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ORIGINAL RESEARCH

Network Pharmacology of Natural Polyphenols for Stroke: A Bioinformatic Approach to Drug Design

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Background: Globally, stroke is a major contributor to disability and a leading cause of death. Stroke is more frequent in underdeveloped countries, where ischemic stroke is one of the most common kinds. Therefore, it is imperative to unravel the processes of ischemic stroke in more depth and develop novel therapeutics to combat the condition. Polyphenols provide a significant preventive role against multiple diseases, including cancer, cardiovascular disorders, atherosclerosis, brain dysfunction, and stroke.

Methods: In the current investigation, computational tools including Swiss Target prediction, DisGeNET, SwissADME, pkCSM, Cytoscape, InterActiVenn, STRING database, and DAVID database were utilized to identify the signaling pathways, putative targets, along with associated genes of the polyphenols for stroke prevention.

Results: This study revealed the possible interactions between the disease targets for Stroke and the selected plant-based polyphenols. Docking results also exhibited the strong to moderate affinity of the selected ligands (Apigenin, Ellagic acid, Ferulic acid, Kaempferol, Genistein, Luteolin, Naringenin, and Quercetin) towards the selected disease target.

Conclusion: This study highlights the neuroprotective role of selected polyphenols through the PI3K/Akt pathway. Further studies are required to investigate additional molecular mechanisms between the polyphenols and their derivatives against pathological targets of Stroke.

Keywords: polyphenols, ischemic stroke, cardiovascular diseases, network pharmacology

Introduction

A stroke, sometimes referred to as a brain attack arises if the blood flow is obstructed to a particular region of the brain or if the blood vessels in the brain rupture. In both cases, the brain either dies or sustains damage. Blood clots in the brain obstruct the arteries which causes blood vessels to rupture which in turn causes bleeding.[1] Rupturing of the arteries can lead to brain damage due to a shortage of oxygen resulting in the demise of the cells in the brain. Stroke can pave the way for several neurological conditions including depression, dementia, etc. The outcomes of Stroke can be permanent brain damage, long-term impairment, or even death.[2]

Statistics show that women die from Stroke at a higher rate than males do. Typically, women account for six out of ten deaths from stroke.[3] Multiple factors contribute to this increased risk. Among these is the fact that women live longer than males do on average and that this prolonged lifespan makes them more vulnerable to stroke. Two more unique risk

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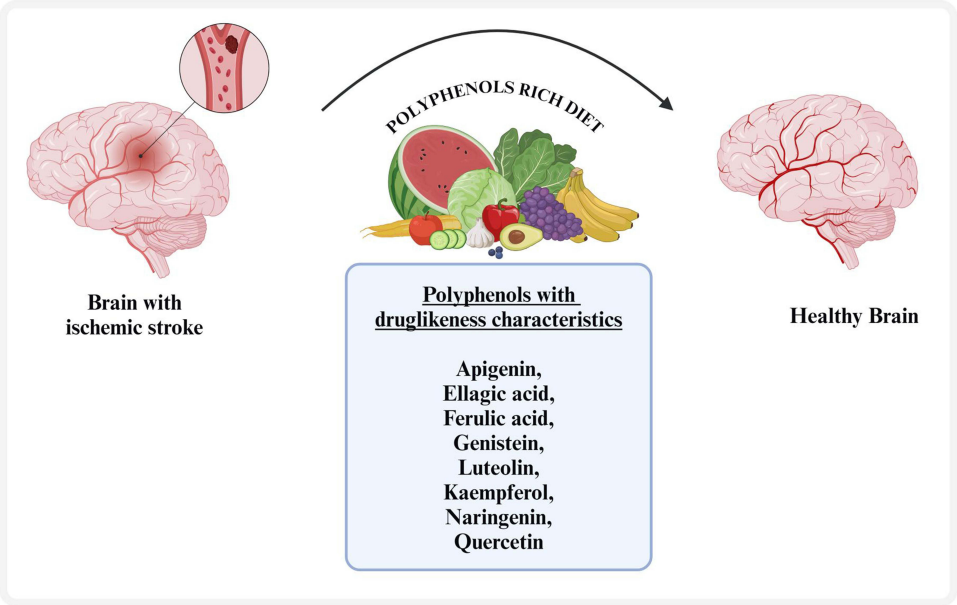
Graphical Abstract



factors that are exclusive to women include a rise in blood pressure during pregnancy and a rise in blood pressure caused by some birth control pills. Women also tend to experience greater stress levels than males, and they are more likely to experience anxiety and depression. When taken as a whole, these factors increase the risk of stroke in women.[4–6] Most stroke patients experience two different types of strokes. The causes of both hemorrhagic and ischemic strokes include internal blood vessel ruptures and artery blockages, which both cause local hypoxia that damages brain tissue.[7]

The blockages that occur in ischemic strokes are often caused by blood clots that become stuck in one of the brain's arteries. To treat ischemic stroke, a typical thrombolytic agent that breaks up a clot, ie tissue plasminogen activator (tPA), is currently the only drug that the FDA has authorized. However, this medication needs to be administered to the stroke patient no later than 4.5 hours after the onset of symptoms.[8] When tPA is administered beyond this therapeutic window, it may cause a hemorrhagic alteration that aggravates pre-existing brain damage. If the clot fails to dissolve by itself or if the individual does not show up at the hospital within the time limit required for tPA therapy, there are other options for treatment, such as a thrombectomy to remove the clot surgically. Since there is an increased chance of having another stroke soon after the first, preventive measures such as anticoagulants, blood pressure, and cholesterol-lowering drugs, may also be administered. By using these treatments as soon as possible, the effects of any difficulties that a stroke may cause can be reduced.[9]

Following a stroke, motor deficits like central facial paresis, hemiplegia (left or right-side paralysis), and hemiparesis (a weakness on either side of the body) - are frequently experienced.[10] Language and speech deficits are also common, including global or mixed aphasia (impairment of language processing) and dysarthria (speech impairment).[11] Additional abnormalities include altered perception, visual difficulties, and decreased blood flow to certain brain areas.[12] Each of these deficits has a significant effect on the standard of life experienced by stroke patients. Hence search for therapeutic agents from the mother nature with multi-target potential against stroke and minimal side effects is of high demand.

Polyphenols are a diverse group of naturally occurring compounds found primarily in plants, known for their neuroprotective, anti-inflammatory, and antioxidant properties.[13,14] Epidemiological studies have linked polyphenol-rich diets with a lower risk of stroke.[15,16] Polyphenols can be classified into various types based on the number and structure of their phenolic rings, with the primary categories being flavonoids, phenolic alcohols, stilbenes, phenolic

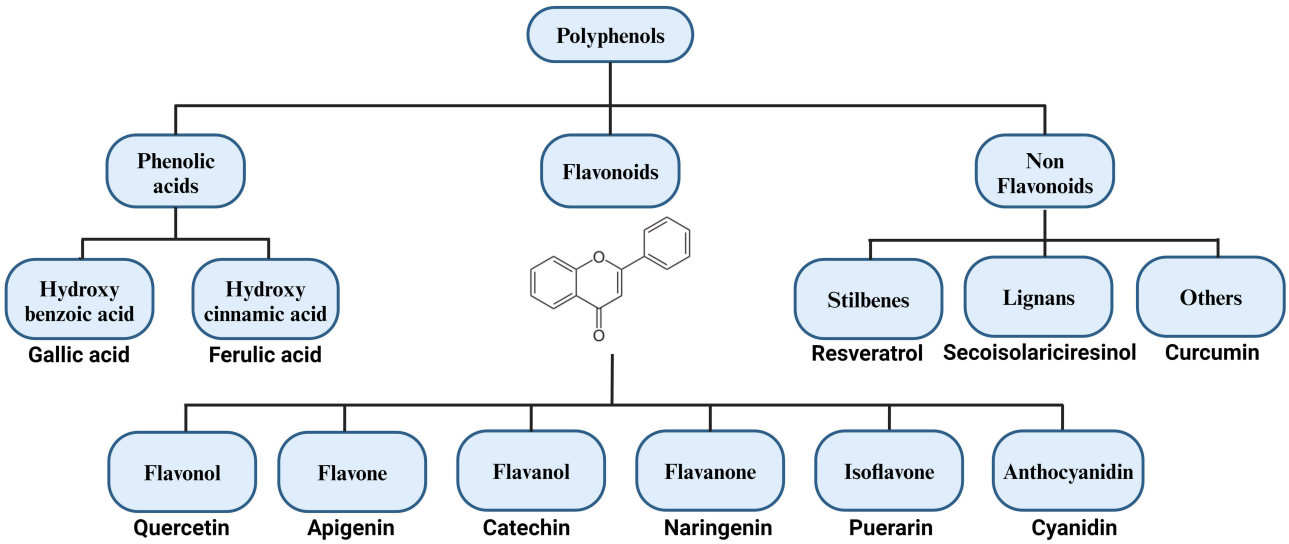


Figure 1 Classification of Polyphenols.

acids, and lignans (Figure 1). Polyphenols play protective role against several chronic conditions such as cardiovascular diseases, Diabetes, Neurodegenerative diseases, Cancer, etc.[17]

In the realm of drug development, network pharmacology is an emerging field because it combines information technology with systematic medicine.[18] The goal of this integrated in silico method is to uncover the factors underlying the therapeutic actions that synergize in case of conventional medications by creating a "disease-gene/protein-compound" network.[19] As a result, the paradigm has changed from the concept of "one drug, one target" to a concept of "network target, multiple component therapeutics." Emerging as a field, network pharmacology is based on the fundamental ideas of systems biology.[18] Furthermore, it has recently been made available for elucidating the molecular processes underlying several complex chronic illnesses, including neurodegenerative, cardiovascular, and cerebrovascular disorders.[20] Consequently, we may generate a basic knowledge of the processes by which multitarget medications cure complicated diseases by network analysis based on several existing databases. Thus, this work employs network pharmacology technique to shed light on the pharmacological effects of polyphenols on stroke and to assess significant target genes for stroke and its possible interaction with the polyphenols.

Materials and Methods

Search Strategy

An increasing body of scientific evidence suggests that natural polyphenols show positive benefits for a range of illnesses. So, a review of the literature was conducted using multiple search engines including PubMed, Scopus, Google Scholar, and ProQuest,[21] with the keywords "Stroke", "neuroprotection", and "polyphenols". The search results showed that 16 compounds (Apigenin, Berberine, Curcumin, Chlorogenic Acid, Ellagic acid, Ferulic acid, Genistein, Kaempferol, Luteolin, Lignan, Naringenin, Quercetin, Resveratrol, Rutin, Rottlerin, Silymarin) were the most prominently associated with anti-aging effects, cerebral vasodilation, neuroprotection and they have shown providing benefits against other neurological diseases.[22–38]

Screening of Compounds

Drug-Likeliness Prediction

A free online tool SwissADME (http://www.swissadme.ch/) is used for assessing the drug-likeliness properties of the 16 selected polyphenolic compounds or ligands.[39,40] Various parameters such as lead likeness, TPSA (Topological Polar Surface Area), brain permeability, bioavailability score, and the gastrointestinal absorption of the selected ligands, ESOL Log S and ESOL class (water solubility), iLogP (lipophilicity), and the violations against the Lipinski rule of five[41,42]

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(hydrogen bond acceptors should be less than ten, hydrogen bond donors should be less than five, octanol-water partition co-efficient should be less than five, and molecular mass should be less than 500 Daltons) were assessed. Further, the bioavailability value should be within the desirable range (F > 30%, where F30 indicates a 30% bioavailability). Bioavailability refers to the proportion of drugs that enter the bloodstream when introduced into the body and are thus available for action.

Using a boiled egg diagram, the predictive analysis for drug-likeness, specifically predicting a molecule's GI (Gastrointestinal) absorption and BBB (Blood-brain barrier) permeability. The white region represents molecules predicted to have high gastrointestinal absorption. The yellow region represents molecules predicted to cross the BBB.[43]

ADMET Profiling

The ADMET (absorption, distribution, metabolism, excretion, and toxicity) profiling was performed using the pkCSM web server (https://biosig.unimelb.edu.au/pkcsm/).[44] The Simplified Molecular Input Line Entry System (SMILES) of the corresponding ligands were extracted from the PubChem database to perform comprehensive pharmacokinetic profiling of the selected ligands.

Prediction of Putative Targets

After evaluating through the SwissADME and the pkCSM tools, the canonical SMILES of the chosen compounds (8 compounds) were retrieved from the PubChem database. Then the Swiss target prediction website was utilized to analyze the possible targets for the ligands. DisGeNET database was used to get the possible targets for the disease (stroke).[17]

Building a Protein–Protein Interaction (PPI) Network

The significance of protein-protein interactions stems from their remarkable diversity, adaptability, and selectivity.[45] The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) was utilized to identify the functional connections among the primary targets. The PPI network obtained by STRING was subjected to analysis of its key regulatory genes and identification of pertinent targets using the CytoHubba plugin for Cytoscape.[46]

KEGG Pathway Enrichment and Gene Ontology Enrichment Analysis

The Database for Annotation, Visualization, and Integrated Discovery (DAVID) database for used to perform the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis and Gene Ontology (GO) enrichment analysis to identify the interactions between the biological processes linked to specific hub genes and the molecules (Figure 2).[47,48]

Docking Study

The docking study was conducted using the AutoDock software between the filtered polyphenols (8 polyphenols) and the selected disease target to see the possible interactions and the binding affinity. The 3D structure of the ligands such as Apigenin, Ellagic acid, Ferulic acid, Kaempferol, Genistein, Luteolin, Naringenin, and Quercetin were downloaded from the PubChem database and downloaded as structural data file. The disease target PI3K (7TZ7) was downloaded from the RCSB-PDB (Research Collaboratory Structural Bioinformatics – Protein Data Bank) database as a PDB file.[49] The protein preparation was done including the removal of water molecules and addition of charges and hydrogen bonds and saved in pdbqt file format.

Results

Screening of Compounds

Drug-Likeliness Prediction

The drug-likeness of the ligands was assessed by the SwissADME web tool using the Lipinski's rule of five filters and the Ghosh filter. The drug-likeness properties such as number of hydrogen-bond donors (HBD), logP, MW, number of hydrogen-bond acceptors (HBA), MLOGP, WLOGP, number of atoms, and MR of each ligand were predicted. 13 of the

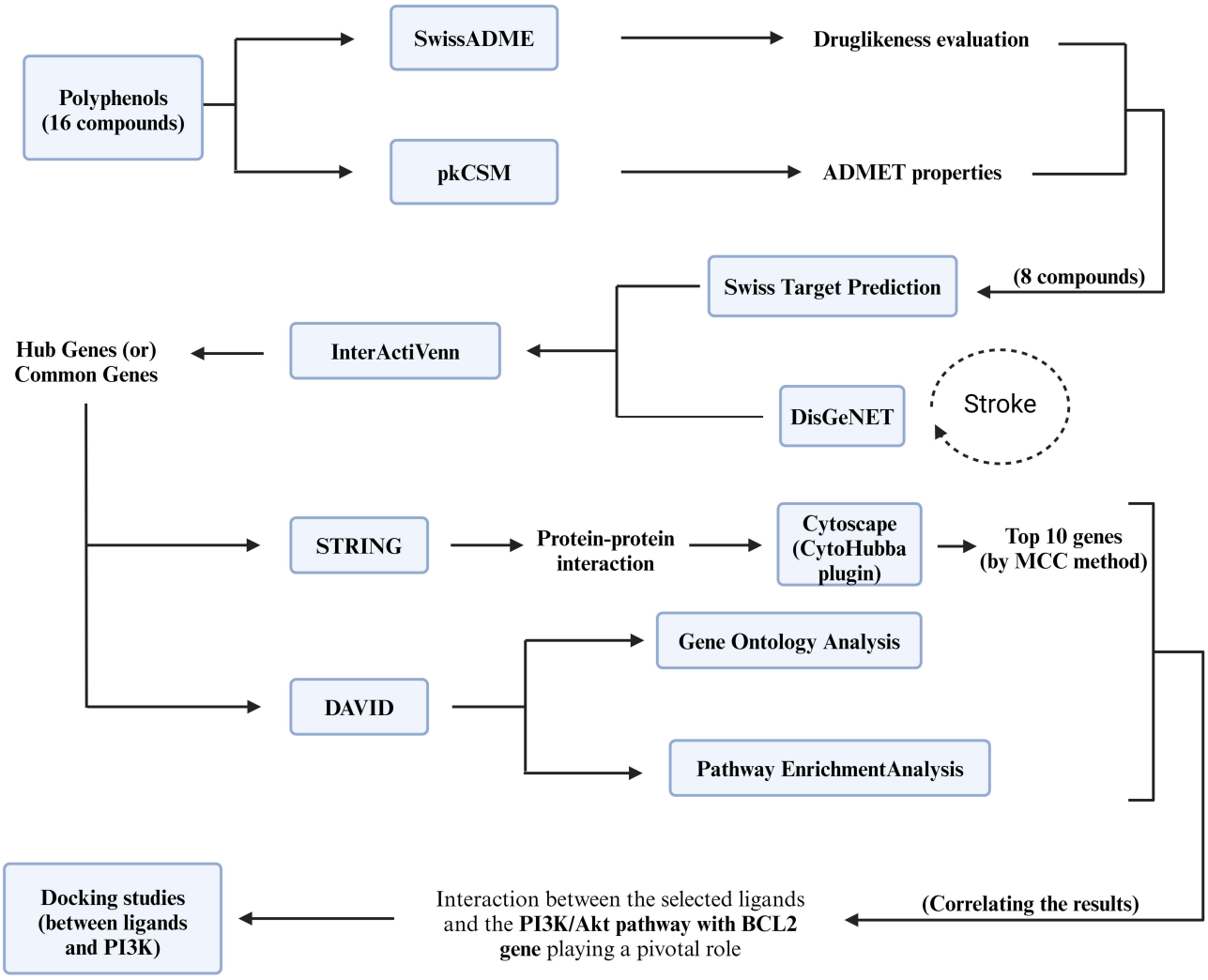

Figure 2 Flowchart showing the Pharmacological Network Analysis Workflow.

16 ligands showed drug-likeness characteristics after passing the Lipinski and Ghosh Filter with no violations. The results of SwissADME drug-likeness are displayed in Table 1.

According to the boiled egg visual depiction (Figure 3), compounds such as Silymarin, Rottlerin, and Chlorogenic acid were found to lack the BBB permeability. The compounds including Berberine, Resveratrol, and Ferulic acid have shown better BBB permeability among the 16 polyphenols.

Table 1 Drug-Likeliness Prediction Using SwissADME Database

Ligands	Molecular Formula	Molecular weight	Hydrogen bond acceptors	Hydrogen bond donors	MLOGP	WLOGP
Chlorogenic Acid	$C_{16}H_{18}O_9$	354.31	9	6	−1.05	−0.75
Ellagic Acid	$C_{14}H_6O_8$	302.19	8	4	0.14	1.31
Curcumin	$C_{21}H_{20}O_6$	368.38	6	2	1.47	3.15
Ferulic acid	$C_{10}H_{10}O_4$	194.18	4	2	1	1.39

(*Continued*)

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Table 1 (Continued).

Kaempferol	$C_{15}H_{10}O_6$	286.24	6	4	−0.03	2.28
Genistein	$C_{15}H_{10}O_5$	270.24	5	3	0.52	2.58
Lignan	$C_{25}H_{30}O_8$	458.5	8	0	1.58	3.87
Luteolin	$C_{15}H_{10}O_6$	286.24	6	4	−0.03	2.28
Naringenin	$C_{15}H_{12}O_5$	272.25	5	3	0.71	2.19
Quercetin	$C_{15}H_{10}O_7$	302.24	7	5	−0.56	1.99
Resveratrol	$C_{14}H_{12}O_3$	228.24	3	3	2.26	2.76
Rottlerin	$C_{30}H_{28}O_8$	516.54	8	5	1.66	5.18
Rutin	$C_{27}H_{30}O_{16}$	610.52	16	10	−3.89	−1.69
Silymarin	$C_{25}H_{22}O_{10}$	482.44	10	5	−0.4	1.71
Apigenin	$C_{15}H_{10}O_5$	270.24	5	3	0.52	2.58
Berberine	$C_{20}H_{18}NO_4^+$	336.36	4	0	2.19	3.10
Ligands	**MR**	**No. of atoms**	**Bioavailability Score**	**Lipinski Filter (violation)**	**Ghose Filter (violation)**	
Chlorogenic Acid	83.5	25	0.11	1	1	
Ellagic Acid	75.31	22	0.55	0	0	
Curcumin	102.8	27	0.55	0	0	
Ferulic acid	51.63	14	0.85	0	0	
Kaempferol	76.01	21	0.55	0	0	
Genistein	73.99	20	0.55	0	0	
Lignan	121.76	33	0.55	0	0	
Luteolin	76.01	21	0.55	0	0	
Naringenin	71.57	20	0.55	0	0	
Quercetin	78.03	22	0.55	0	0	
Resveratrol	67.88	17	0.55	0	0	
Rottlerin	145.1	38	0.55	1	2	
Rutin	141.38	43	0.17	3	4	
Silymarin	120.55	35	0.55	0	1	
Apigenin	73.99	20	0.55	0	0	
Berberine	94.87	25	0.55	0	0	

Notes: Lipinski filter: Molecular Weight (MW) ≤500, MLogP (LogP calculated by the Moriguchi method) ≤4.15, N or O ≤10, NH or OH≤5. Ghosh filter:160≤MW≤480, −0.4≤WLOGP (Water partition co-efficient) ≤5.6, 40 ≤ Molar Refractivity (MR) ≤130, 20 ≤atoms ≤70, F ≥ 30%.

ADMET Profiling

The pkCSM web server was utilized to analyze the SMILES of the corresponding ligands to make predictions. Understanding the pharmacokinetics of these ligands in humans through ADMET profiling is crucial for their potential

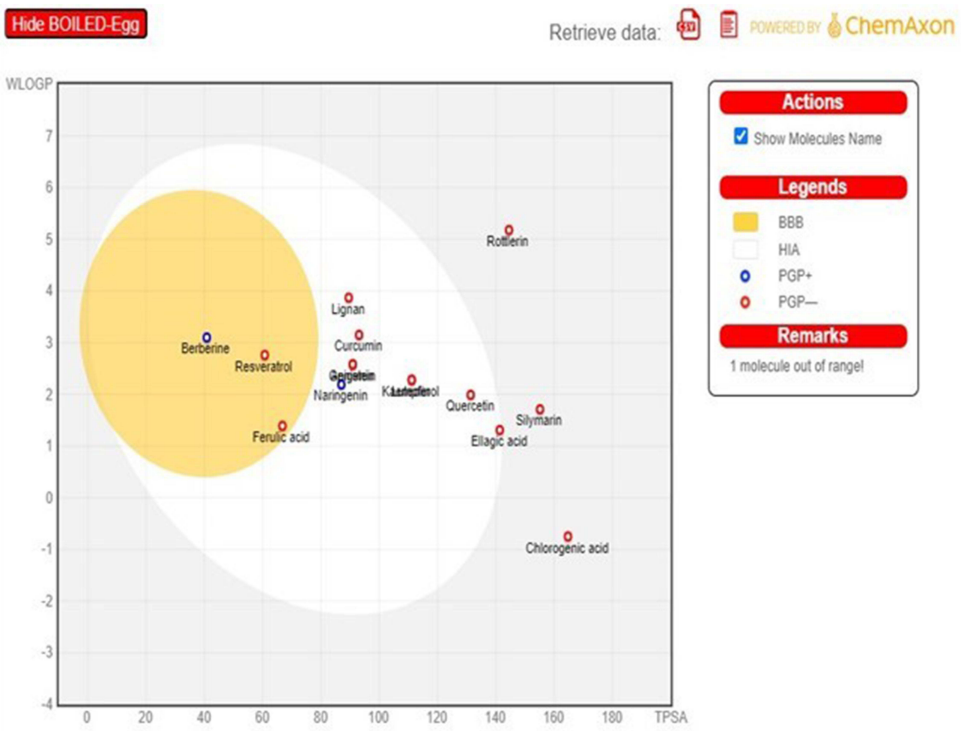

Figure 3 Boiled egg graphical representation of polyphenols using SwissADME.

development as lead compounds in the medical field. Table 2 presents the findings from the ADMET investigation of the corresponding ligands. Most ligands were anticipated to be soluble in water since their water solubility (log S) values were larger than −5.

A noteworthy finding in pharmacokinetics research is that, although Berberine is an inhibitor of P-glycoprotein II, our data indicated that Curcumin, Lignan, Rottlerin, and Silymarin are inhibitors of P-glycoprotein I/II. The BBB permeability was assessed using the logarithmic ratio of the concentration of the

Table 2 ADMET Profiling Using pkCSM Software

Ligands	Water solubility (log mol/L)	$Caco_2$ permeability (log Papp in 10–6 cm/s)	Intestinal absorption (human) (% Absorbed)	Skin Permeability (log Kp)	P-glycoprotein I inhibitor	P-glycoprotein II inhibitor	VDss (human) (log L/kg)
Chlorogenic Acid	−2.449	−0.840	36.377	−2.735	No	No	0.581
Ellagic Acid	−3.181	0.335	86.684	−2.735	No	No	0.375
Curcumin	−4.010	−0.093	82.190	−2.764	Yes	Yes	−0.215
Ferulic acid	−2.817	0.176	93.685	−2.720	No	No	−1.367
Kaempferol	−3.040	0.032	74.290	−2.735	No	No	1.274
Genistein	−3.595	0.900	93.387	−2.735	No	No	0.094
Lignan	−5.670	1.283	100	−2.736	Yes	Yes	−0.730
Luteolin	−3.094	0.096	81.130	−2.735	No	No	1.153
Naringenin	−3.224	1.029	91.310	−2.742	No	No	−0.015
Quercetin	−2.925	−0.229	77.207	−2.735	No	No	1.559

(*Continued*)

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Table 2 (Continued).

Resveratrol	−3.178	1.170	90.935	−2.737	No	No	0.296
Rottlerin	−2.972	−0.306	72.957	−2.735	Yes	Yes	−0.414
Rutin	−2.892	−0.949	23.446	−2.735	No	No	1.663
Silymarin	−3.204	0.435	61.861	−2.735	Yes	Yes	0.369
Apigenin	−3.329	1.007	93.250	−2.735	No	No	0.822
Berberine	−3.973	1.734	97.147	−2.576	No	Yes	0.580
Ligands	**BBB permeability (log BB)**	**CNS permeability (log PS)**	**CYP2D6 substrate**	**CYP3A4 substrate**	**CYP2D6 inhibitor**	**CYP3A4 inhibitor**	**Total clearance (log mL/min/kg)**
Chlorogenic Acid	−1.407	−3.856	No	No	No	No	0.307
Ellagic Acid	−1.272	−3.533	No	No	No	No	0.537
Curcumin	−0.562	−2.990	No	Yes	No	Yes	−0.002
Ferulic acid	−0.239	−2.612	No	No	No	No	0.623
Kaempferol	−0.939	−2.228	No	No	No	No	0.477
Genistein	−0.710	−2.048	No	No	No	No	0.151
Lignan	−1.170	−3.273	No	Yes	No	Yes	0.341
Luteolin	−0.907	−2.251	No	No	No	No	0.495
Naringenin	−0.578	−2.215	No	No	No	No	0.060
Quercetin	−1.098	−3.065	No	No	No	No	0.407
Resveratrol	−0.048	−2.067	No	Yes	No	No	0.076
Rottlerin	−1.472	−2.903	No	Yes	No	No	0.017
Rutin	−1.899	−5.178	No	No	No	No	−0.369
Silymarin	−1.207	−3.639	No	No	No	No	−0.103
Apigenin	−0.734	−2.061	No	No	No	No	0.566
Berberine	0.198	−1.543	No	Yes	Yes	Yes	1.270
Ligands	**Renal OCT2 substrate**	**AMES toxicity**	**Max. tolerated Dose (human) (log mg/kg/day)**	**hERG I inhibitor**	**hERG II inhibitor**	**Hepatotoxicity**	**Skin Sensitization**	**Minnow toxicity (log mM)**
Chlorogenic Acid	No	No	−0.134	No	No	No	No	5.741
Ellagic Acid	No	No	0.476	No	No	No	No	2.110
Curcumin	No	No	0.081	No	No	No	No	−0.081
Ferulic acid	No	No	1.082	No	No	No	No	1.825
Kaempferol	No	No	0.531	No	No	No	No	2.885
Genistein	No	No	0.478	No	No	No	No	1.941
Lignan	No	No	0.365	No	No	No	No	2.086
Luteolin	No	No	0.499	No	No	No	No	3.169
Naringenin	No	No	−0.176	No	No	No	No	2.136
Quercetin	No	No	0.499	No	No	No	No	3.721

(*Continued*)

Table 2 (Continued).

Resveratrol	No	Yes	0.331	No	No	No	No	1.522
Rottlerin	No	No	0.453	No	Yes	No	No	1.967
Rutin	No	No	0.452	No	Yes	No	No	7.677
Silymarin	No	No	0.650	No	Yes	No	No	2.543
Apigenin	No	No	0.328	No	No	No	No	2.432
Berberine	No	Yes	0.144	No	No	Yes	No	−0.277

Abbreviations: VDss, Steady state of volume of distribution; CYP2D6, cytochrome P450 2D6; CYP3A4, cytochrome P450 3A4; Renal OCT2, Organic cation transporter 2; hERG I, human ether-A-go-go related gene I; hERG II, human ether-A-go-go related gene II.

drug in the brain to the concentration of the drug in the plasma (log BB), is used to compute the blood-brain barrier (BBB) permeability. If the log BB value is greater than 0.3, ligands can pass the blood-brain barrier; however, ligands with a log BB value of less than −1 hardly make it to the brain.[29] Similarly, ligands with a log PS value greater than −2 are thought to be able to enter the central nervous system, but those with a log PS less than −3 cannot.

Skin permeability, crucial for transdermal drug delivery, is assessed by log Kp, where values greater than −2.5 cm/h mean that a molecule will barely pass through the skin.[29] The Caco-2 permeability value imitates the in vitro model of the human intestinal mucosa to access the absorption of oral medicines. Higher permeability is indicated by log Papp values greater than 0.90.[29]

The dose of medication required to distribute evenly in blood and plasma is represented by the steady-state volume of distribution (VDss), which is regarded as low if log VDss is less than −0.5 and high if it is greater than 0.45. The blood plasma's uniform distribution is represented by the lower VDss value. The elimination of drugs from blood or plasma by the kidneys and liver is shown by the total clearance values. The total clearance value shows the elimination of drugs either from the blood or from the plasma. Renal drug clearance is significantly influenced by renal OCT2 (organic cation transporter 2). When OCT2 substrates interact with the OCT2 protein transporter, adverse effects may result.

Of the 16 ligands listed in Table 2, Resveratrol and Berberine were shown to be carcinogenic and mutagenic in an AMES mutagenic test, indicating their potential for cancer. Berberine exhibits hepatotoxicity, whereas the other ligands were non-skin sensitizing and non-hepatotoxic. The hepatotoxicity prediction verifies that the ligands will not interfere with regular liver activities. Skin sensitization tests demonstrated that the ligands do not cause allergic side effects if applied topically. Except for Rutin, Rottlerin, and Silymarin, none of the ligands were anticipated to inhibit the human ether-A-go-go gene (hERG) I or II. Many medications typically fail to make it off the market due to hERG I and II inhibition, which is the blockage of the potassium ion (K+) channel.

In Phase I clinical studies, the maximum recommended human tolerated dose (log mg/kg/day) is calculated to assess the threshold for toxicity, classified as low if ≤0.477 log mg/kg/day and high if >0.477 log mg/kg/day. In toxicity assays like the Minnow test, a chemical is considered to show high acute toxicity if LC50 is less than 0.5mM, meaning that 50% of the Flathead Minnows tested die at this concentration.

Taking all these criteria into account, 8 ligands including (Ellagic acid, Ferulic acid, Kaempferol, Genistein, Luteolin, Naringenin, Quercetin, and Apigenin) can be considered as the safest among the selected 16 polyphenols.

Prediction of Putative Targets

273 putative target genes of 8 chosen active components were obtained discarding the duplicated genes using the Swiss Target Prediction database. Following this, from the DisGeNET database 1159 genes linked to ischemic stroke were obtained. Then using InterActiVenn, a Venn diagram was plotted to predict the common targets between the compound-related genes and ischemic stroke (Figure 4). 87 putative genes that protect against ischemic stroke were identified as hub targets.

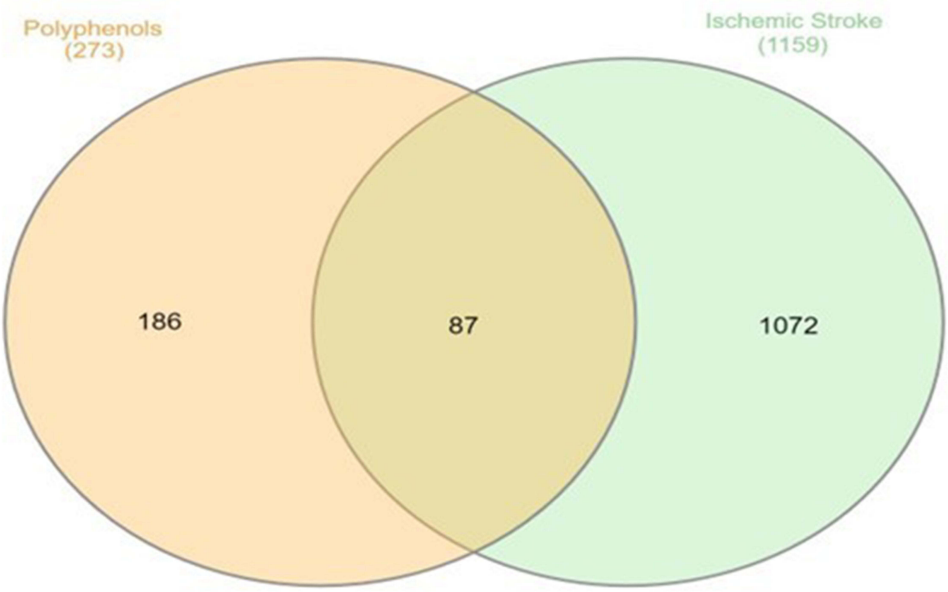

Figure 4 The hub genes or common genes between the ischemic stroke and the selected polyphenols were envisioned on a Venn diagram by using InteractiVenn (http://www.interactivenn.net/).

Building a Protein-Protein Interaction (PPI) Network

STRING database is the one useful resource for predicting protein-protein interactions. The PPI network of gene lists was predicted using STRING database and is depicted in Figure 5.[50] Cytoscape (v3.10.1) was employed to show the predicted PPI network (Figure 6). Using the Cytoscape plugin CytoHubba, by Maximal Clique Centrality (MCC) topological analysis, the top 10 core genes (STAT3, BCL2, NFKB1, ESR1, MMP9, PPARG, PTGS2, CTNNB1, SIRT1, RELA) were identified (Figure 7).[51]

GO (Gene Ontology) Enrichment Analysis

GO enrichment analysis were carried out using the DAVID database to assess the function of the primary targets at three different levels namely molecular function (MF), cellular component (CC), and biological process (BP). The GO enrichment analysis (Figure 8) showed that most of the target genes were involved in Prostaglandin biosynthesis, prostaglandin metabolism, and plasminogen activation in particular dominated the enriched BP ontologies. In the CC analysis, the amyloid and microsome account for the majority (87 target genes). Tyrosine-protein kinase, dioxygenase, peroxidase, and other enzymes dominated the enriched MF ontologies.

KEGG (Kyoto Encyclopedia of Genes and Genomes) Enrichment Analysis

Using the DAVID database, simultaneously KEGG enrichment analysis of the 87 target genes was carried out. The KEGG pathway enrichment analysis results showed that 139 signal pathways and 87 putative target genes had significant associations (FDR < 0.05). In Figure 9, the top 10 pathways with the highest enrichment ratios are shown.

According to the KEGG enrichment analysis of the top 10 pathways associated between the selected polyphenols and ischemic stroke, the most relevant pathway is the PI3K-Akt Signaling Pathway involving the hub genes (Figure 10).

Using Cytoscape plugin CytoHubba, by MCC topological analysis, the top 10 target genes involved between the disease and the drug was predicted previously. By correlating these two results, it was concluded that the PI3K-AKT signaling pathway with its key target BCL2 gene together can play a vital role in stroke.

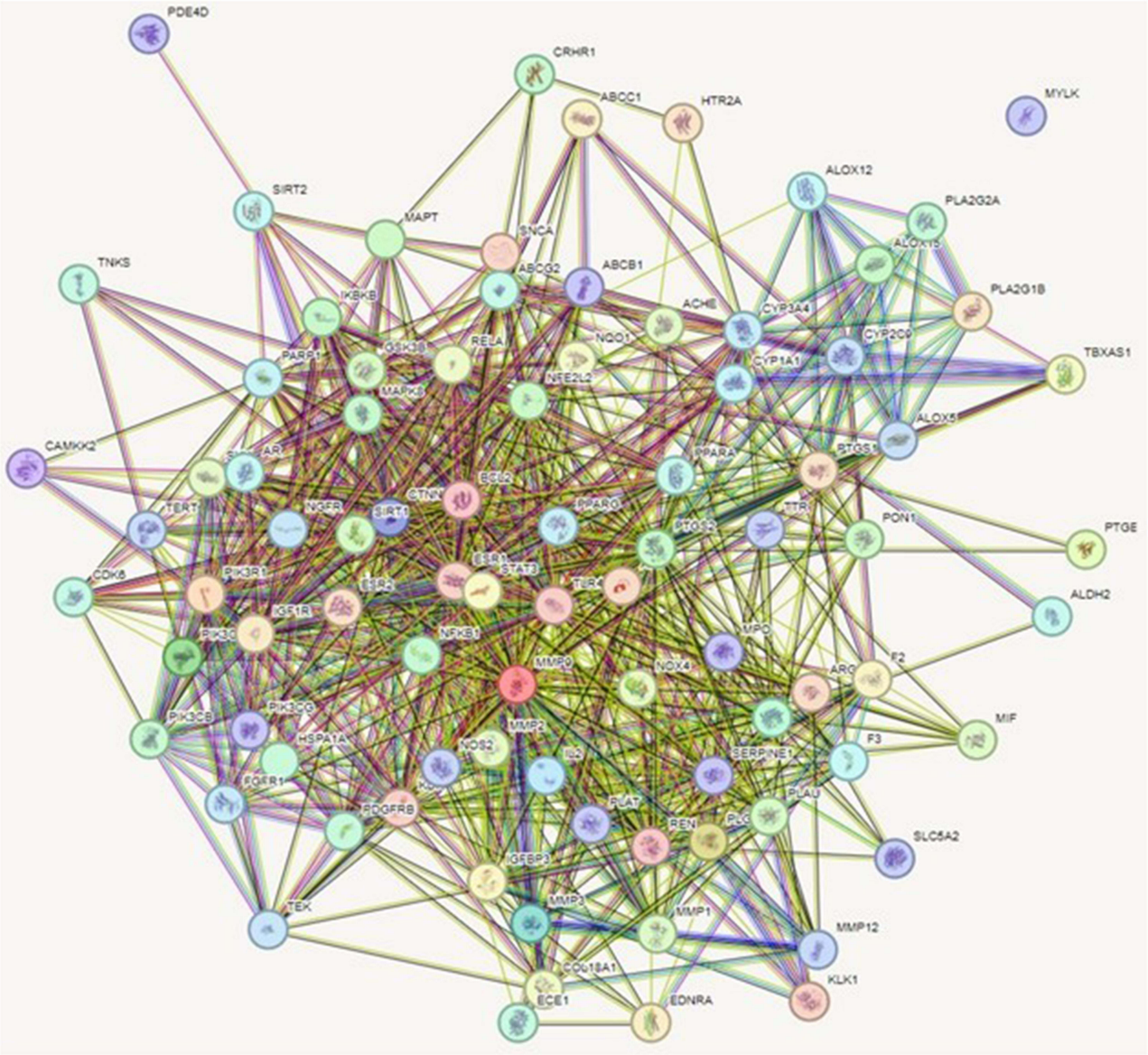

Figure 5 PPI Network analysis between selected polyphenols and targets for Ischemic Stroke obtained using STRING Database (12.0).

Docking Study

To further validate the connection between the filtered polyphenols and the PI3K pathway, docking study was performed. The results of docking study, showing interactions between the ligands and the disease target, is displayed in Table S1 and Figures S1–S8. All 8 ligands including Ellagic acid, Ferulic acid, Kaempferol, Genistein, Luteolin, Naringenin, Quercetin, and Apigenin showed a binding affinity value from −6.60 (for Ellagic acid) to −3.83 kcal/mol (for Ferulic acid), indicating a high to moderate interaction between the ligands and the protein.[52,53]

Discussion

This study provides a baseline for the initial screening of some polyphenols as well as a novel therapeutic concept for further investigation into the mechanisms underlying the use of polyphenols in the treatment of stroke. The ADMET characteristics of putative substances for various models, such as gastrointestinal absorption, BBB penetration, and

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Figure 6 PPI Network using Cytoscape (v3.10.1).

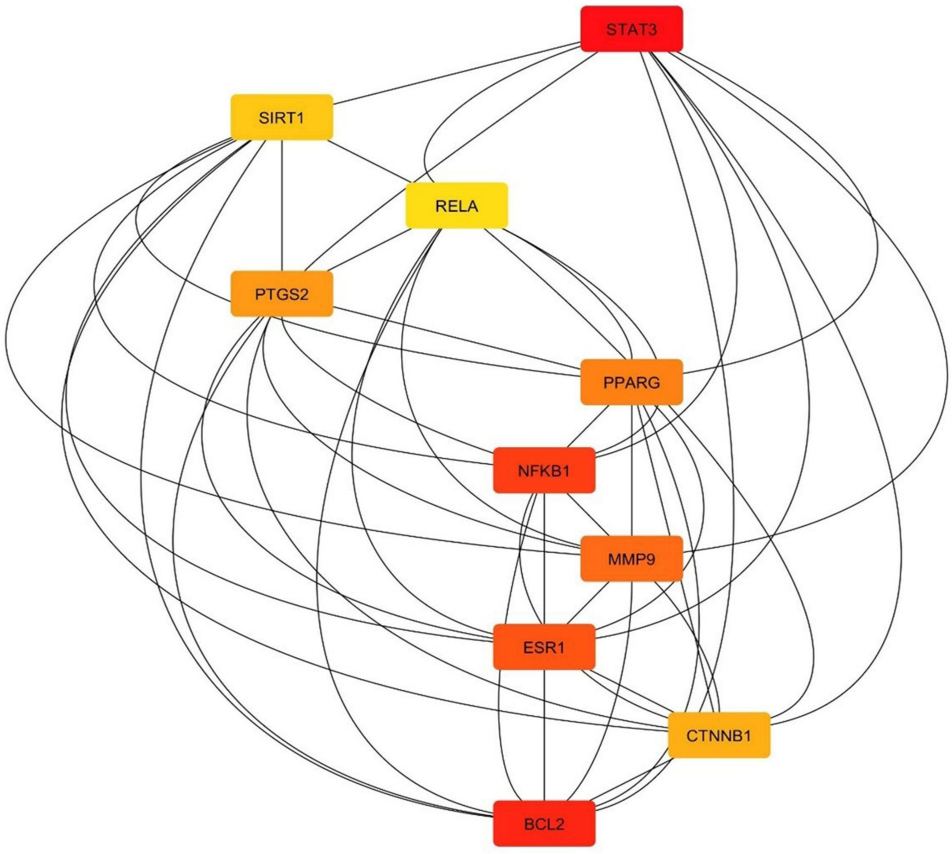

Figure 7 Top 10 genes sorted by MCC method using CytoHubba plug-in. The node colour changes from red to yellow reflecting the rank from high to low in the network.

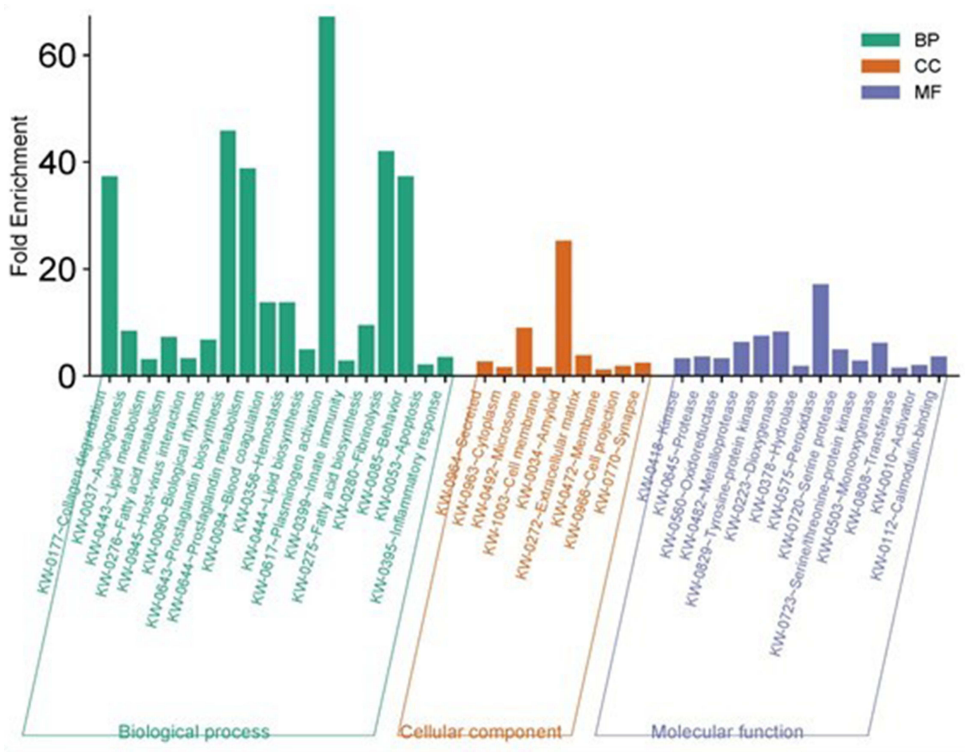

Figure 8 Target protein GO enrichment analysis. The quantity of GO entries ($P < 0.05$) in the functional categories of biological process (BP), molecular function (MF), and cell composition (CC).

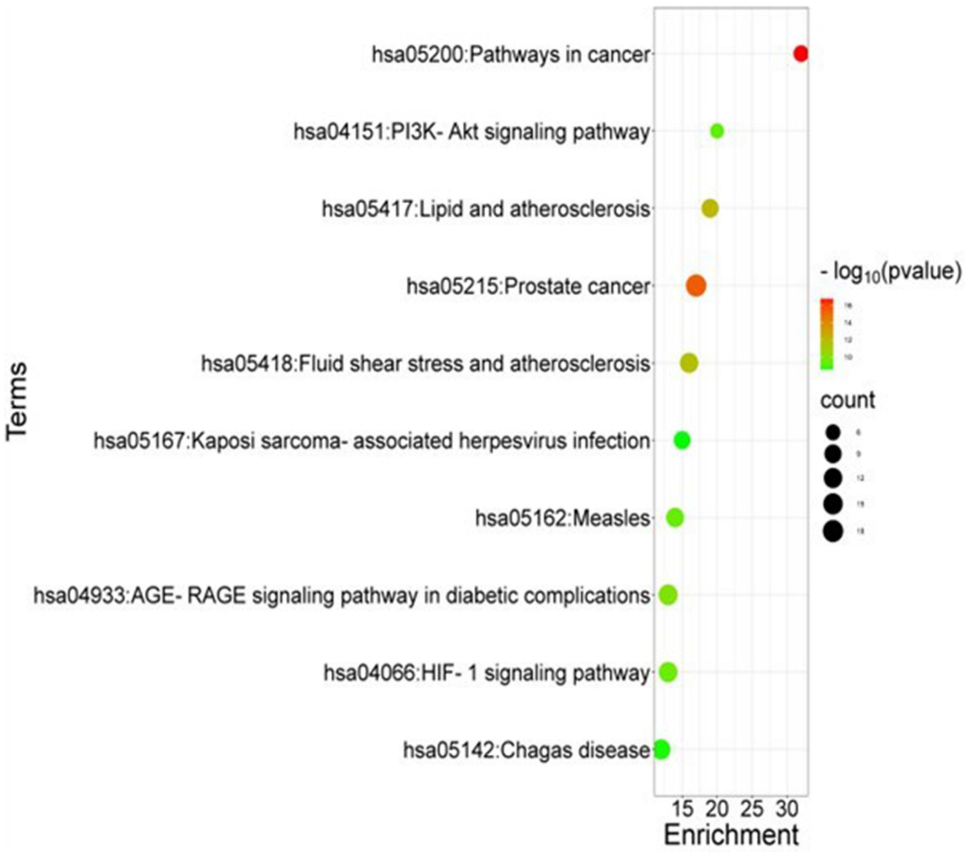

Figure 9 Top 10 KEGG terms of hub genes.

https://doi.org/10.2147/AABC.S470861

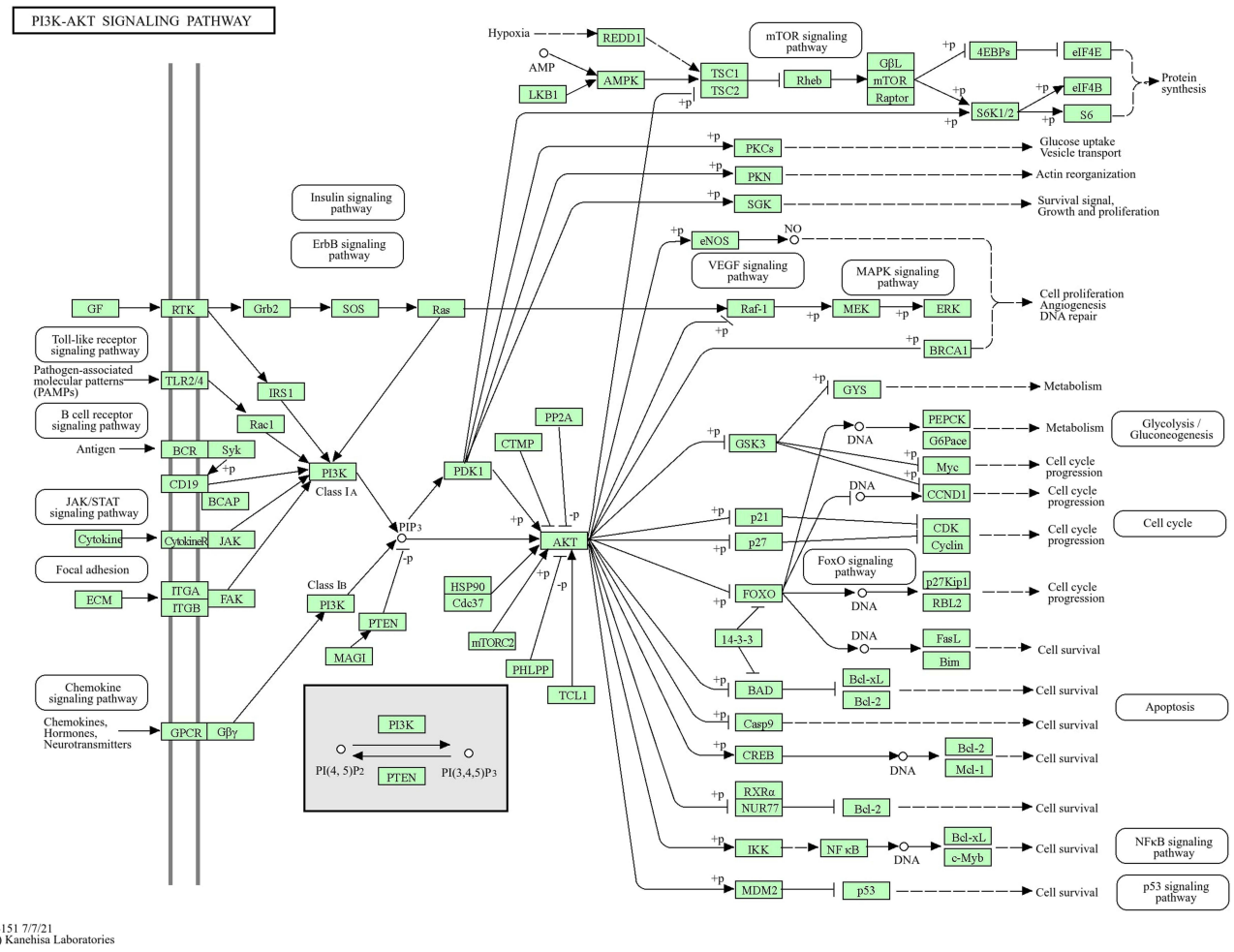

Figure 10 PI3K-Akt Signaling Pathway (KEGG map). The activation of PI3K-AKT signaling pathway downregulates the expression of cysteinyl aspartate specific proteinases-9 (Caspase-9) and-3 (Caspase-3) and can also act on downstream targets like the mammalian target of rapamycin (mTOR) and apoptosis-related protein B lymphocyte tumor-2 (BCL-2) associated x protein (BAX), which ultimately leads to anti-apoptosis mechanism.

P-glycoprotein substrates, demonstrated favourable outcomes that substantially reinforce the polyphenols' relevance.[17] The anti-stroke targets of certain polyphenols were mostly related to prostaglandin production, prostaglandin metabolism, and plasminogen activation, as per GO functional analysis. Studies on the KEGG pathway showed that targets were linked to the pathways relevant to stroke. Further docking study confirmed the substantial interactions between the selected ligands and the disease target (PI3K) which were found to be in good agreement with the previous reported study.[54] Thus, this current research explored an interconnection between the selected polyphenols, the PI3K-AKT signaling pathway, and the BCL2 gene. An intricate network of molecular signal transduction pathways interacts in the pathogenic process of ischemic stroke with the PI3K/Akt signaling pathway playing an essential role in anti-neuronal apoptosis and also other associated mechanisms (Figure 10). Apoptosis, which mostly can be observed in ischemic penumbra, starts a few hours following a cerebral ischemic episode. Ischemic neuronal apoptosis is a mitochondrial-centered mechanism that involves genes from the BCL-2 and cysteine protease (Caspase) families. When brain ischemia induces stress, BCL-2 gene expression falls while BAX gene expression rises. It results in an apoptosis-dependent sequence and activation of caspase. Early ischemia-induced apoptosis is mostly mediated by activated caspase, particularly caspase-1 and caspase-3, which can alter proteins.[55,56] The PI3K/Akt signaling system and apoptosis are inextricably linked. The upregulation of BCL2 activates the PI3K-AKT signaling pathway through which apoptosis can be inhibited, producing a protective effect against ischemic injury.[57] The current study provides an analytical basis for the use of polyphenols in the management of stroke by elucidating the association between certain polyphenols and the

PI3K/Akt signaling pathway and focusing on the pivotal role of BCL2 gene in the genesis of ischemic stroke,[58] offering a basic framework for the use of polyphenols for the management of ischemic stroke.

Limitations

The present study focused on limited polyphenols. Other potential compounds such as Piceatannol, Pterostilbene, as well as natural polyphenols from marine sources should be taken into consideration for future research works.

Conclusion

Stroke is the second leading cause of death and disability worldwide, with significant financial ramifications. Therefore, improving post-stroke care and developing more effective therapeutic interventions. The challenge of transferring research into clinical settings has severely impeded advancements in stroke research, despite the abundance of research exploring various paths leading to stroke and a growing understanding of the etiology of stroke. In comparison to single natural or pharmaceutical chemical substances, consuming foods or natural products with a higher and more varied concentration of polyphenols can produce far more beneficial results faster. Further research is required in the future to investigate additional pathways and genes to provide comprehensive knowledge in the prevention of stroke. Additional laboratory studies are warranted to further explore the pharmacological potential of the selected compounds and their derivatives against stroke in the near future.

Abbreviations

RPTK, Receptor protein tyrosine kinase; PIP2, Phosphatidylinositol 4.5-bisphosphate; PIP3, Phosphatidylinositol (3,4,5)-triphosphate; FADD, Fas-associated death domain; BID, BH3 interacting-domain death agonist.

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Disclosure

The authors declare that they have no conflicts of interest in this work.

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