood glucose levels by improving the function of the pancreas in producing insulin [4]. The use of natural ingredients, both in medicinal and other forms, tends to increase. Traditional medicine is still popular among Indonesians, because it is considered effective and affordable [5–7].

Diabetes Mellitus (DM) is a metabolic disorder characterized by hyperglycemia resulting from insulin abnormalities, where insulin production is not maximized by the pancreas [8, 9]. According to the International Diabetes Federation (IDF), in 2022, 8.75 million people worldwide were classified as having DM. In Indonesia in 2022, there were 41,817 individuals with DM, including 13,311 individuals aged <20 years, 26,781 individuals aged between 20-59 years, and 1,721 individuals aged 60 years or older [10].

Indonesia ranks fourth globally in terms of the highest number of diabetes mellitus patients. From 2019 to 2020, the number of diabetes mellitus patients increased by 33,751 individuals, from 841,994 in 2019 to 875,745 in 2020 [11]. The prevalence of diabetes mellitus by gender in 2019 was 9.65% in males and 9% in females. It is estimated that the prevalence of diabetes mellitus patients will increase with the aging population, reaching 19.9% or 111.2 million in the 65-79 age group. This increase is projected to rise to 576 million by 2030 and 700 million by 2045 [12]. Generally, diabetes treatment involves oral antidiabetic drugs and insulin injections to control blood sugar levels and maintain normalcy [13]. The use of safe antidiabetic drugs with minimal side effects is crucial due to the long-term nature of diabetes treatment. Currently, diabetes treatment largely relies on synthetic drugs, which can lead to permanent organ damage. Therefore, there is an increasing tendency to utilize natural substances, whether in medicine or otherwise [14, 15].

The use of traditional medicine is needed as an alternative diabetes therapy in long-term treatment, one of which is using yacon leaves. One strategy that can be employed to ascertain the antidiabetic activity of yacon leaves is by predicting protein targets. Pharmacological network studies can be utilized to elucidate the relationship between molecular proteins and diabetes mellitus, which exhibits multi-component, multi-target, and multichannel characteristics. Moreover, they offer better guidance for subsequent steps and propose new suggestions and approaches for the mechanisms and drug development in treating diabetes mellitus [16].

The research aimed to explore the potential of yacon leaf compounds as antidiabetic agents, pharmacokinetics, biological pathways, the pharmacological network profile of compound proteins, and the molecular protein predicted to be the target compound in yacon leaves against Type 2 Diabetes Mellitus (T2DM) through network pharmacology analysis. This method facilitates the identification of bioactive compound collections within plants and maps drug targets and molecules associated with syndromic disease phenotypes into biomolecular networks [17]. This investigation seeks to elucidate the molecular mechanisms of yacon leaf extract's antidiabetic role and provide insight into developing new therapeutic interventions for diabetes mellitus.

2. Materials and Methods

2.1. Bioactive Compounds of Yacon Leaf Extract

The bioactive compounds of yacon leaves were obtained from previous findings and analyzed using the Gas Chromatography-Mass Spectrometry (GC-MS) method. GCMS testing used yacon leaf samples harvested at the end of the Kharkiv region, Merefa growing season. Sample preparation was carried out by analyzing carboxylic acid methyl ether compounds, and testing using hexane solvent. The GC-MS results were tested using yacon leaf samples [18].

2.2. Preparation of Yacon Bioactive Compounds Profile

The Simplified Molecular-Input Line-Entry System (SMILES) profiles, 2D structures in sdf format, and three-dimensional (3D) structures of each component contained in Gas Chromatography-Mass Spectrometry (GC-MS) analysis of yacon leaves were obtained from the PubChem website [19].

2.3. Prediction of Bioactive Compounds' Activity

The compounds in the yacon leaf extract are valuable for developing anti-diabetic drugs that specifically target Type 2 Diabetes Mellitus (DMT2). Therefore, an analysis of potential activity was conducted using the prediction tool WAY2DRUG PASS (http://www.pharmaexpert.ru/passonline/predict.php). This tool employs Structure-Activity Relationship (SAR) analysis to compare input compounds

with known ones exhibiting specific potential [20]. The potential activity of compounds on this website will be indicated by a Pa score (Probability activity). If the Pa value exceeds 0.7 (Pa > 0.7), the compound has very high biological activity, and the results are not significantly different from tests on a laboratory scale. On the other hand, if the Pa value is above 0.5 but less than 0.7 (0.5 < Pa < 0.7), the compound exhibits quite high biological activity. It is considered a bioactive compound in both in vitro and in vivo experimental tests and shows potential for developing new medicinal compounds with appropriate bioactivity. Compounds with a predicted value of insulin increase > 0.5 will be continued for network analysis [21].

2.4. Pharmacokinetic Analysis

In drug development, a series of pharmacokinetic parameters known as ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) is required to assess how a drug behaves in the body, including its absorption into the bloodstream, distribution to tissues, metabolism by enzymes, excretion, and potential toxic effects. Lipinski's Rule of 5 (Ro5) is used as a parameter to examine the drug characteristics determined for each ligand. The SMILES notation of each ligand is entered into the Protox II database (https://toxnew.charite.de/protox_II/index.php?site=compound_input) and the ADMETLab 2.0 database (https://admetmesh.scbdd.com/service/evaluation/index) [22, 23]. Compounds that were further analyzed are those that belong to toxicity classes IV-VI and meet the Ro5 criteria.

2.5. Identification and Analysis of Protein Targets

Identification and analysis of protein targets were performed on compounds that had Pa values > 0.5, met the Ro5 criteria and toxicity classes IV-VI, using the Swiss Target Prediction analysis tool (http://www.swisstargetprediction.ch/) and Super Pred (https://predik.amal.de/), prediction results can be obtained by entering the identified SMILES notation. Genes and proteins related to T2DM were obtained from the Gene cards database (https://www.genecards.org/), disease targets and yacon leaf extract targets were then mapped using Draw Venn Diagram (https://bioinformatics.psb.ugent.be/webtools/Venn/) to determine the target the target cutoff point. Annotation of insulin leaf extract targets focused on biological processes and pathways from the Kyoto Encyclopedia of Genes and Genomes (KEGG) and was performed using the DA-VID web server (https://david.ncifcrf.gov/) [21], then annotation of gene ontology biological processes for insulin leaf extract targets was performed using ShinyGO 0.76 (http://bioinformatics.sdstate.edu/go76).

2.6. Analysis of Network Pharmacology

Analysis of interactions between target proteins obtained from insulin leaf extract and their relationship with T2DM was carried out using the STRING (Search Tools for the Search of Interacting Genes/Proteins) database version 11.5. The input consists of the intersection of target proteins derived from insulin leaf extract and T2DM-related proteins, including the insulin receptor (INSR). The organism chosen for analysis is Homo sapiens (human), the resulting analysis data is presented in TSV format from the STRING database, and further processed using Cytoscape version 3.10 for detailed examination [21].

3. Results and Discussion

3.1. Bioactive Compounds of Yacon Extract

The yacon extract's bioactive compound profile was derived through GC-MS analysis [18]. The SMILES profiles and three-dimensional (3D) structures were obtained from PubChem. There were 41 compounds from the GCMS results of yacon leaf extract, 18 of which were identified as carboxylic acids, and the corresponding SMILES data were compiled, as presented in Table 1.

3.2. Potential of Yacon Leaf Bioactive Compounds for DMT2 Treatment

The parameters utilized to assess the potential of yacon leaf extract as an antidiabetic agent encompass its effects as an insulin promoter, a trigger for nuclear factor erythroid 2-related factor 2 (Nrf2), and a wound healing agent, as depicted in Figure 1 [20]. The prediction of compounds exhibiting antidiabetic activity primarily hinges on their capacity as insulin promoters due to their consistent ability to enhance insulin levels. Among these compounds, seven were identified with Pa values > 0.5, and compound C28 emerged with the highest Pa value as an insulin promoter, reaching 0.662, as detailed in Table 2.

Based on the insulin promoter SAR profile, seven secondary metabolite compounds with Pa values > 0.5 were identified, as illustrated in Figure 2. Among these, Bicyclo[3.1.1]heptane, 2,6,6-trimethyl, (1.alpha, 2.beta, 5.alpha)-, m-menth-1(7)-ene, and Eicosane belong to the terpenoid group. The compound 2-pyrrolidinone, 1-methyl- falls within the alkaloid category, while several compounds are classified as lipid or fatty acid esters, namely Hexadecanoic acid, methyl ester, Hexadecanoic acid, 14-methyl-, methyl ester, and Octadecanoic acid, methyl ester.

Table 1. GC-MS analysis results and compounds profile of yacon leaf from PubChem database.

No.	Molecular Formula	Compound	Canonical SMILES
1	$C_8H_{12}O_5$	2-pentenedioic acid, 3-methoxy-, dimethyl ester	COC(=CC(=O)OC)CC(=O)OC
2	$C_9H_{14}O_7$	Citric acid, trimethyl ester	COC(=O)CC(CC(=O)OC)(C(=O)OC)O
3	$C_{15}H_{30}O_2$	Methyl tetradecanoate	CCCCCCCCCCCCCC(=O)OC
4	$C_{10}H_{18}$	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, (1.alpha, 2.beta, 5.alpha)-	CC1CCC2CC1C2(C)C
5	$C_{11}H_{18}O$	5,6,7,7-tetramethyl-octa-3,5-dien-2-one	CC(=C(C)C(C)(C)C)C=CC(=O)C
6	$C_8H_{12}O$	Cyclohexanol, 1-ethynyl-	C#CC1(CCCCC1)O
7	$C_{22}H_{22}FN_3O$	1-(4-fluorophenyl)-2-[(4-hydroxy-6-methylpyrimidin-2-yl)	CC1=CC(=O)NC(=N1)SCC(=O)C2=CC=C(C=C2)F
8	$C_{20}H_{40}O$	3,7,11,15-tetramethyl-2-hexadecen-1-ol	CC(C)CCCC(C)CCCC(C)CCCC(=CCO)C
9	$C_{10}H_{18}$	m-menth-1(7)-ene	CC(C)C1CCCC(=C)C1
10	$C_{10}H_{16}O$	2-cyclopenten-1-one, 2-pentyl-	CCCCCC1=CCCC1=O
11	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester	CCCCCCCCCCCCCCCC(=O)OC
12	$C_{18}H_{28}O_3$	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	CC(C)(C)C1=CC(=CC(=C1O)C(C)(C)C)CCC(=O)OC
13	$C_{12}H_{27}O_4P$	Phosphoric acid, monododecyl ester	CCCCCCCCCCCCOP(=O)(O)O
14	$C_{11}H_{18}O$	2-pyrrolidinone, 1-methyl-	CN1CCCC1=O
15	C_5H_9NO	2-n-heptylfuran	CCCCCCCC1=CC=CO1
16	$C_{18}H_{36}O_2$	hexadecanoic acid, 14-methyl-, methyl ester	CCC(C)CCCCCCCCCCCCC(=O)OC
17	$C_{10}H_{16}O$	2,4-dodecadienal	CCCCCCCC=CC=CC=O
18	$C_{18}H_{32}O_2$	Valeric acid, tridec-2-ynyl ester	CCCCCCCCCCC#CCOC(=O)CCCC
19	$C_{18}H_{32}O$	9,12,15-octadecatrien-1-ol	CCC=CCC=CCC=CCCCCCCCCO
20	$C_{19}H_{36}O_2$	10-octadecenoic acid, methyl ester	CCCCCCCC=CCCCCCCCCC(=O)OC
21	$C_{19}H_{34}O_2$	Octadecanoic acid, methyl ester	CCCCCCCCCCCCCCCCCC(=O)OC
22	$C_{21}H_{46}OSi$	6,10,14-trimethyl-pentadecan-2-ol, o-trimethylsilyl	CC(C)CCCC(C)CCCC(C)CCCC(C)O[Si](C)(C)C
23	$C_{10}H_{14}O$	1(2h)-pentalenone, hexahydro-5-methyl-4-methylene	CC1CC2C(C1=C)CCC2=O
24	$C_{19}H_{32}O_2$	9,12,15-octadecatrienoic acid, methyl ester	CCC=CCC=CCC=CCCCCCCCC(=O)OC
25	$C_{21}H_{42}O_2$	Eicosanoic acid, methyl ester	CCCCCCCCCCCCCCCCCCCC(=O)OC
26	$C_{22}H_{44}O_2$	Heneicosanoic acid, methyl ester	CCCCCCCCCCCCCCCCCCCCC(=O)OC
27	$C_{23}H_{46}O_2$	Docosanoic acid,methyl ester	CCCCCCCCCCCCCCCCCCCCCC(=O)OC
28	$C_{20}H_{42}$	Eicosane	CCCCCCCCCCCCCCCCCCCC
29	$C_{24}H_{48}O_2$	Tricosanoic acid, methyl ester	CCCCCCCCCCCCCCCCCCCCCC(=O)OC
30	$C_{14}H_{22}N_2O_2S$	Diisopropylketone p-tosylhydrazone	CC1=CC=C(C=C1)S(=O)(=O)NN=C(C(C)C)C(C)C
31	$C_{25}H_{50}O_2$	Tetracosanoic acid, methyl ester	CCCCCCCCCCCCCCCCCCCCCCCC(=O)OC
32	$C_{14}H_{44}O_6Si_7$	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13, 13-tetradecamethyl-	C[Si](C)O[Si](C)(C)O[Si](C)(C)O[Si](C)(C)O[Si](C)(C)O[Si](C)(C)O[Si](C)C
33	$C_{10}H_{30}O_3Si_4$	Tetrasiloxane, decamethyl-	C[Si](C)(C)O[Si](C)(C)O[Si](C)(C)O[Si](C)(C)C
34	$C_{12}H_{22}Si_2$	1,3-bis(trimethylsilyl)benzene	C[Si](C)(C)C1=CC(=CC=C1)[Si](C)(C)C
35	$C_{27}H_{54}O_2$	Hexacosanoic acid, methyl ester	CCCCCCCCCCCCCCCCCCCCCCCCCC(=O)OC
36	$C_{13}H_{22}OSi_2$	2,4,6-cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-	C[Si](C)(C)C1=CC(=CC(=O)C=C1)[Si](C)(C)C
37	$C_{10}H_{30}O_3Si_4$	Tetrasiloxane, decamethyl-	C[Si](C)(C)O[Si](C)(C)O[Si](C)(C)O[Si](C)(C)C
38	$C_6H_{20}O_4Si_4$	Cyclotrisiloxane, hexamethyl-	C[Si]1(O[Si](O[Si](O1)(C)C)(C)C)C
39	$C_{17}H_{14}O_4$	2-(acetoxymethyl)-3-(methoxycarbonyl) biphenylene	CC(=O)OCC1=CC2=C(C=C1C(=O)OC)C3=CC=CC=C32
40	$C_{22}H_{44}O_2$	Heneicosanoic acid, methyl ester	CCCCCCCCCCCCCCCCCCCCC(=O)OC
41	$C_{11}H_{22}O_2$	Decanoic acid, methyl ester	CCCCCCCCCC(=O)OC

3.3. Pharmacokinetic Evaluation and Drug Similarity Assessment

The development of drugs encounters significant challenges, such as suboptimal pharmacokinetic properties and safety issues, which hinder drug discovery. ADMET (absorption, distribution, metabolism, excretion, and toxicity) represents a set of crucial pharmacokinetic parameters in drug development, assessing how a drug behaves within the body, including absorption into the bloodstream, distribution to tissues, metabolism by enzymes, excretion, and potential toxic effects [23]. Table 3 presents the results of ADMET and the analysis of drug similarity for potential compounds from Yacon leaf extracts using AdmetLab 2.0. Red indicates discrepancies against ADMET and Lipinski criteria.

Overall, the toxicity classes of these compounds range from class IV to class VI, as depicted in Figure 3,

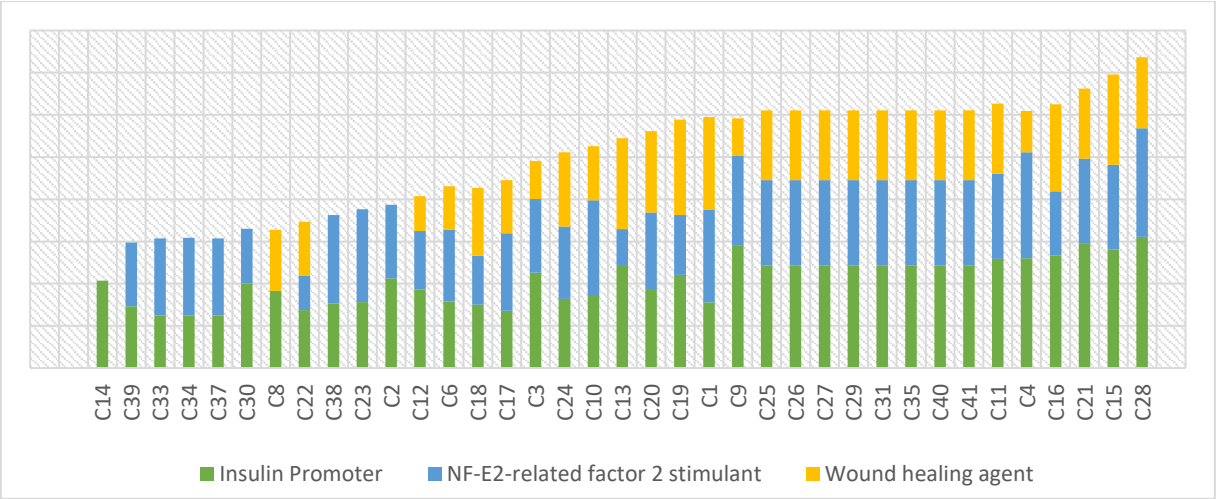

Figure 1. Evaluation of the potential of yacon leaf extract as an antidiabetic based on predicted structure-activity relationships (SAR).

Table 2. SAR profile of insulin promoter (Pa score > 0.5) from bioactive compounds from yacon leaf extract.

Compound Identity	IUPAC Compound Name	Insulin Promoter Score
C4	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, (1.alpha, 2.beta, 5.alpha)-	0.521
C9	m-menth-1(7)-ene	0.582
C11	Hexadecanoic acid, methyl ester	0.518
C15	2-pyrrolidinone, 1-methyl-	0.562
C16	Hexadecanoic acid, 14-methyl-, methyl ester	0.533
C21	Octadecanoic acid, methyl ester	0.589
C28	Eicosane	0.662

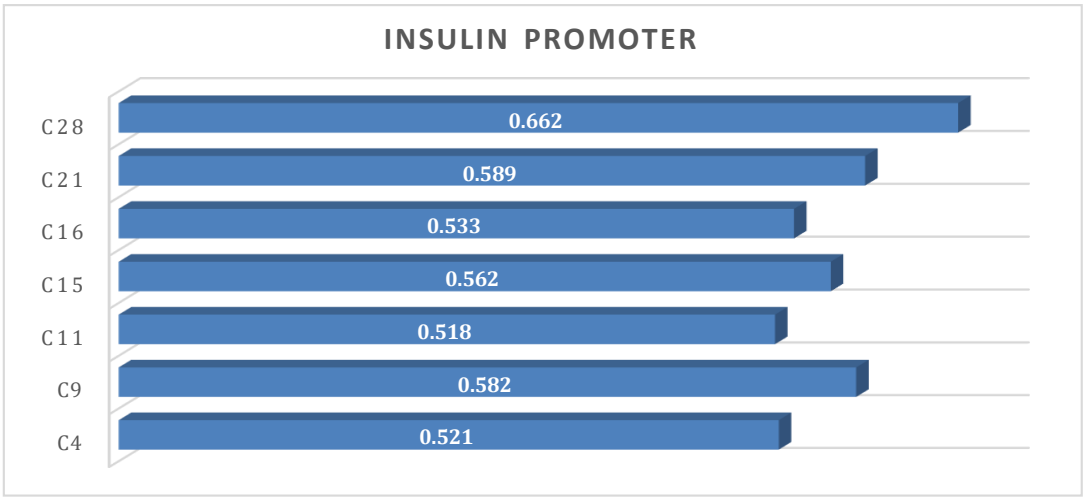

Figure 2. SAR-based prediction regarding the potential of yacon leaf extract to treat T2DM, especially as an insulin promoter.

suggesting that these compounds are predicted to have low toxicity levels and can be considered safe, including the seven compounds that are the focus of further analysis. Compounds C15 and C28 fall into class 4, compounds C9, C11, C16, and C21 belong to class 5, and compound C4 is classified under class 6. Based on Lipinski's Ro5 assessment for 41 secondary metabolite compounds from Yacon leaves, 40 meet the Ro5 criteria, indicating their potential as drug-like compounds. Furthermore, the Protox II database was utilized to assess the compliance of compounds with Lipinski's Ro5 and their potential toxicity. Bars outlined in black indicate the seven selected compounds for additional analysis.

3.4. Potential Protein Targets and Biological Pathways

Following identifying compounds with high bioavailability predictions, the subsequent step involves searching for protein targets. The search results indicate the presence of 129 proteins predicted to interact with the secondary metabolites of Yacon leaves. Subsequently, the search continued to identify proteins associated with DMT2 by leveraging the online GeneCards database, where 5112

Table 3. ADMET results and drug similarity analysis of potential compounds from yacon leaf extract. Bold indicates discrepancies against ADMET and Lipinski criteria.

Compounds	Pgp-inhibitor	Pgp-substrate	HIA	F20%	F30%	Blood Brain Barrier	H- HT	DILI	FDAMDD	Drug Likeness Lipinski
C1	0.026	0.004	0.022	0.304	**0.99**	**0.988**	**0.803**	0.241	0.13	Approved
C2	0.015	0.001	0.478	0.419	**0.995**	**0.702**	0.53	0.589	0.062	Approved
C3	0.149	0.002	0.003	**0.985**	**0.993**	**0.76**	0.027	0.285	0.019	**Declined**
C4	0.001	0	0.003	0.029	0.455	**0.882**	0.071	0.091	0.122	Approved
C5	**0.966**	0.006	0.026	0.385	0.026	0.112	0.269	0.095	0.443	Approved
C6	0	0	0.003	0.007	0.002	**0.998**	0.081	0.087	0.059	Approved
C7	0.003	0.003	0.007	0.001	0.003	0.093	0.368	**0.965**	0.463	Approved
C8	0.034	0.001	0.002	0.687	0.357	0.227	0.098	0.051	0.026	Approved
C9	0	0	0.002	**0.787**	**0.778**	**0.995**	0.076	0.233	0,054	Approved
C10	0.245	0.001	0.003	0.036	0.065	**0.991**	0.582	0.042	**0.823**	Approved
C11	0.033	0.002	0.002	**0.98**	**0.996**	0.388	0.026	0.328	0.017	Approved
C12	**0.975**	0.004	0.232	**0.995**	**0.969**	**0.753**	0.124	0.049	0.313	Approved
C13	0.033	0.001	0.002	**0.939**	**0.783**	0.129	0.02	0.028	0,246	Approved
C14	0.001	0.062	0.009	0.019	0.02	**0.99**	0.119	0.061	0.106	Approved
C15	0.002	0.018	0.002	0.653	**0.965**	0.433	0.066	0.231	0,034	Approved
C16	0.092	0.001	0.002	**0.969**	**0.986**	0.292	0.035	0.279	0.027	Approved
C17	0.001	0.007	0.006	0.494	**0.888**	**0.992**	0.586	0.035	**0.873**	Approved
C18	**0.815**	0.002	0.004	**0.998**	**0.997**	0.434	0.127	0.237	0.535	**Declined**
C19	0	0.199	0.006	0.009	**0.94**	0.406	0.002	0.009	0,015	Approved
C20	0.002	0.006	0.004	**0.882**	**0.977**	0.179	0.018	0.056	0.017	Approved
C21	0.006	0.001	0.002	**0.973**	**0.998**	0.197	0.025	0.375	0.016	Approved
C22	0.487	0	0.002	0.474	**0.776**	0.027	0.085	0.18	0.011	Approved
C23	0	0.001	0.006	0.046	0.003	**0.98**	0.511	0.285	**0.737**	Approved
C24	0	0.019	0.008	0.002	**0.913**	0.317	0.006	0.016	0.028	Approved
C25	0.001	0.001	0.003	**0.955**	**0.999**	0.109	0.023	0.426	0.016	Approved
C26	0	0.001	0.003	**0.942**	**0.999**	0.081	0.022	0.452	0.015	Approved
C27	0	0.001	0.003	**0.923**	**1.0**	0.06	0.021	0.475	0.016	Approved
C28	0	0	0.02	0.205	**0.999**	0.037	0.006	0.334	0.035	Approved
C29	0	0.001	0.003	**0.902**	**1.0**	0.044	0.02	0.492	0.016	Approved
C30	0.002	0.001	0.024	0.264	0.002	0.161	0.106	**0.985**	0.018	**Declined**
C31	0	0.001	0.003	**0.88**	**1.0**	0.033	0.019	0.505	0.016	Approved
C32	0.096	**0.757**	**0.991**	0.014	0.009	0	0.004	0.014	0.215	**Declined**
C33	0.034	0.04	**0.944**	0.026	0.099	0	0.01	0.008	0.298	Approved
C34	0.007	0.005	0.136	0.012	0.009	0.018	0.047	0.047	0.062	Approved
C35	0	0	0.003	**0.828**	**1.0**	0.019	0.016	0.524	0.017	Approved
C36	0.01	0.007	0.645	0.012	0.006	0.01	0.052	0.057	0.069	Approved
C37	0.034	0.04	**0.944**	0.026	0.009	0	0.01	0.008	0.298	Approved
C38	0.052	0.007	0.074	0.006	0.061	0	0.021	0.036	0.151	Approved
C39	0.019	0.001	0.003	0.027	**0.979**	0.259	0.326	**0.922**	0.046	Approved
C40	0	0.001	0.003	**0.942**	**0.999**	0.081	0.022	0.452	0.015	Approved
C41	0.592	0.005	0.003	**0.975**	**0.971**	**0.985**	0.031	0.185	0.024	Approved

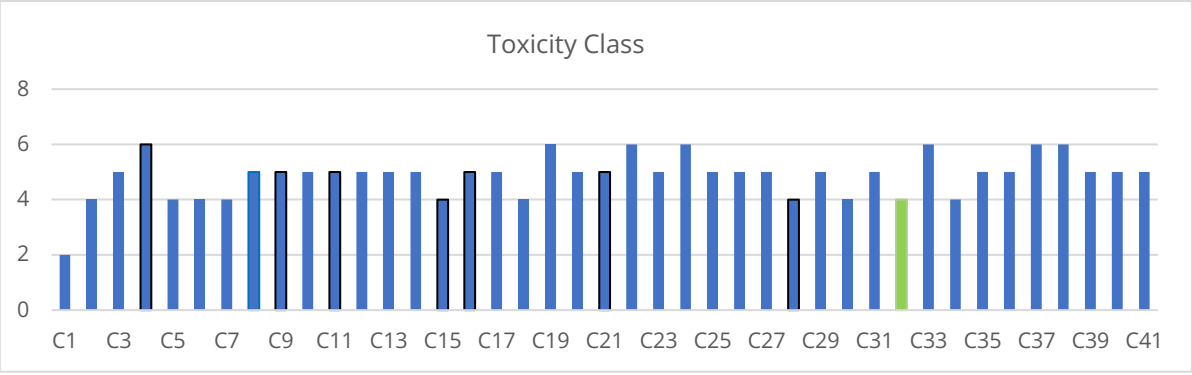

Figure 3. Prediction of similarity of drug compounds in yacon leaf extract using Lipinski's Ro5 and prediction of toxicity classes. The black-lined bars indicate the seven compounds selected for additional analysis. The green bar indicates non-compliance with the Ro5 criteria.

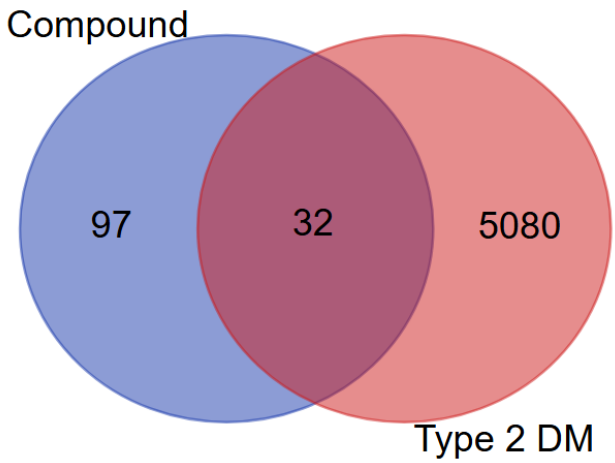

Figure 4. Venn diagram showing shared targets between Yacon leaf extract and genes related to T2DM.

Table 4. List of protein code names resulting from intersections.

Protein code	Protein name
NOS2	Nitric Oxide Synthase 2
SLC22A2	Solute Carrier Family 22 Member 2
SHBG	Sex Hormone Binding Globulin
CYP19A1	Cytochrome P450 Family 19 Subfamily A Member 1
PPARD	Peroxisome Proliferator Activated Receptor Delta
PAM	Peptidylglycine Alpha-Amidating Monooxygenase
G6PD	Glucose-6-Phosphate Dehydrogenase
FABP2	Fatty Acid Binding Protein 2
GRIN2B	Glutamate Ionotropic Receptor NMDA Type Subunit 2B
ACHE	Acetylcholinesterase (Yt Blood Group)
PTPN1	Protein Tyrosine Phosphatase Non-Receptor Type 1
AR	Adrenergic Receptor
ADRA2A	Adrenoceptor Alpha 2A
GPR119	G Protein-Coupled Receptor 119
ADRB1	Adrenoceptor Beta 1
CTSB	Cathepsin B
FYN	FYN Proto-Oncogene,
CTSK	Src Family Tyrosine Kinase Cathepsin K
ESR1	Estrogen Receptor 1
HMGCR	3-Hydroxy-3-Methylglutaryl-CoA Reductase
MC4R	Melanocortin 4 Receptor
CA4	Carbonic Anhydrase 4 PHD
PHF8	Finger Protein 8
BRD2	Bromodomain Containing 2
DCK	Deoxycytidine
KDM5C	Kinase Lysine Demethylase 5C
PFKFB3	6-Phosphofructo-2-Kinase/Fructose-2,6-Biphosphatase
PPAR-α	3
GRIN1	Peroxisome Proliferator Activated Receptor Alpha
CCR5	Glutamate Ionotropic Receptor NMDA Type Subunit 1
CYP24A1	C-C Motif Chemokine Receptor 5
SQLE	Cytochrome P450 Family 24 Subfamily A Member 1
	Squalene Epoxidase

proteins related to DMT2 were found (Figure 4). Targets related to DMT2 and targets from the Yacon leaf extract were then mapped using a Venn diagram to determine the intersection of targets. The intersection results between proteins predicted to interact with the secondary metabolites of Yacon leaves and proteins associated with DMT2 yielded 32 proteins (Table 4).

Through analysis conducted using the KEGG Pathway, high fold enrichment in pathways such as the cAMP signaling pathway, metabolic pathways, and the PPAR signaling pathway suggests their crucial role in the biological context studied, potentially as targets for therapeutic intervention, all of which contribute to DMT2 (Figure 5). Various protein targets implicated in DMT2 and associated with these pathways include carbonic anhydrase 4 (CA4), cytochrome P450 family 24 subfamily A member 1 (CYP24A1), cytochrome P450 family 19 subfamily A member 1 (CYP19A1), Squalene Epoxidase (SQLE), 3-Hydroxy-3-Methylglutaryl CoA Reductase (HMGCR), 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3), Glucose-6-phosphate dehydrogenase (G6PD), Fatty acid binding protein 2 (FABP2), Peroxisome proliferator-activated receptor alpha (PPARA), Peroxisome proliferator-activated receptor delta (PPARD), G protein-coupled receptor 119 (GPR119), Adrenoceptor beta 1 (ADRB1), and glutamate ionotropic receptor NMDA type subunit 2B (GRIN2B). In Figure 5, red indicates high activity, whereas blue indicates low activity.

3.5. Network Pharmacology Analysis

The network pharmacology analysis undertaken in this study has effectively pinpointed bioactive components implicated in DMT2 activity. Examination utilizing the KEGG pathway has demonstrated that the protein targets contained within the Yacon leaf extract are engaged in pathways such as the cAMP signaling pathway, metabolic pathways, PPAR signaling pathway, and insulin resistance pathway. Additionally, insulin receptors were integrated into the pharmacological network analysis to evaluate the protein targets' ability to interact with insulin receptors (INSR) (Table 5).

Based on the findings of the pharmacological network analysis, nine proteins are implicated in the metabolic pathway: CA4, CYP24A1, CYP19A1, SQLE, HMGCR, PFKFB3, G6PD, NOS2, and DCK, although 2 of them are not connected. Five interconnected proteins are present in the cAMP signaling pathway: ADRB1, GPR119, PPARA, GRIN2B, and GRIN1. involved in the PPAR signaling pathway: PPARD, FABP2, and PPARA. Furthermore, the protein targets involved in the insulin resistance pathway include PPARA and PTPN1 (Figure 6). Nodes represent all proteins produced by a single gene locus encoding DMT2-related proteins, while edges denote interactions between two proteins. Explanations accompanying nodes representing proteins also offer their

interpretations. Colored nodes signify key interaction elements, whereas white nodes indicate intermediary roles. Nodes containing information suggest known or predicted 3D structures of the proteins, while empty nodes indicate unknown 3D structures. Information on protein-protein interactions (PPI) of all target proteins is amalgamated and visualized, and their biological networks are scrutinized using Cytoscape. This analysis aims to categorize protein targets based on degree values and visualize and pinpoint essential proteins in the protein network, considering parameters such as degree value, betweenness centrality, and closeness centrality.

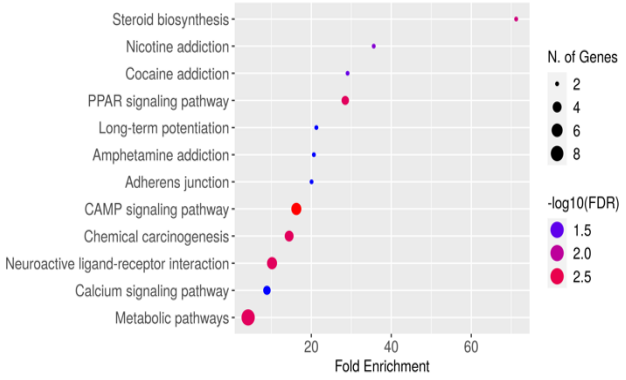

Figure 5. Gene ontology biological process annotation for Yacon leaf extract targets.

Table 5. Pathways associated with T2DM using KEGG analysis.

No	Pathway Code	Pathway Name
1	hsa01100	Metabolic pathways
2	hsa04024	cAMP signaling pathway
3	hsa04931	Insulin resistance
4	hsa03320	PPAR signaling pathway

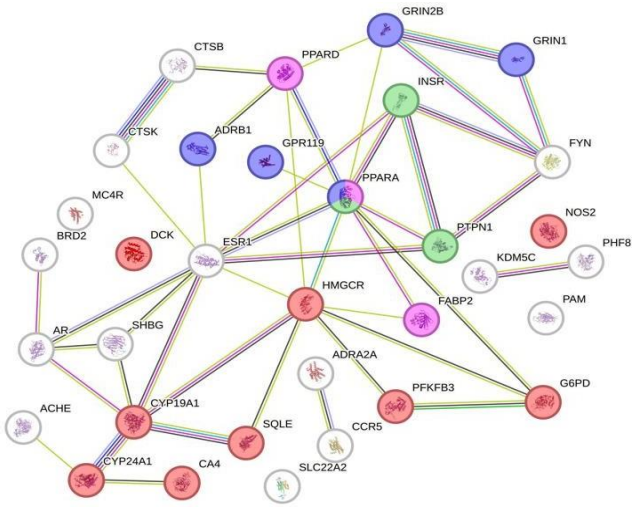

Figure 6. PPI of Yacon leaf extract targets in DMT2, red= metabolic pathway, blue= cAMP pathway, purple= PPAR pathway, green= insulin resistance pathway**.**

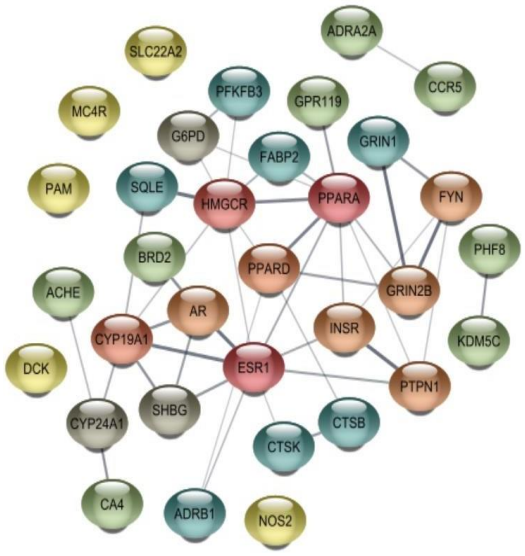

Figure 7. Classification of target proteins based on degree.

All protein targets are classified into six categories based on degree values. Red-colored nodes, namely PPAR-α, ESR1, and HMGCR, indicate high degree values ranging from 7 to 9, while orange-colored nodes (4-6), including CYP19A1, PPARD, PTPN1, GRIN2B, FYN, and AR, represent intermediate degree values. Gray-colored nodes, such as SHBG, CYP24A1, and G6PD, indicate a degree value of 3, whereas blue-colored nodes (degree value of 2), including ADRB1, CTSK, FABP2, PFKFB3, CTSB, GRIN1, and SQLE. Green-colored nodes with a degree value of 1 include ACHE, KDM5C, PHF8, BRD2, GPR119, CCR5, CA4, and ADRA2A, while yellow-colored nodes, including MC4R, PAM, DCK, SLC22A2, and NOS2, have a degree value of 0 (Figure 7)**.**

The analysis of PPI networks reveals ten core protein targets based on node degree: ESR1, PPAR-α, HMGCR, CYP19A1, PPARD, PTP1N, GRIN2B, FYN, AR, and SHBG (Table 6). These proteins play pivotal roles in developing DMT2, insulin sensitivity, insulin secretion, and glucose metabolism processes. In the context of the PPI network analysis, three proteins, ESR1 (score: 9.941095), PPAR-α (score: 9.783138), and HMGCR (score: 8.53488372), exhibit notably high overall scores. However, it's noteworthy that ESR1 and HMGCR don't display significant potential for DMT2; ESR1 shows a high potential for cancer in the insulin-resistant endocrine pathway, while HMGCR demonstrates a strong potential for reducing cholesterol levels.

ESR1 is a gene responsible for encoding the estrogen receptor alpha, which primarily functions to interact with estrogen hormones. These hormones influence breast cancer growth and evolution [24]. Estrogen impacts various aspects, including glucose tolerance, lipid metabolism, and blood pressure regulation. ESR1, estrogen receptor subtype, is associated with insulin

Table 6. Network analysis results of the top ten PPIs.

Protein	Degree	Beetweeness centrality	Closeness centrality	Overall score	Pathway
ESR1	9	0.57500000	0.36609574	9.9410957	Endocrine signaling pathway
PPARγ	9	0.24825428	0.53488372	9.783138	PPAR signaling pathway, CAMP signaling pathway, Insulin resistance
HMGCR	8	0.25171277	0.53488372	8.53488372	Metabolism pathway
CYP19A 1	6	0.28063241	0.5	6.78063241	Metabolism pathway
PPARD	5	0.10126262	0.46	5.56126262	PPAR signaling pathway
PTPN1	4	0.03791172	0.45098039	4.48889211	Insulin resistance signaling pathway
GRIN2B	4	0.07437417	0.39655172	4.47092589	CAMP signaling pathway
FYN	4	0.03069828	0.34848484	4.37918312	CAMP signaling pathway
AR	4	0.08695652	0.4259259	4.51288242	CAMP signaling pathway
SHBG	3	0.0	0.4181818	3.04181818	Insulin resistance

sensitivity and acts as a co-modulator alongside insulin receptor substrate 1 (IRS1) to regulate insulin signaling [25]. Therefore, ESR1 may also contribute to T2DM.

PPAR-α (peroxisome proliferator-activated receptor alpha) exhibits significant and promising potential as a potential target based on network analysis employing several parameters. Moreover, PPAR-α may be involved in three pathways of DMT2: the cAMP signaling pathway, the PPAR signaling pathway, and the insulin resistance pathway. In the PPAR signaling pathway, PPAR-α degrades fatty acids, particularly those involved in lipid metabolism. Fatty acid degradation can influence insulin sensitivity in cells. PPAR-α is a key regulator in the adipogenesis process, triggering the formation of small adipocytes highly responsive to insulin. Beyond its crucial role in adipogenesis, PPAR-α also significantly controls lipid metabolism within mature adipocyte cells. Adipogenesis triggers related to the capacity to capture fatty acids have proven essential factors in maintaining overall insulin sensitivity in the body's system [26].

PPAR-α is a protein target that interacts with five secondary metabolite compounds from yacon leaves, namely Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, (1.alpha, 2.beta, 5.alpha) (Pinane), m-menth-1(7)-ene, Hexadecanoic acid, methyl ester, hexadecanoic acid, 14-methyl-, methyl ester, and Eicosane. PPAR-α represents an isoform of the PPAR family, which controls the gene activities that regulate glucose and lipid metabolism. PPAR-α is also a primary regulator of energy metabolism [27].

PPAR-α is a component in adipose differentiation and hypertrophy, as well as mediating the effects of insulin. PPAR-α also influences the transcriptional regulation of the GLUT4 gene by directly and specifically suppressing GLUT4 promoter transcriptional activity through the binding of PPAR/RXR heterodimers to the GLUT4 promoter along 66/163 bp, with the assistance of cell phosphorylation [28]. Activation of PPAR-α within mature adipocytes leads to the expression of numerous genes involved in the insulin signaling cascade, thereby enhancing insulin sensitivity. PPAR-α can also impact insulin signaling at various pathway stages, enhancing overall insulin sensitivity and improving glucose and lipid metabolism [29]. Several parameters used to determine the top ten PPIs are degree value, betweenness centrality, and closeness centrality. This parameter is used to identify nodes (proteins) that have an important role in a biological network. The degrees provide insight into how much a protein interacts with a particular node. Closeness centrality estimates how fast information flows through a node or how short the shortest path from node x to all other nodes is. On the other hand, betweenness centrality is based on communication flow, where nodes with high betweenness centrality have a significant role in controlling the flow of information [29].

4. Conclusions

This study concluded that yacon leaf extract targets proteins related to the cAMP signaling pathway, the PPAR signaling pathway, and the insulin resistance pathway as antidiabetics, consisting of 10 proteins, namely PPAR-α, ESR1, HMGCR, CYP19A1, PPARD, PTP1N, GRIN2B, FYN, AR, and SHBG, where PPAR-α is a potential protein as an antidiabetic. However, there is a protein that shows its potential in breast cancer, namely ESR-1, so this study can pave the way to explore the activity of insulin leaf extract in cancer therapy.

Author Contributions: Conceptualization, A.E.W. and T.E.T.; methodology, A.E.W.; software, H.E.I.S. and F..; validation, T.E.T., H.E.I.S. and F.; formal analysis, A.E.W., T.E.T., H.E.I.S..; investigation, A.E.W.; resources, T.E.T.; data curation, A.E.W. F.; writing—original draft preparation, A.E.W., T.E.T., H.E.I.S; writing—review and editing, A.E.W., T.E.T., H.E.I.S.; visualization, F.; supervision, T.E.T.; project administration, T.E.T. All authors have read and agreed to the published version of the manuscript.

Funding: This work received no external funding.

Ethical Clearance: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data used in this study are available upon request from the corresponding author by applicable data protection and privacy regulations.

Conflicts of Interest: The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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