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Original Research Article

Network Pharmacology and Docking of *Nephrolepis cordifolia* as Type-2 Antidiabetic Agent

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ABSTRACT

Type 2 diabetes mellitus (T2DM) is a chronic disease characterized by disturbances in insulin secretion or action. Impaired insulin secretion causes blood glucose to increase continuously, resulting in potential complications of the disease, such as diabetic neuropathy, coronary heart disease, and hypertension. Therefore, this study aims to identify compounds from *N. cordifolia* with potential for T2DM and their mechanisms using network pharmacology and docking. The screening results showed that ellagic acid was the active compound with the potential to bind to T2DM proteins. Ellagic acid also helped in the treatment of T2DM by interacting with PI3K-Akt, Rap1, mTOR signalling, endocrine resistance, focal adhesion, and FOXO signalling. Based on the docking of ellagic acid against the FOXO signalling protein, namely; CDK2, EGFR, INSR; SMAD3, IGF1; BRAF, AKT1, and IGF1R, the binding energy values obtained were -8.56; -7.77; -7.43; -7.05; -7.68; -6.48; -7.82; -8.9 kcal/mol, respectively. This indicates that ellagic acid had a strong binding affinity with the target protein. In conclusion, ellagic acid was identified as a major compound from *N. cordifolia* that could be used to treat T2DM by alteration of FOXO signalling.

***Keywords*:** Diabetes, *Nephrolepis cordifolia*, Endocrine, Ellagic acid.

Introduction

Diabetes mellitus (DM) is a chronic disease that affects a significant number of people and is characterized by issues with insulin secretion or action resulting in elevated blood sugar levels leading to nerve damage, heart disease, high blood pressure, and other complications when insulin secretion is disrupted.[1] DM has emerged as a major global health concern impacting approximately 285 million people worldwide according to the World Health Organization, and this figure is expected to reach 438 million by the year 2030.[2] It also ranks fifth among the leading causes of mortality causing about 6.7 million deaths globally in recent times. Consequently, countries have intensified their efforts to develop new treatments both natural options and synthetic products.[3,4] Kalimantan Island located in Indonesia presents an opportunity for producing effective medicinal plant-related remedies that are similar to modern medicines.[5]

Kinca fern (*Nephrolepis cordifolia* (L.) K. Presl) is a plant native to Kalimantan with many medicinal uses, belonging to the Nephrolepidaceae family. Locally, it is used to lower blood sugar, reduce swelling, and fight bacterial infections.[6]

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The plant contains various compounds including flavonoids, tannins, alkaloids, triterpenoids, and steroids.[7] According to other studies, it contains the compounds *β*-sitosterol, oleanolic acid, Fern-9(11) ene, Myristic acid, Octadecylester, Nonanol, *β*-ionone and Eugenol.[8,9] The water extract and ethyl acetate fraction of *N. cordifolia* have antibacterial and antifungal properties.[10]Specifically, the water extract can inhibit the growth of various bacteria (*Bacillus cereus, Bacillus megaterium, Burkholderia symbiont, Serratia marcescens*) and fungi (*Alternaria alternata, Curvularia lunata, Fusarium oxysporum*), thanks to active compounds like phloroglucinol, gallic acid, and catechin.[11] The ethanol extract of its tuber has an antioxidant capacity of 93.898 ± 0.923 g/mL and a flavonoid content of 3.119 ± 0.091 EA.[12]Studies suggest that the antioxidant properties of *N. cordifolia* extract help reduce oxidative stress, which is a key factor in diabetes complications.[13,14]

The ethanol extract of *N. cordifolia* can protect the liver from damage caused by paracetamol by lowering liver biomarkers (Alkaline Phosphatase, Serum Glutamic Pyruvic Transaminase, serum glutamic oxaloacetate transaminase, and bilirubin) and cholesterol levels (TG, LDL-C, TC, and VLDL-C) while increasing levels of protective enzymes (CAT, SOD, and GSH).[15] This extract also has strong antibacterial and antioxidant effects, supported by compounds like methyl ester, hexadecanoic acid, and 5-Phenyl-2,4-pyrimidinediamine identified through GC-MS and docking studies.[16] The DPPH method shows that *N. cordifolia* leaf extract can reduce inflammation with an IC_{50} of 120.94 µg/mL and has an antioxidant capacity of 21.36 µg/mL. The red blood cell membrane permeability method confirms its anti-inflammatory effect with an IC_{50} of 21.36 µg/mL.[17] Essential oils from *N. cordifolia* leaves can kill various cancer cells, including HCT-116 (IC_{50} 40.8 ppm), MCF-7 (IC_{50} 37.6 ppm), and A-549 (IC_{50} 23.6 ppm).[9] Water and methanol extracts of *Nephrolepisauriculata*, which are high in flavonoids and phenolics, have been shown to help fight diabetes in other species.[18] The ethyl acetate fraction of *Nephrolepisundurata* can lower blood sugar by preventing the breakdown of sugar into glucose

and increasing SOD and catalase levels.[19] These findings are supported by *in vitro* tests on amylase and glucosidase enzymes.[20]
Network pharmacology and molecular docking studies are now important tools for understanding how natural products work and finding potential treatments for complex diseases, such as diabetes.[21]Therefore, this study aims to identify active compounds from *N. cordifolia* and explore their mechanisms for combating diabetes using network pharmacology.

Materials and Methods

Materials
Kinca Fern (*N. cordifolia* (L.) K. Presl) sample was collected on November 14, 2023, from Sungai Baru, Banjarmasin, South Kalimantan with coordinates 3.322833°S 114.601194°E, and identified at the basic laboratory of FMIPA, Lambung Mangkurat University by Dr. Gunawan as a taxonomist. Voucher number IV-21-2-18 with deposit code 108/TS-11/2023 was assigned.

Software
AdmetSAR (2.0; 2022) (http://lmmd.ecust.edu.cn/admetsar2/), Swisstargetprediction (2D and 3D; 2023) (http://www.swisstargetprediction.ch/), Similarity ensemble approach (v. 3.0; 2022) (https://sea.bkslab.org/), NCBI (2023) (https://www.ncbi.nlm.nih.gov/), Gencards (5.21: 2022) (https://www.genecards.org/), DataWarior (5.2.1; 2020)(https://openmolecules.org/datawarrior/), chemaxon (https://chemaxon.com/), Cytoscape (v3.9.1; 2022) (https://Cytoscape.org/), venn diagram (Venny 2.1.0; 2017)(https://bioinfogp.cnb.csic.es/tools/venny/), Enrichr (2023) (https://maayanlab.cloud/Enrichr/), Discovery Studio (2.0; 2008) studio (https://www.3ds.com/products/biovia/discovery-studio) and yasara (view; 2019).[22]

Screening of N. cordifolia compounds
A total of 10 g of *N. cordifolia* plant powder was extracted by maceration in 1 L of ethanol. The extract was filtered and concentrated to dryness using a rotary evaporator at 40°C to obtain 1.2 g of a thick crude extract. This thick extract was analyzed by LC-MS/MS. The LC-MS/MS conditions used were 5 mg of *N. Cordifolia* extract dissolved in 1 mL of methanol and filtered with a 0.2 µm nylon membrane. Flow rate 0.2 mL/min. Mobile phase water and acetonitrile. Gradient 0-1 minute, column temperature 30°C. Injection volume 2 µL. The compounds obtained were selected for pharmacophore using dataWarrior from the ChEMBL database. The selected compounds were selected using AdmetSAR to select the probability of compounds being absorbed in the intestine and their bioavailability.

Collection and Screening of N. cordifolia Related Target Proteins
The SwissTarget Prediction and SEA databases were utilized to collect target proteins associated with *N. cordifolia* compounds, employing canonical SMILES from PubChem. The search was restricted to human targets with a probability and Tanimoto coefficient of 0.5.[23] The target proteins obtained from these databases were subsequently merged, and any duplicates were eliminated.
Collection and Screening of Target Proteins Related to Type 2 Diabetes Mellitus (T2DM)
Proteins associated with Type 2 Diabetes (T2DM) were gathered from the GeneCards and NCBI databases. The NCBI data encompassed all findings, whereas GeneCards was restricted to the top 500 proteins.[24] The proteins from both databases were merged, and any duplicates were eliminated.[25]The search term employed was "type 2 diabetes".
The process involved creating both a component-target network and a general-target network.
To illustrate the interactions between compounds from *N. cordifolia* and target proteins, a network was constructed using Cytoscape v3.9.1. The network consisted of "nodes" representing the compounds and proteins, and "edges" indicating the connections between the proteins. To identify common targets between *N. cordifolia* and T2DM, a Venn diagram was utilized to determine overlapping target proteins. Cytoscape v3.9.1 was employed to compare the target proteins from both sources and create the common-target network.

The process involves creating a Protein-Protein Interaction Network (PPI Network).
Using the STRING database, a protein interaction network was constructed for *N. cordifolia* proteins and those associated with Type 2 Diabetes (T2DM). The network, which focused on human proteins with medium confidence, was downloaded in TSV format and visualized using Cytoscape v3.9.1. The two networks were merged using Cytoscape to identify common proteins. Clusterone, a Cytoscape tool, was utilized to analyze the network and identify key proteins. Target proteins were selected from the first Clusterone group with a degree centrality over 5. Proteins were considered core if they were not removed.[26]Higher scores indicated greater importance in T2DM.[27]

Enrichment analysis
The Enrichr and KEGG databases were utilized to analyze proteins that have an overlap with those connected to Type 2 Diabetes (T2DM). The analyses had a significance level of 0.05, which was conducted to gain insight into the biological processes, molecular functions, cellular components as well as signalling pathways related to these specific proteins.[28]

Docking
To assess the potential efficacy of various phytochemical components in treating Type 2 Diabetes Mellitus (T2DM) via the FOXO pathway mechanism, Autodock 4 software was utilized for molecular docking. A total of 8 well-known protein targets - including CDK2 (6gue), EGFR (1m17), INSR (5e1s), SMAD3 (1mjs), IGF1 (5u8q), BRAF (3og7), and AKT1(4ekl)- were downloaded from Protein Data Bank (PDB). Before docking, unnecessary water molecules and ligands that did not impact the reaction were eliminated from proteins. Additionally, YASARA software[22] was used to minimize all existing protein structures. This protocol used the binding energy between the native ligand and the target protein complex as a reference value. The amino acid residues of the target proteins that bind to *N. cordifolia* compounds were evaluated and compared with the native ligand and docking binding energies. When the docking process resulted in binding similarity, it was considered effective and could be regarded as pharmacophore similarity.[29]

Results and Discussion

The experimental methodology (plant extraction, LC-MS/MS analysis, Datawarrior and AdmetSAR physicochemical parameters identification is presented in Figure 1. Following the LC-MS/MS analysis of *N. cordifolia*, a total of 62 compounds were obtained through the MS dial database (Figure 2 and Table 1). LC-MS/MS data interpretation was similar to GC-MS data analysis because it involved mass fragmentation.[30] DataWarrior, using the ChEMBL database for analysis in this study, yielded 39 compounds. The subsequent analysis process used AdmetSAR to observe which compounds were absorbed and passed through the intestine, resulting in 26 compounds. The Swiss Target Prediction proteins related to *N. cordifolia* compounds got 16 proteins, and the SEA target obtained 75 proteins. After getting rid of duplicates, 75 proteins were obtained. Whereas using STRING to view the network, 67 proteins out of the 75 total proteins were acquired, as illustrated in Figure 3. The proteins released were BHMT, CA, CACNA2D1, CAD, FOLA, GPR35, NAAA, PSBA, and SLC22A6. The number of nodes created was 73, with a total of 375 edges. Only 6 of the 26 *N. cordifolia* compounds, bonded with the 67 proteins. Furthermore, using the keyword "Type 2 Diabetes" as the search term, the NCBI database search yielded 2204 proteins while limiting the results from Gencard to the 500 proteins with the highest scores. A total of 2417 proteins was obtained after removing the duplicates.

In predicting Protein-protein Interaction Network (PPI Network), the 2 interaction networks involved in T2DM were combined and taken slices to produce 25 target proteins that interact with each other, as shown in Figure 4A. The STRING database was used to view the interaction models for the intersecting proteins. In this study, only ellagic acid interacted with these 25 proteins. Subsequently, the network using Cytoscape v3.9.1 was analyzed to determine the degree centrality, average shortest path length, betweenness centrality, and closeness centrality scores. Based on Figure 4A, a total of 25 proteins that formed the intersection between the target and the T2DM protein were analyzed. However, these interactions were further examined using STRING in this study. As shown in Figure 4B, carbonic anhydrase 1 (CA1) and betaine-homocysteine S-methyltransferase 1 (BHMT) were 2 proteins that did not interact with each other, forming 24 nodes and 145 edges. A cystoscape analysis using the clustering model of 23 proteins yielded 21 proteins with a quality value of 0.983, density of 0.567, and a P-value of 2.856E-9. In Figure 5, 2 proteins were the outliers namely HSPA1A and POLI. With degree centrality > 5, 19 proteins were obtained, and the released proteins were PLK1 and BACE1. The number of nodes formed was 26 and 196 edges. With degree centrality > 5, an Average Shortest Path Length, betweenness centrality, and closeness centrality of 1.8636, 0.01, and 0.53658 were obtained, respectively.

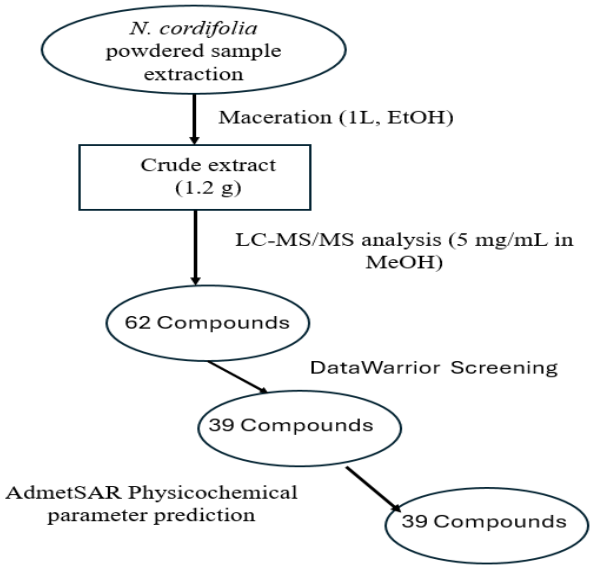


Figure 1: A flow chart showing extraction LC-MS/MS identification and ligands

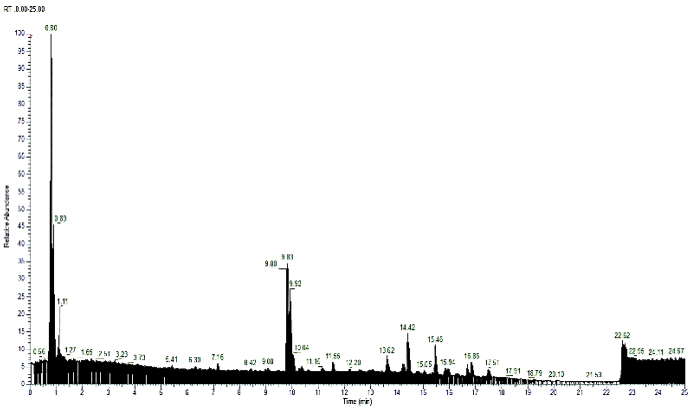


Figure 2: LCMS/MS profile of *N. cordifolia*

For enrichment analysis, a total of 19 proteins obtained were further analyzed using Enrichr to observe the biological processes, molecular function, cellular components, and signal pathways related to *N. cordifolia* and T2DM compounds. With a P-value of 0.05, the enrichment analysis with Enrichr showed that there were 579 biological processes, 29 cellular components, 52 molecular functions, and 103 Kegg pathways (Figure 6). Subsequently, the results of this enrichment analysis were interacted with using Cytoscape v3.9.1. The number of nodes formed was 26 and 109 edges, as shown in Figure 5. Figure 7 shows the Foxosignalling pathway that the *N. cordifolia* compound showed effectiveness in treating T2DM.

Staged screening for potential T2DM mechanisms yielded only a compound, ellagic acid, out of the 62 compounds found in *N. cordifolia*. Ellagic acid was based on a network pharmacology approach, using cluster 1 from Cytoscape, and continued with enrichment using Enrichr. EGFR, INSR, BRAF, AKT1, SMAD3, IGF1, and IGF1R were some of the proteins that interact directly with ellagic acid in this pathway. In this study, this protein docked with ellagic acid. The RCSB CDK2 protein database search showed that EGFR, INSR, BRAF, and AKT1 had native ligands, necessitating docking validation to determine the optimal coordinates for docking ellagic acid. The values for CDK2 native ligands EGFR, INSR, BRAF, and AKT1 were 1.4114 Å, 1.8241 Å, 1.3342 Å, 1.9365 Å, and 1.2653 Å, respectively. These values were less than 2 Å and met docking validation requirements.[31] Consequently, docking coordinates could be used for test ligands. CDK2, EGFR, INSR, BRAF, and AKT1 coordinates were respectively X:-6.24388, Y: -22.2288, Z: 22.6673; X: 22.0137, Y: 0.252828, Z: 52.794; X:4.04472, Y: 20.6813, Z: 20.9674; X:1.86852, Y: -2.63767, Z: -19.9177; X:28.0319, Y: 5.22134, Z: 10.8912. It was impossible to use playmolecule.org coordinates for SMAD3, IGF1, and IGF1R because no native ligand was exhibited. The coordinates for these molecules were X: 9.98427, Y: 13.403, Z: 6.25336; X: 5.05455, Y: 25.2878, Z: 33.6212; and X: 18.1516, Y: 18.44, and Z: 14.0852. There were more accurate docking results for ellagic acid with all of the target proteins in Table 2 compared to the native ligand.

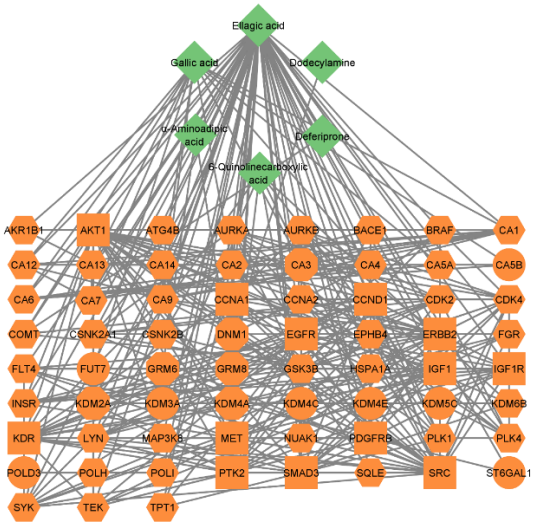


Figure 3: Compounds from *N. cordifolia* and target proteins
Description: Green diamond is a compound of *N. cordifolia*. We obtained three orange-colored protein groups from AverageShortestPathLength 1.00-1,99 (round rectangle), 2,00 – 2.49 (hexagon), 2.50-2.99 (Octagon) dan 3,00-3.64 (Ellipse)

However, for EGFR, it bound similarly to the native ligand, at -7.77 kcal/mol, and the native ligand ([6,7-bis(2-methoxy-ethoxy)quinazoline-4-yl]-(3-ethynylphenyl)amine) at -7.78 kcal/mol). When a protein had a native ligand, one of the docking criteria was the similarity of the amino acid residues that bound to the ligand.[32] Apart from comparison with the native ligand, ellagic acid was bound to CDK2, sharing amino acids such as LYS33, LEU83, VAL18, LEU134, ALA31, VAL64, and ILE10. Ellagic acid was bound to EGFR interacting with the following amino acids ALA719, LEU820, LYS721, and MET742. Ellagic acid was also bound to INSR with the same amino acids: MET1079, LEU1002, GLU1077, LYS1030, LEU1002, VAL1010, and ALA1028. When binding to BRAF, ellagic acid had

similar binding properties to TRP531, IL463, ALA481, CYS532, and ASP594. Ellagic acid combined with AKT1 and formed the same bond as the native ligand. These bonds include LYS179, GLU228, MET227, MET281, VAL164, LYS179, ALA177, and ALA230. Figure 8 showed that hydrogen bonds were important in the interaction of the ligands with the proteins. Hydrogen bonds were important in determining the strength of the binding energy, the more hydrogen bonds there were between the ligand and the protein, the stronger the bond affinity.[33] The hydrogen bonds between ellagic acid and CDK2 were TYR15, LYS33, LEU83, GLU51, and PHE82. EGFR formed hydrogen bonds with LYS721, THR766, GLU738, and THR830. The hydrogen bonds that occurred with INSR were MET1079, LEU1002, GLU1077, LYS1030, and GLY1082. HIS248, GLU245, SER263, and PHE247 formed hydrogen bonds with SMAD3. IGF1 formed a hydrogen bond with THR41, MET59, GLN40, and TYR60. Interaction with BRAF formed a hydrogen bond with CYS532 and GLN530. When interacting with AKT1, the amino acid residues were LYS179, ALA230, ASP292, GLU228. Glu499, ALA500, ASN608, LEU606, and GLN546 were the residues of IGF1R that form hydrogen bonds with ellagic acid.

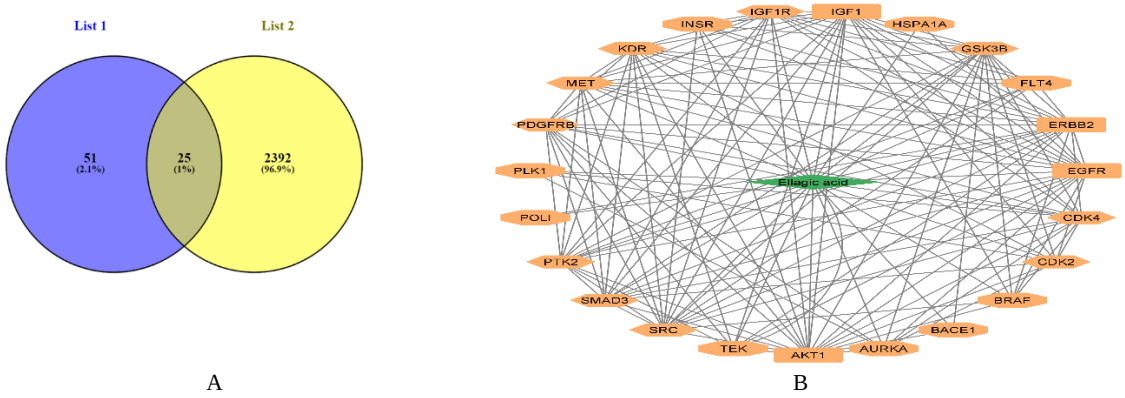


Figure 4: A general target network. A. Venn diagram of *N. cordifolia* and T2DM's 25 target proteins. B. Visualization of 25 target proteins in the T2DM network.
Description: The green diamond is the active compound of *N. cordifolia*, and yellow is its direct target. From AverageShortestPathLength, three groups were obtained, namely 1-1,3 (round rectangle), 1,31 – 1,6 (hexagon) dan 1,61 -2 (Octagon).

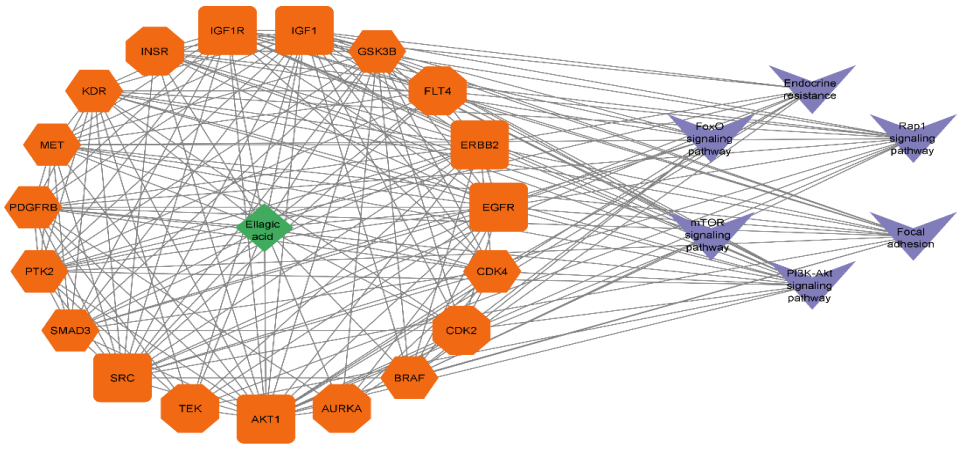


Figure 5: Cluster one of the common target networks with pathway
Description: The green diamond is a selected compound from *N. cordifolia*, namely ellagic acid. From AverageShortestPathLength, three groups of proteins with orange colours are obtained, namely 1-1.3 (round rectangle), 1.31 - 1.6 (hexagon) and 1.61 -2 (octagon). The pathway is a purple round rectangle.

Table 1: Prediction of compounds contained in *N. cordifolia* using the LC-MS/MS method

No	Name	Formula	Calc. MW	RT [min]	Area (Max.)
1	Methyl isonicotinate	$C_7H_7NO_2$	137.0474	0.812	2.22E+09
2	2-Amino-1,3,4-octadecanetriol	$C_{18}H_{39}NO_3$	317.2922	9.933	1.51E+09
3	L-α-PALMITIN	$C_{19}H_{38}O_4$	330.2761	14.434	6.67E+08
4	1-Stearoylglycerol	$C_{21}H_{42}O_4$	358.3075	15.473	4.37E+08
5	Betaine	$C_5H_{11}NO_2$	117.079	0.801	3.54E+08
6	Choline	$C_5H_{13}NO$	103.0998	0.771	3.49E+08
7	L-alpha-Glycerylphosphorylcholine	$C_8H_{20}NO_6P$	257.1025	0.777	1.7E+08

8	N-(3-Carboxypropanoyl)-5-hydroxynorvaline	$C_9H_{15}NO_6$	233.0897	0.775	1.26E+08
9	D-(+)-Pyroglutamic Acid	$C_5H_7NO_3$	129.0425	1.11	1.14E+08
10	Muramic acid	$C_9H_{17}NO_7$	251.1003	0.774	1.03E+08
11	9(Z),11(E),13(E)-Octadecatrienoic Acid methyl ester	$C_{19}H_{32}O_2$	292.2399	15.974	87710436
12	L-alpha-Glycerylphosphorylcholine	$C_8H_{20}NO_6P$	257.1025	0.865	66522322
13	6-(alpha-D-glucosaminyl)-1D-myo-inositol	$C_{12}H_{23}NO_{10}$	341.1316	0.736	56927022
14	5-Hydroxymethyl-2-furaldehyde	$C_6H_6O_3$	126.0316	0.804	55038071
15	α-Eleostearic acid	$C_{18}H_{30}O_2$	278.2244	14.21	54551607
16	Ascopyrone M	$C_6H_8O_4$	144.0421	0.795	53904030
17	2,2,6,6-Tetramethyl-1-piperidinol	$C_9H_{19}NO$	157.1466	9.078	46503406
18	(+/-)-Muscone	$C_{16}H_{30}O$	238.2294	14.434	45535567
19	1-[(3-Carboxypropyl)amino]-1-deoxy-beta-D-fructofuranose	$C_{10}H_{19}NO_7$	265.1159	0.843	43904589
20	Glycylglutamic acid	$C_7H_{12}N_2O_5$	204.0744	0.801	40461472
21	Ascopyrone M	$C_6H_8O_4$	144.0422	0.9	37694830
22	Hexadecanamide	$C_{16}H_{33}NO$	255.2561	10.359	35909875
23	Oleamide	$C_{18}H_{35}NO$	281.2716	14.855	31265884
24	Ellagic acid	$C_{14}H_6O_8$	302.0059	5.419	30346179
25	Pipecolic acid	$C_6H_{11}NO_2$	129.0789	0.814	29833267
26	Phosphoric acid	H_3O_4P	97.97707	0.871	28295745
27	5α-Dihydrotestosterone	$C_{19}H_{30}O_2$	290.2242	15.522	24324669
28	α-Aminoadipic acid	$C_6H_{11}NO_4$	161.0687	0.739	22132676
29	Dodecylamine	$C_{12}H_{27}N$	185.214	9.886	20972189
30	6-Oxo-pipecolinic acid	$C_6H_9NO_3$	143.0583	1.674	20456416
31	N-acetyl-L-2-aminoadipic acid	$C_8H_{13}NO_5$	203.0793	0.789	20396275
32	D-(+)-Proline	$C_5H_9NO_2$	115.0634	0.796	20305988
33	N-Acetylglucosaminitol	$C_8H_{17}NO_6$	223.1054	0.763	19070721
34	9-Oxo-10(E),12(E)-octadecadienoic acid	$C_{18}H_{30}O_3$	294.2193	13.073	19067034
35	2-(2-thienyl)-4H-chromen-4-one	$C_{13}H_8O_2S$	228.0245	1.314	18953463
36	6-Methyl-2-pyridinemethanol	C_7H_9NO	123.0685	0.805	18637347
37	1-Linoleoyl glycerol	$C_{21}H_{38}O_4$	354.2764	14.218	16959151
38	Hydrolyzed fumonisin B1	$C_{22}H_{47}NO_5$	405.3446	10.074	16187756
39	6-Oxo-pipecolinic acid	$C_6H_9NO_3$	143.0582	1.299	15794982
40	2-Tetradecylcyclobutanone	$C_{18}H_{34}O$	266.2606	15.473	15623353
41	Stearamide	$C_{18}H_{37}NO$	283.2874	15.814	15571412
42	Dihydrothymine	$C_5H_8N_2O_2$	128.0585	0.781	14950518
43	Desethylatrazine	$C_6H_{10}ClN_5$	187.0632	7.753	14666359
44	Stearidonic acid	$C_{18}H_{28}O_2$	276.2089	13.733	14650724
45	Navenone A	$C_{15}H_{15}NO$	225.1152	10.604	14101602
46	Sphinganine	$C_{18}H_{39}NO_2$	301.2978	10.96	13546398
47	1-*O*-Phosphonopentitol	$C_5H_{13}O_8P$	232.0347	0.815	13453280
48	Gallic acid	$C_7H_6O_5$	170.0216	1.288	13353363
49	Armillaramide	$C_{34}H_{69}NO_4$	555.5217	16.898	12577890
50	Glucosamine	$C_6H_{13}NO_5$	179.0793	0.871	12539791
51	5α-Dihydrotestosterone	$C_{19}H_{30}O_2$	290.2242	15.387	11815623
52	Pyroglutamylglycine	$C_7H_{10}N_2O_4$	186.064	0.809	10997788

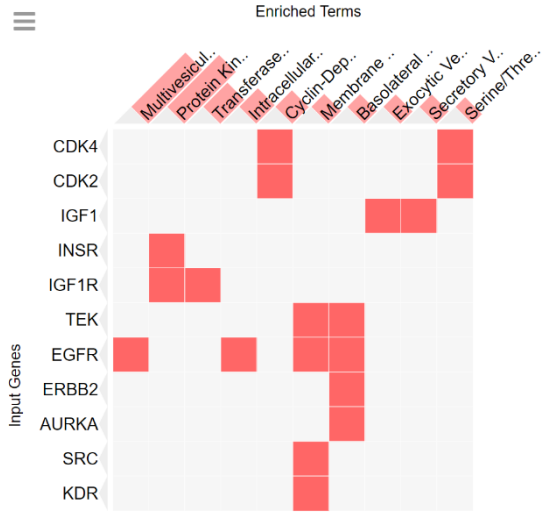
53	Stearidonic acid	$C_{18}H_{28}O_2$	276.2089	13.607	10128885
54	Deferiprone	$C_7H_9NO_2$	139.0627	0.813	9614883
55	DL-Stachydrine	$C_7H_{13}NO_2$	143.0944	0.816	7929778
56	Linoleamide	$C_{18}H_{33}NO$	279.2558	14.211	7257303
57	alpha-Methylstyrene	C_9H_{10}	118.0782	11.562	6895237
58	cis-12-Octadecenoic acid methyl ester	$C_{19}H_{36}O_2$	296.2712	17.404	6312929
59	Methyl (3,5-dihydroxy-2-oxo-2,3-dihydro-1H-indol-3-yl)acetate	$C_{11}H_{11}NO_5$	237.0634	0.817	6052892
60	6-Quinolinecarboxylic acid	$C_{10}H_7NO_2$	173.0477	1.185	5305445
61	1-Aminocyclohexanecarboxylic acid	$C_7H_{13}NO_2$	143.0944	0.923	4791983
62	Stachydrine	$C_7H_{13}NO_2$	143.0944	1.112	2998144

N. cordifolia influenced the PI3K-Akt signalling pathway, which had the highest score on T2DM through protein enrichment, as shown in Figure 6. This pathway was the primary pathway involved in the regulation of glucose uptake and metabolism in various tissues, including muscle, liver, and adipose tissue.[34] Disruptions in this pathway could disrupt insulin signalling, resulting in reduced translocation of the glucose transporter GLUT4 to the cell membrane and decreased glucose absorption.[35] Problems with the PI3K/Akt pathway cause the liver to produce more glucose and the body to form more fat tissue, which makes high blood sugar and insulin resistance worse.[36] Focal adhesion kinase (FAK) is a protein that helps control how cells stick together and move. This protein is important for diabetic heart problems. Glucose can affect FAK and paxillin, which help signal insulin release.[37]In hyperglycemic conditions, there is an increase in FAK phosphorylation, so the use of inhibitors can suppress TAK1 phosphorylation and NF-κB activation.[38] Proteins in the Rap1 signalling pathway also play a role in type 2 diabetes.[39] Decreased Rap1 activity in the hypothalamus protects against hyperglycemia, and increased Rap1 activity is influenced by cAMP2.[40] Furthermore, chemicals that cause endocrine resistance induce insulin resistance.[41] Activation of mTORC1 in pancreatic β cells reduced blood glucose, increased insulin secretion, and had a positive impact on glucose tolerance.[42] Deregulation of mTORCI by drugs could worsen the incidence of hyperglycemia.[43] In the liver, FoxO1 contributed to T2DM by increasing hepatic glucose production.[44] In skeletal muscle, FoxO1 reduced glucose uptake and oxidation, increased lipid uptake and oxidation, and improved muscle atrophy.[45] Furthermore, increased FoxO1 in the hypothalamus increased the risk of T2DM.[46]

Network pharmacology study was instrumental in identifying ellagic acid as an active compound from *N. cordifolia*, which played a significant role in T2DM. This compound had the potential to affect 19 protein targets in T2DM. Through this method, the mechanism of action of ellagic acid could be described at the molecular level more comprehensively from upstream to downstream in the signalling pathway. Specifically reducing FoxO1 expression through inhibition of EGFR, RAF, CDK2, IGF1, IGF1R, INSR, AKT, and SMAD3 mechanisms.

Different types of Nephrolepis, like *Nephrolepisauriculata*, were studied for their anti-diabetic properties, due to the flavonoids and phenolics contained.[18]The ethyl acetate fraction from *Nephrolepisunduranta* reduced blood sugar levels by inhibiting the formation of glucose from complex carbohydrates through inhibitors of the enzymes α-amylase and α-glucosidase[20], decreasing MDA levels, and increasing SOD and catalase levels.[19] A recent investigation revealed that ellagic acid derived from *N. cordifolia* has beneficial effects on diabetes management, specifically controlling glucose transporter 4 and elevating insulin levels while boosting a protein associated with insulin.[47] Several have established that ellagic acid exhibits strong binding affinity to its target proteins through hydrogen and hydrophobic bonds resulting in an optimum bond energy range between -5 to -10 kcal/mol, hence it affords great stability.[48] Furthermore, there are similarities in how natural ligands bind as compared to how ellagic acid binds onto its intended receptor due to matching shape configurations.[49]

A



B

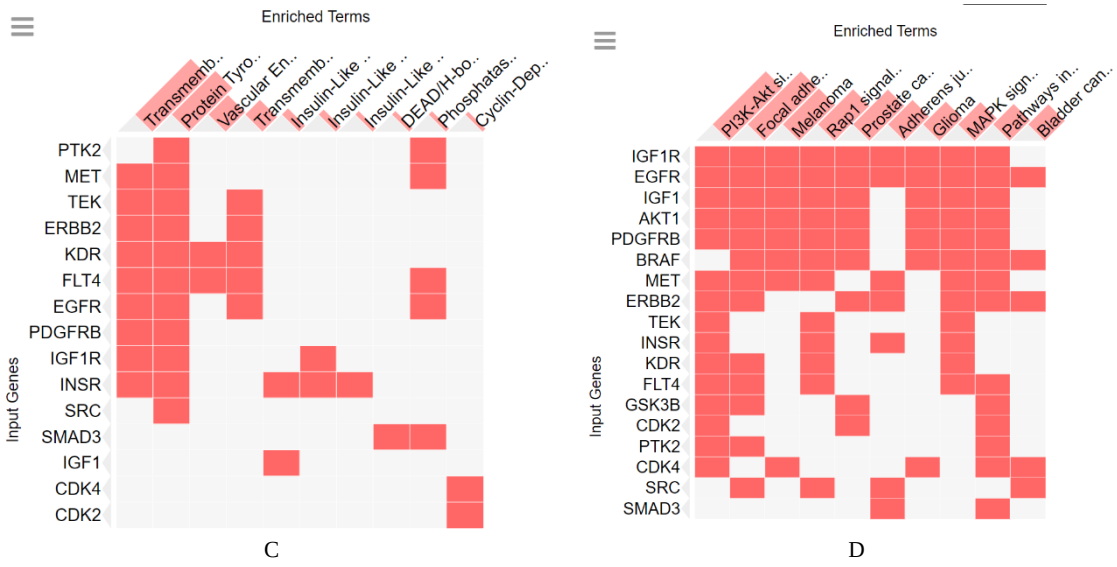


Figure 6: GO analyses; **A.** biological process; **B.** cellular component; **C.** molecular function; **D.** kegg pathway

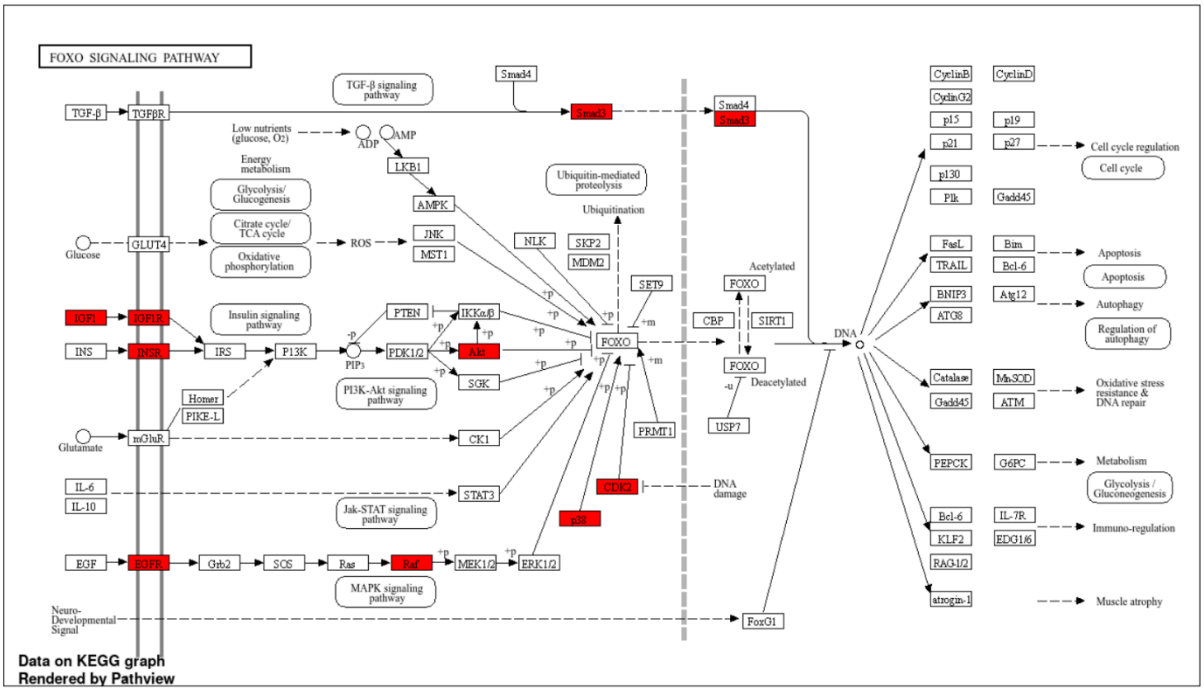


Figure 7: FOXO signalling is one of the potential targets of *N. cordifolia* as an antidiabetic type 2.
Description: The red colour is the pathway influenced by ellagic acid.

Table 2: Docking of proteins involved in the FOXO signalling pathway

Protein	ligand	Binding energy	Dissoc. constant [pM]	Interacting residues
CDK2	Native ligand	-7.32	4300000	LYS33; LEU83; ASP86; LYS89; GLU81; PHE80; TYR15; VAL18; ILE10; LEU134; ALA31; VAL64
	Ellagic acid	-8.56	534990	TYR15; LYS33; LEU83; GLU51; PHE82; VAL18; LEU134; ALA31; VAL64; ILE10
EGFR	Native ligand	-7.78	2000000	LEU768; LEU694; MET769; PRO770; GLN767; MET742; LEU753; LEU764; LYS721; ALA719; LEU820
	Ellagic acid	-7.77	2010000	LYS721; THR766; GLU738; THR830; ALA719; CYS751; LEU820; VAL702; ALA719; MET742; CYS751

INSR	Native ligand	-8.81	350540	MET1079; HIS1081; GLU1077; ASN1097; SER1090; LEU1002; VAL1010; ALA1028; LYS1030; VAL1060; VAL1010; LEU1002; ALA1028; MET1139
	Ellagic acid	-7.43	3580000	MET1079; GLU1077; LYS1030; GLY1082; LEU1002; VAL1010; ALA1028; MET113
SMAD3	Ellagic acid	-7.05	6840000	HIS248; GLU245; SER263; PHE247
IGF1	Ellagic acid	-7.68	2360000	THR41; MET59; GLN40; MET59; TYR60; TYR24
BRAF	Native ligand	-9.49	110170	CYS532; GLN530; LYS483; TRP531; ILE513; LEU514; ILE463; ALA481; LEU514; ALA481
	Ellagic acid	-6.48	17770000	TRP531; ILE463; VAL471; ALA481; CYS532
AKT1	Native ligand	-9.98	48630	GLU228; GLY157; LYS179; TYR229; LEU156; GLU278; MET281; LYS158; VAL164; MET227; ALA177; ALA230
	Ellagic acid	-7.82	1840000	LYS179; ALA230; ASP292; GLU228; MET227; MET281; VAL164; ALA177
IGF1R	Ellagic acid	-8.9	300450	TRP544; ARG575; ASN608; GLU499; ALA500; ASN608; LEU606; GLN546; THR545; LYS542; PRO607

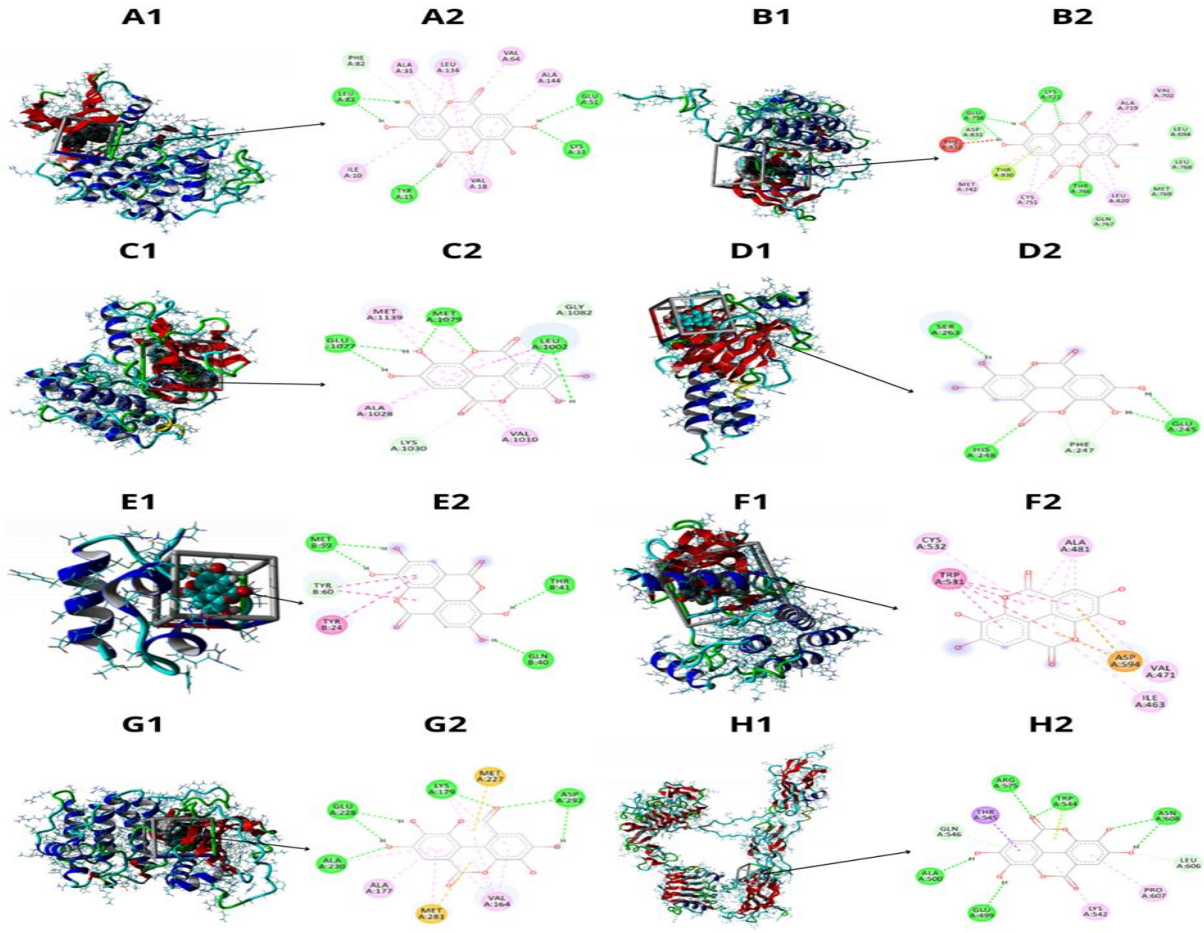


Figure 8: Interaction between Ellagic acid and target proteins in the FOXO pathway
Description: A. CDK2; **B.** EGFR; **C.** INSR; **D.** SMAD3; **E.** IGF1; **F.** BRAF; **G.** AKT1; **H.** IGF1R. **1.** Binding area ; **2.** Interaction 2D.

Conclusion

The findings from this study revealed the potential of *N. cordifolia* phytoconstituents (ligands) interactions with T2DM target proteins (CDK2, EGFR, INSR; SMAD3, IGF1; BRAF, AKT1, and IGF1R) and their inhibition of the FOXO signalling pathway involved in blood glucose upregulation. The FOXO signalling pathway inhibition results in blood glucose reduction in T2DM patients. In particular ellagic acid was identified as the major phytochemical in *N. cordifolia* with potential antidiabetic activity and could further be explored through *in vitro* and *in vivo* studies towards the development of pharmaceutical leads.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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