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Systematic analysis of the molecular mechanisms mediated by coffee in Parkinson's disease based on network pharmacology approach

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ABSTRACT

Many epidemiological studies have associated coffee consumption with a reduced Parkinson's disease (PD) risk, but the molecular mechanisms remain unclear. In this study, systematic pharmacological and bioinformatics approaches were employed to explore the bioactive components and potential mechanisms mediated by coffee in PD. We identified 12 active compounds in coffee associated with 47 PD-related targets, which might exert synergism because some targets were enriched in multiple signaling pathways and biological processes. The compound–target network and protein–protein interaction network exemplified the multi-component and multi-target effect mediated by coffee in PD. Furthermore, a molecular docking assessment verified the great activity between active compounds identified in coffee and PD-related targets. These results showed that the bioactive components of coffee exerted synergistic effects against PD-associated targets through numerous pathways and provided a new perspective for scientific research exploring the multi-component and multi-target mechanisms mediated by coffee in PD.

1. Introduction

Parkinson's disease (PD) is recognized as the second-most common neurodegenerative disease of aging after Alzheimer's disease. PD is characterized by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNc) of the midbrain (Kalia & Lang, 2015; Poirier & Marchand, 1988). The resulting lack of dopamine in motor control areas of the brain leads to the characteristic manifestations of tremor, rigidity, bradykinesia, and postural instability. PD can also present with non-motor symptoms, such as dementia, sleep disturbance, anosmia, autonomic dysfunction, and depression (Poewe et al., 2017; Wermuth et al., 2005; Zheng et al., 2012). To date, the primary therapy for PD targets the dopaminergic pathway to ameliorate PD symptoms, but the long-term use of these drugs may lead to undesirable side effects, including the commonly reported levodopa-induced involuntary movements (Olanow et al., 2004). No current intervention can slow or halt the progressive disease course (Obeso et al., 2017). Therefore, avoiding risk factors and reducing disease risk represent potentially useful strategies for preventing the occurrence and development of PD.

Coffee is one of the most commonly consumed beverages worldwide and its consumption has been negatively correlated with the risks of several chronic diseases, such as diabetes, cardiovascular disease, neurodegenerative disorders, and cancer (Cano-Marquina et al., 2013; Gokcen & Sanlier, 2019). Coffee consumption has also consistently been associated with a reduced risk of PD. Several important early studies

Abbreviations: PD, Parkinson's disease; SNc, substantia nigra pars compacta; NP, network pharmacology; ADME, absorption, distribution, metabolism, and excretion; GI, gastrointestinal; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; C–T, compound–target; PPI, protein–protein interaction; DC, degree centrality; BC, betweeness centrality; CC, closeness centrality; MCODE, Molecular Complex Detection; MCC, maximal clique centrality; MAOB, monoamine oxidase B; MAPK, mitogen-activated protein kinase; HIF, hypoxia-inducible factor; TNF, tumor necrosis factor; LRRK2, leucine-rich repeat kinase 2; AKT1, AKT serine/threonine kinase 1; CASP3, caspase 3; APP, amyloid precursor protein; HSP90AA1, heat shock protein 90 alpha family class A member 1; TLR4, toll-like receptor 4; GSK3B, glycogen synthase kinase 3 beta; BACE1, beta-secretase 1; SLC6A3, solute carrier family 6 member 3; PTGS2, prostaglandin-endoperoxide synthase 2; STAT3, signal transducer and activator of transcription; HMOX1, heme oxygenase 1; MMP9, matrix metalloproteinase 9; ESR1, estrogen receptor 1; ABCB1, ATP-binding cassette subfamily B member 1; DRD4, dopamine receptor D4; EHT, eicosanoyl-5-hydroxytryptamide; NF, nuclear factor; AP, activator protein; BBB, blood–brain barrier; BAX, Bcl-2-associated protein X; PINK1, PTEN-induced kinase 1; PI3K, phosphoinositol 3-kinase.

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demonstrated that increased coffee consumption significantly decreased PD risk in a dose-dependent manner (Hernan et al., 2002; Ross et al., 2000; Tan et al., 2003). Caffeine represents the most commonly investigated component among coffee's constituents and has been associated with neuroprotective effects, mediated by the inhibition of lipid peroxidation and the reduction of reactive oxygen species production (Kolahdouzan & Hamadeh, 2017). Our previous study found that lower absolute levels of caffeine and caffeine metabolite profiles served as promising diagnostic biomarkers for early PD, which was consistent with the neuroprotective effects of caffeine that were previously reported by epidemiologic and experimental studies (Fujimaki et al., 2018; Hatano et al., 2016; Takeshige-Amano et al., 2020). However, an increasing number of studies have focused on the health-related effects of decaffeinated bioactive compounds and their positive implications for neurodegenerative diseases. Decaffeinated compounds may play important roles as antioxidants (Colombo & Papetti, 2020; Hu et al., 2019; Islam et al., 2018; Limaa et al., 2017), such as quercetin, chlorogenic acid, ferulic acid, kahweol, and cafestol. Various active compounds in coffee may exert synergistic effects against diseases, but current research tends to focus on single components in isolation. PD is a multifactorial disorder associated with oxidative stress, neuroinflammation, mitochondrial dysfunction, α-synuclein proteostasis, calcium homeostasis, autophagy, and neurotransmitter abnormalities (Poewe et al., 2017). Therefore, the systematic and comprehensive analysis of potential mechanisms mediated by the components in coffee, which may result in the combination of multiple targets, is a reasonable aim.

Network pharmacology (NP) is an innovative method used to study the mechanisms of drugs at the systemic level, based on computer science, molecular biology, pharmacology, and other disciplines (Hopkins, 2007). Many effective drugs act by modulating multiple proteins rather than acting against a single target. Unlike earlier "one drug, one target" approaches, NP updates the research paradigm to a new "multi-component, network target" model (Azmi, 2012). NP can integrate various drug and biological databases, systematically and holistically elaborating the disease–target protein–drug pathway through a bioinformatics analysis, which provides a deeper understanding of drug interactions in complex diseases (von Eichborn et al., 2011; S. D. Zhang et al., 2014).

Coffee beverages are typically prepared from the roasted beans of the genus *Coffea*, which is a flowering plant in the family Rubiaceae (Davis et al., 2006). The roasting process may enhance the activity of bioactive compounds in coffee (Sulaiman et al., 2011). Although coffee has never been used as a medicine, it is one of the most commonly consumed beverages. Approximately 2.3 billion cups of coffee are consumed worldwide every day (Deshpande et al., 2014), which has the potential to have significant impacts on the health of coffee drinkers on a population scale. However, the specific mechanisms mediated by coffee in PD remain unclear. In this study, we aimed to apply an NP approach to explore key active compounds, core target molecules, and the potential relationships between coffee and PD, and provide ideas and a theoretical basis for further elucidating the synergistic effects and mechanisms of multi-component, multi-target agents found in coffee against PD.

2. Materials and methods

2.1. Screening active compounds and pharmacokinetic predictions

The primarily chemical compounds in coffee were mined by searching literature indexed by PubMed and the Web of Science. The compound structures were identified in PubChem (https://pubchem.ncbi.nlm.nih.gov/). Information regarding the absorption, distribution, metabolism, and excretion (ADME) properties of each compound identified with potential biological activity were acquired from SWISSADME (Daina et al., 2017) (http://www.swissadme.ch/), and we applied the gastrointestinal (GI) absorption and bioavailability scores as screening parameters to reflect the absorption and similarity with existing drugs. We set the thresholds for candidate compounds as GI absorption = high and bioavailability score $\geq$ 0.3 (Mi et al., 2020).

2.2. Target prediction

For the current study, we used the SwissTargetPrediction to predict prospective targets for the active compounds identified in coffee. We set probability $>$ 0 as the threshold for candidate targets, integrating large-scale chemical, genomic, and pharmaceutical data (Daina et al., 2019). Using "Parkinson disease" as the search term, PD-related targets were mined from two existing databases, the DisGeNet database (http://www.disgenet.org/) (Pinero et al., 2017) and GeneCards database (https://www.genecards.org/) (Safran et al., 2010). Official gene names were obtained from the UniProt database (http://www.uniprot.org/) (Bateman et al., 2019) by restricting the types to "*Homo sapiens*." Venn diagrams (http://bioinformatics.psb.ugent.be/webtools/Venn/) were generated to perform overlap analysis to obtain overlapping PD-related targets associated with the various compounds identified in coffee.

2.3. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis

The overlapping PD-related targets associated with the various coffee compounds were input into the DAVID database (https://david.ncifcrf.gov/home.jsp) (Jiao et al., 2012), a web-based service that facilitates gene/protein list analysis and produces enriched GO terms and KEGG pathways. A threshold of $P < 0.05$ was applied, and a chart was plotted by http://www.bioinformatics.com.cn, an online platform for data analysis and visualization.

2.4. Network construction

A compound–target (C–T) network was constructed for the compounds identified in coffee, which was able to systematically describe multiple interactions between the compounds in coffee and their related targets. Protein–protein interaction (PPI) data were obtained from the STRING database (version 11.0) (https://string-db.org/) (Szklarczyk et al., 2017). We selected a confidence score of $>$ 0.4, with the species restricted to "*Homo sapiens*," to construct the PPI network (Ge et al., 2018). All visualized network models were generated using Cytoscape (version 3.7.1) (Demchak et al., 2014), an open software package project for visualizing, integrating, modeling, and analyzing interaction networks. The topological feature of each node in the network was assessed by calculating three parameters with Centiscape2.2 in Cytoscape software, including "degree centrality (DC)," "betweenness centrality (BC)," and "closeness centrality (CC)" (Scardoni et al., 2009). DC reflects the relative importance of a node in a network, while BC represents the probability that a signal passes through the nodes; higher DC and BC values indicate more important nodes. CC reflects the network tightness; a tighter network has higher efficiency. Clustering subnetworks were produced using the Molecular Complex Detection (MCODE) algorithm (Bader & Hogue, 2003) in Cytoscape software, with K-core = 2, to identify the most significant modules in the PPI networks. Hub genes were screened by CytoHubba in Cytoscape software, using the maximal clique centrality (MCC) method (Chin et al., 2014). MCC represents a topological analysis method in CytoHubba for identifying featured nodes and hub genes in PPI networks.

2.5. Molecular docking

We performed molecular docking via AutoDock Vina (Trott & Olson, 2010) to explain the mechanism and binding activity between active components and target proteins. The structures of compounds were downloaded in SDF format from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/), and the SDF format was transformed into

a mol2 format file using Chem 3D software. Receptor structures were downloaded from the RCSB Web site (http://www.rcsb.org/pdb) in PDB format. PyMol software was used to remove solvent molecules and ligands, and AutoDock Tools (version 1.5.6) was used to add hydrogen atoms and charges, with default settings selected for all other parameters, and the results were saved in pdbqt format. Finally, AutoDock Vina (version 1.1.2) was used to perform molecular docking analysis. The theoretical binding affinities were predicted based on the docking scores. The protein–ligand interactions were identified using Discovery Studio Visualizer 2020 (https://discover.3ds.com/discovery-studio-visualizer-download). We also performed molecular docking between positive drugs (selegiline and rasagiline) and the target protein monoamine oxidase B (MAOB) to demonstrate the rationality of the molecular docking model.

3. Results

3.1. Screening of active compounds in coffee

Through literature mining, we obtained identified 15 key active compounds in roasted coffee, which could primarily be classified into the following three types (Table 1): purine alkaloids (caffeine, theobromine, trigonelline, trigonelline, and nicotinic acid), polyphenols (chlorogenic acid, caffeic acid, quinic acid, ferulic acid, hydroxyhydroquinone, quercetin, phenylindane, and secoisolariciresinol), and diterpenoids (kahweol and cafestol). We screened these active compounds according to their ADME properties, which resulted in the exclusion of chlorogenic acid, quinic acid, and phenylindane based on their low GI absorption or bioavailability scores. Owing to thermal instability, chlorogenic acid may be almost completely degraded into volatile phenolic compounds when submitted to intense roasting conditions (Wei et al., 2012). Although the roasting process can also induce the generation of some important bioactive compounds, such as melanoidins, these compounds are generally considered to be poorly absorbed and, therefore, have low bioavailability, even if the low-molecular-weight water-soluble fraction appears to be partially absorbed by the intestinal tract (Tagliazucchi & Bellesia, 2015). We maintained 12 key active compounds with excellent ADME properties for use in the NP analysis (Fig. 1).

3.2. Compound–Target network

Coffee exerts a wide range of pharmacological and biological effects through a variety of compounds and targets. For the current study, we performed target fishing for the 12 selected compounds based on SwissTargetPrediction, collecting 249 candidate targets. We constructed a C–T network and systematically described the multiple interactions between compounds identified in coffee and their related targets. We obtained 261 nodes and 470 edges in our initial network and screened for nodes that were larger than the median values of "DC," "BC," and "CC" for use as hub nodes in the analysis. These three parameters are the most important parameters for measuring the criticality of a node within a network and represent important indicators for new drug discovery and target prediction (Scardoni et al., 2009). We screened out the core network by limiting the parameters to DC $>$ 2, CC $>$ 0.001147, and BC $>$ 4.358372. Higher degree values represent closer relationships between compounds and targets, indicating the importance of each compound in this network. Fig. 2A reveals that the core C–T network consisted of 51 nodes and 165 edges. Diamond nodes represent compound nodes, whereas ellipse nodes represent the coffee targets. The C–T network suggested that active compounds in coffee may regulate entire biological network systems rather than single targets, indicating the complexity of multi-component and multi-target relationships. As shown in Fig. 2B, quercetin (degree = 103) has the most potential targets, followed by secoisolariciresinol (degree = 69), ferulic acid (degree = 61), caffeic acid (degree = 50), and caffeine (degree = 50). These high-degree compounds are likely to represent the core pharmacodynamic substances in coffee.

3.3. Identifying PD-related targets in coffee

For the current study, we performed target fishing using the 12 selected compounds, based on SwissTargetPrediction, collecting 249 candidate targets. Then, we mined 351 known PD-related targets from two existing databases by drawing a Venn diagram (Fig. 3A). Finally, we matched the targets of coffee and PD and chose the 47 overlapping targets as potential PD-related targets of coffee (Fig. 3B).

3.4. GO and KEGG pathway enrichment analysis

A total of 47 overlapping genes were identified by matching potential targets of coffee with disease-associated genes. To further elucidate the multiple mechanisms of coffee against PD, we conducted GO enrichment and KEGG pathway analysis. The leading 10 enriched terms, including biological process, cellular component, and molecular function, are presented in Fig. 4A. Particularly, the enriched biological process ontologies were dominated by peptidyl-threonine phosphorylation, the positive regulation of nitric oxide biosynthetic process, response to drug, protein autophosphorylation, and the activation of mitogen-activated protein kinase (MAPK) activity. The enriched molecular function ontologies were dominated by identical protein binding, protein binding, enzyme binding, monoamine transmembrane transporter activity, and nitric-oxide synthase regulator activity. The plasma membrane accounted for the largest proportion in the cellular component analysis (33 target genes). Among them, 20 significant KEGG pathways were identified, as shown in Fig. 4B. The hypoxia-inducible

Table 1
Active compounds and ADME parameters.

Compound	Formula	Weight (g/mol)	GI absorption	Bioavailability score	Reference
Caffeine	C8H10N4O2	194.19	0.55	High	Butt & Sultan, 2011; Ludwig et al., 2014; Mancini et al., 2018
Theobromine	C7H8N4O2	180.16	0.55	High	Gasscht et al., 2015; Onatibia-Astibia et al., 2017; van Dam et al., 2020
Theophylline	C7H8N4O2	180.16	0.55	High	Camandola et al., 2019; Gasscht et al., 2015; Onatibia-Astibia et al., 2017
Trigonelline	C7H7NO2	137.14	0.55	High	Butt & Sultan, 2011; Colombo & Papetti, 2020; Ludwig et al., 2014
Nicotinic acid	C6H5NO2	123.11	0.85	High	Gasscht et al., 2015; Ludwig et al., 2014; Wierzejska, 2017
Chlorogenic acid	C16H18O9	354.31	0.11	Low	Colombo & Papetti, 2020; Gokcen & Sanlier, 2019; Mancini et al., 2018
Caffeic acid	C9H8O4	180.16	0.56	High	Butt & Sultan, 2011; Colombo & Papetti, 2020; Mancini et al., 2018
Quinic acid	C7H12O6	192.17	0.56	Low	Colombo & Papetti, 2020; Mancini et al., 2018
Ferulic acid	C10H10O4	194.18	0.85	High	Colombo & Papetti, 2020; Grosso et al., 2017
Hydroxyhydroquinone	C6H6O3	126.11	0.55	High	Butt & Sultan, 2011; Gasscht et al., 2015; Yenisetti & Muralidhara, 2016
Quercetin	C15H10O7	302.23	0.55	High	Colombo & Papetti, 2020; Lee et al., 2016; Mancini et al., 2018
Phenylindane	C15H14	194.27	0.55	Low	Kumar et al., 2019; Ludwig et al., 2014; Mancini et al., 2018
Secoisolariciresinol	C20H26O6	362.4	0.55	High	Butt & Sultan, 2011; Mazur et al., 1998; Reddy et al., 2020
Kahweol	C20H26O3	314.4	0.55	High	Butt & Sultan, 2011; Gokcen & Sanlier, 2019; Ludwig et al., 2014
Cafestol	C20H28O3	316.4	0.55	High	Butt & Sultan, 2011; Gokcen & Sanlier, 2019; Ludwig et al., 2014

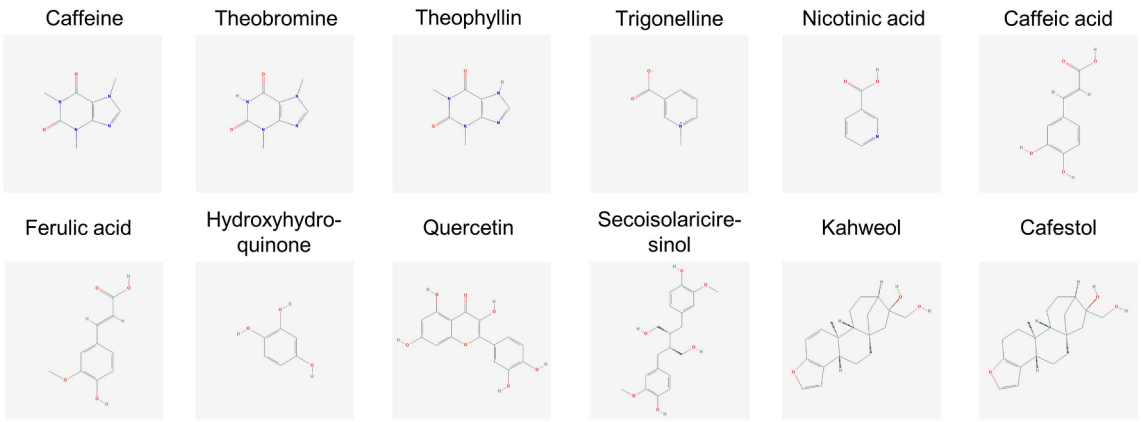

Fig. 1. Key active compounds and chemical structures.

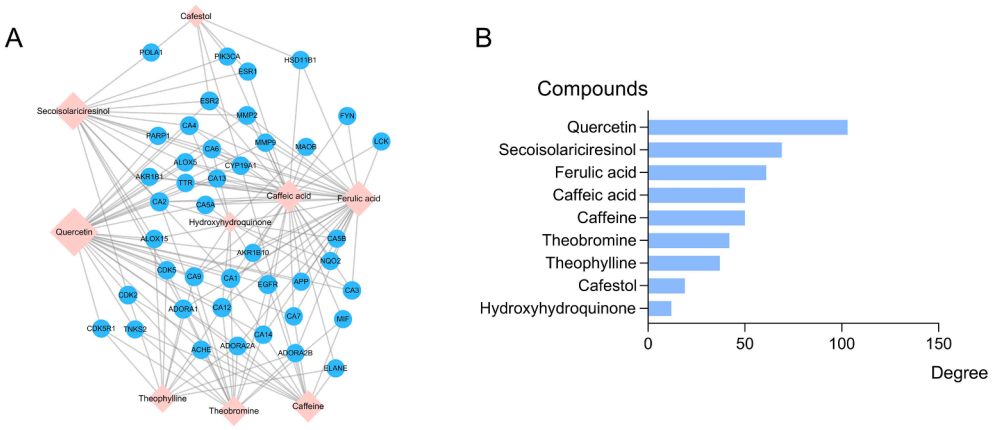

Fig. 2. (A) Compound–Target Network. (B) The core compounds in coffee.

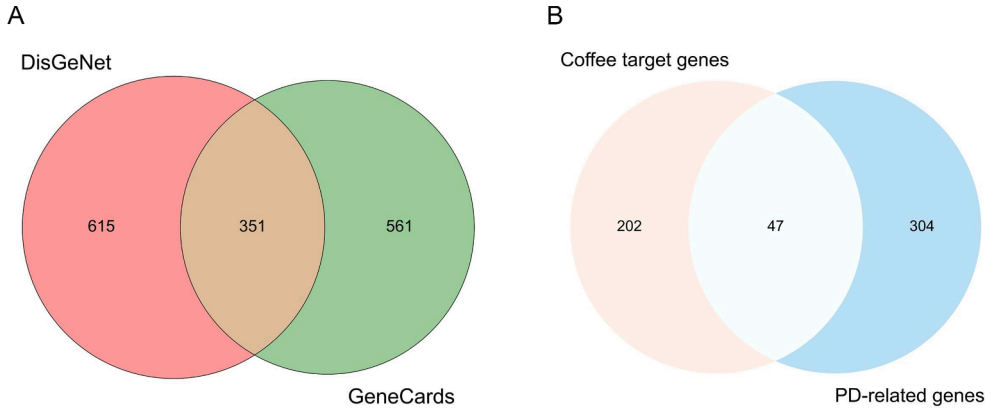

Fig. 3. (A) Venn diagram showing the overlap analysis of PD-related genes. (B) Venn diagram showing the overlap analysis between coffee-targeted and PD-related genes.

factor (HIF)-1 signaling pathway, tumor necrosis factor (TNF) signaling pathway, and insulin resistance pathway may represent key interaction pathways that apply synergistic effects against PD. Furthermore, the serotonergic synapse and dopaminergic synapse were also important interaction pathways. The main pathways of coffee against the development of PD are integrated in Fig. 5.

3.5. Protein–Protein interaction network

The PPI network was constructed and analyzed using the STRING database and Cytoscape software and consisted of 47 nodes and 313 interaction lines. In the PPI core network (Fig. 6A), a node represents one protein, and the large orange nodes to the small blue nodes correspond to the degree values (from large to small). Each line represents the interaction between proteins, and the thick orange lines to the thin blue lines correspond to the combined scores (from high to low). Higher scores represent closer interactions between proteins in the PPI network. We also created a PPI network centered on leucine-rich repeat kinase 2 (LRRK2). The results revealed that coffee may affect PD in LRRK2 mutation carriers through AKT serine/threonine kinase 1 (AKT1), caspase 3

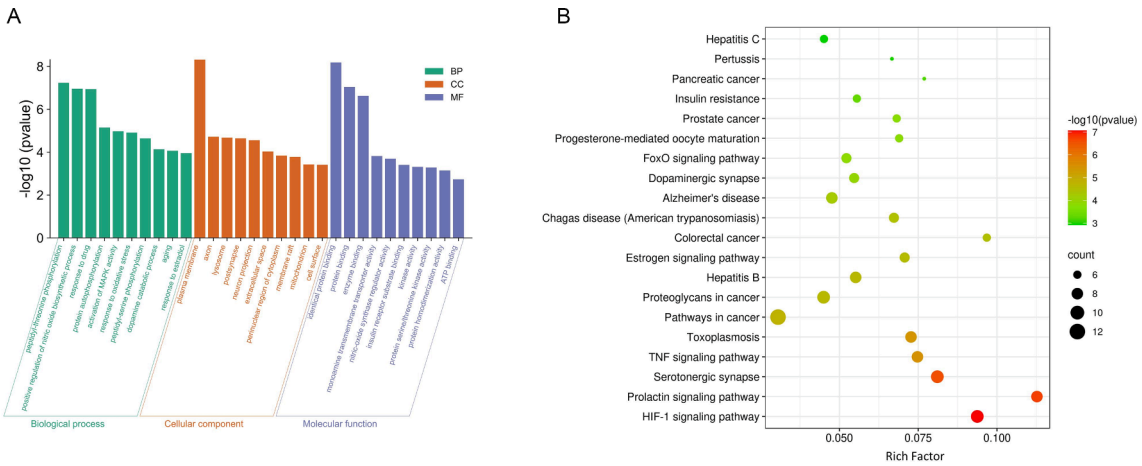

Fig. 4. (A) The top 10 GO terms for biological process, cellular component, and molecular function, respectively. (B) The top 20 signaling pathways identified by KEGG enrichment analysis.

(CASP3), amyloid precursor protein (APP), heat shock protein 90 alpha family class A member 1 (HSP90AA1), toll-like receptor 4 (TLR4), glycogen synthase kinase 3 beta (GSK3B), MAPK, MAOB, beta-secretase 1 (BACE1), and solute carrier family 6 member 3 (SLC6A3) (Fig. 6B). Among these nodes, hub genes were identified using plugcluster Cytohubba using the MCC method. The top 10 hub genes were identified as AKT1, CASP3, MAPK1, prostaglandin-endoperoxide synthase 2 (PTGS2), signal transducer and activator of transcription (STAT3), heme oxygenase 1 (HMOX1), TLR4, matrix metalloproteinase 9 (MMP9), estrogen receptor 1 (ESR1), and HSP90AA1 (Fig. 6C), with darker colors indicating higher scores. To study the PPI network further, the MCODE plugin in Cytoscape software was introduced to generate clusters that might represent molecular complexes, defined as topological modules [42]. Four clusters were found through MCODE (K-core = 2) (Fig. 7). Cluster 1 contained 19 nodes and 95 edges, with a score of 10.556; Cluster 2 contained 10 nodes and 24 edges, with a score of 5.333; Cluster 3 contained 5 nodes and 7 edges, with a score of 3.500; and Cluster 4 contained 3 nodes and 3 edges, with a score of 3.000. Cluster analysis showed that HMOX1, ATP-binding cassette subfamily B member 1 (ABCB1), SLC6A3, and dopamine receptor D4 (DRD4) were the seeds of these four major clusters, which might represent the key target molecules of each cluster.

3.6. Molecular docking analysis

The top nine compounds by the degree ranking and 11 targets obtained from the combined analysis of the top 10 identified by the MCC algorithm and the top 10 identified by the PPI network were verified by molecular docking analysis. A lower binding affinity indicates better binding activity between ligands and receptors. The results revealed that the docking scores ranged from −4.1 kcal/mol to −9.4 kcal/mol, as shown in Fig. 8A. The positive drugs and MAOB (PDB ID: 4CRT) docking scores were –8 kcal/mol (selegiline) and –7 kcal/mol (rasagiline). An affinity < −7 kcal/mol indicates a strong binding activity (Trott & Olson, 2010). Furthermore, on the basis of the average binding free energy between targets and compounds, the targets, ranked from low to high, were PTGS2 (PDB ID: 5F19), HSP90AA1 (PDB ID: 4BQG), AKT1 (PDB ID: 4GV1), MAPK1 (PDB ID: 1PME), ESR1 (PDB ID: 1UOM), APP (PDB ID: 5AMB), STAT3 (PDB ID: 5AX3), CASP3 (PDB ID: 4PS0), HMOX1 (PDB ID: 3CZY), TLR4 (PDB ID: 2Z66), and MMP9 (PDB ID: 4JIJ). The average docking values between compounds and targets showed that quercetin, cafestol, and secoisolariciresinol were the lowest among the nine compounds, suggesting that quercetin, cafestol, and secoisolariciresinol combine more easily with PD-related targets. The combinations between quercetin and the top four core targets—AKT1, CASP3, PTGS2, and MAPK1—are shown in Fig. 8B–E, while quercetin and LRRK2 are shown in Fig. 8F and rasagiline and MAOB are shown in Fig. 8G.

4. Discussion

In this study, in view of the complexity of the active ingredients found in coffee and the diversity of potential regulatory targets identified in humans, for the first time, we employed NP approaches, which integrated chemical, pharmacokinetic, and pharmacological data mined from several databases, to explore the bioactive components and mechanisms underlying the effects of coffee on PD. We identified 12 active compounds in coffee that potentially act on 47 PD-related targets to exert synergism and performed enrichment analysis to identify multiple signaling pathways and biological processes. The C–T network and the PPI network exemplified the characteristics of the multi-component and multi-targeted effects of coffee on PD. These results are consistent with the reported complexity of PD pathogenesis, and allow for a better understanding of the interactions that occur among the target genes.

4.1. Active compounds in coffee against PD

According to the C–T network, we found that caffeine is not the most important compound in coffee, and our results suggested that quercetin, secoisolariciresinol, ferulic acid, and caffeic acid all displayed higher DC values than caffeine. A large quantity of experimental evidence has suggested that quercetin can be considered a supplemental therapy for PD, with pharmacological functions through the control of various molecular pathways, including reducing lipid peroxidation, inducing autophagy, decreasing the level of oxidative stress, and reducing apoptosis (Ashrafizadeh et al., 2019; Sriraksa et al., 2012; Tamtaji et al., 2020). The results of the molecular docking experiments performed in the present study indicated that quercetin easily binds with PD-related targets, and another *in silico* study (Baul & Rajiniraja, 2018) demonstrated that quercetin was a comparatively strong ligand for α-synuclein. Some studies have found that flavonoids can affect α-synuclein degradation, and quercetin, as an important flavonoid, was found to play a key role in the prevention of α-synuclein prion-like dissemination and aggregation (Eskandari et al., 2020; Yu & Lee, 2020). Combined with our results, these findings suggest that coffee may slow or halt the progressive course of PD, although more scientifically designed epidemiological and experimental studies remain necessary to verify this hypothesis. Secoisolariciresinol is a primary component of lignans, which are phenolic compounds found in coffee (Butt & Sultan, 2011; Mazur et al., 1998). The antioxidant activities of lignans and their

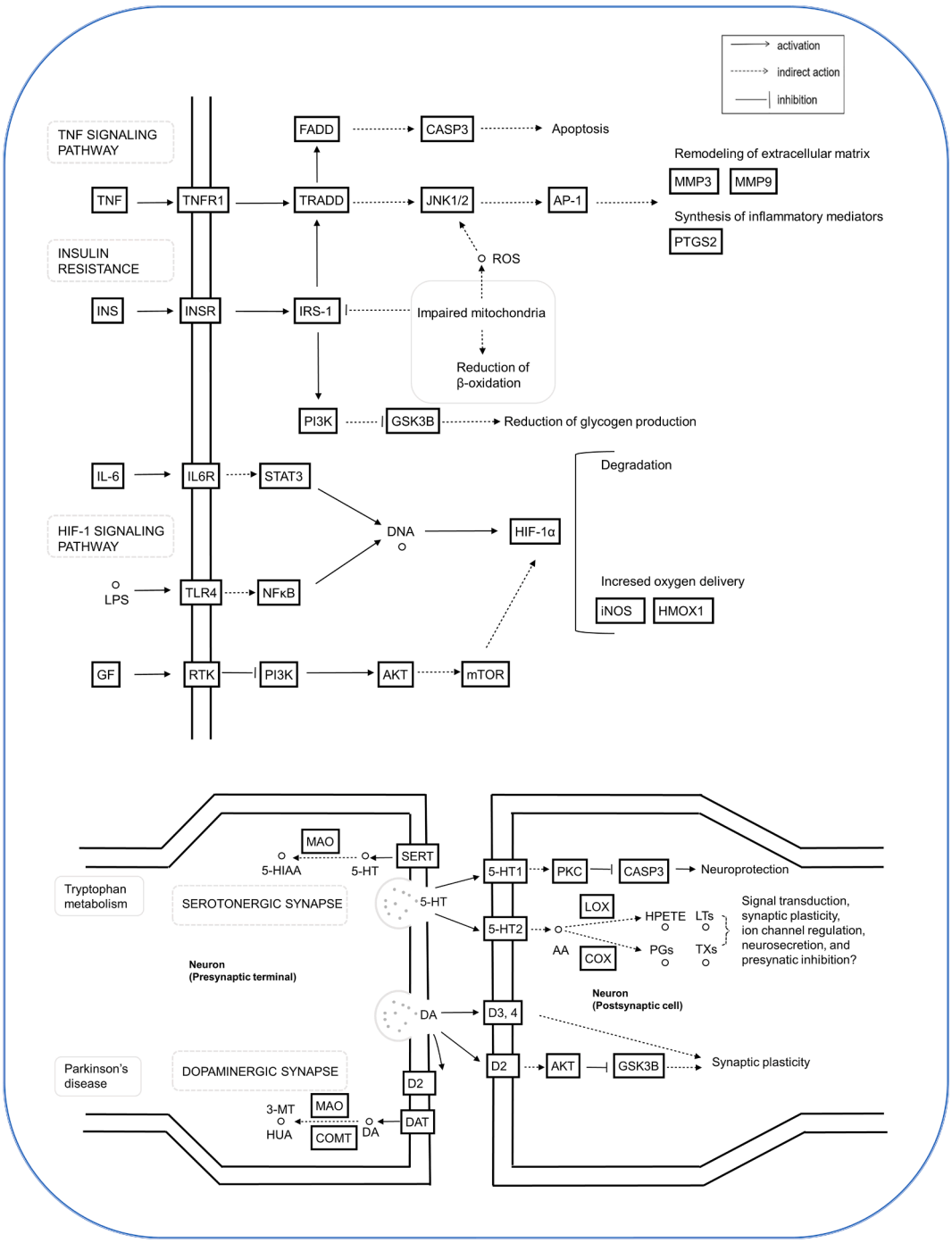

Fig. 5. The integrated pathways of coffee against the development of PD.

metabolites have also been suggested to contribute to reductions in serum cholesterol, lipid peroxidation, and the development of atherosclerosis in animals (Ikeda et al., 2003; Kitts et al., 1999), and antioxidant activity is likely an additional positive effect of secoisolariciresinol on PD. Caffeine represents the most investigated component in coffee. *In vitro* and *in vivo* studies have shown that caffeine is associated not only with the activation of antioxidant pathways, but also with their interactions with transcription factors that induce apoptosis (Park et al., 2017). Diterpenes, such as cafestol and kahweol, exhibit important antioxidant and chemoprotective properties and can increase serum cholesterol levels (Penson et al., 2018). However, these molecules are largely removed from coffee when it is brewed using a paper filter. Our study included cafestol and kahweol, representing coffee brewed without percolation, such as espresso. Recently, some active compounds with therapeutic potential for PD, such as eicosanoyl-5-hydroxytryptamide (EHT), have been found in coffee. EHT has been reported to have synergy with caffeine in models of α-synucleinopathy (Yan et al., 2018). However, because the amount of EHT required to produce a therapeutic effect cannot be obtained by simply drinking coffee each day, we did not include EHT in our analysis.

4.2. Pathways in coffee against PD

We found several key signaling pathways in coffee that act against PD, such as the HIF-1 signaling pathway, the TNF signaling pathway, and insulin resistance, serotonergic synapse, and dopaminergic synapse

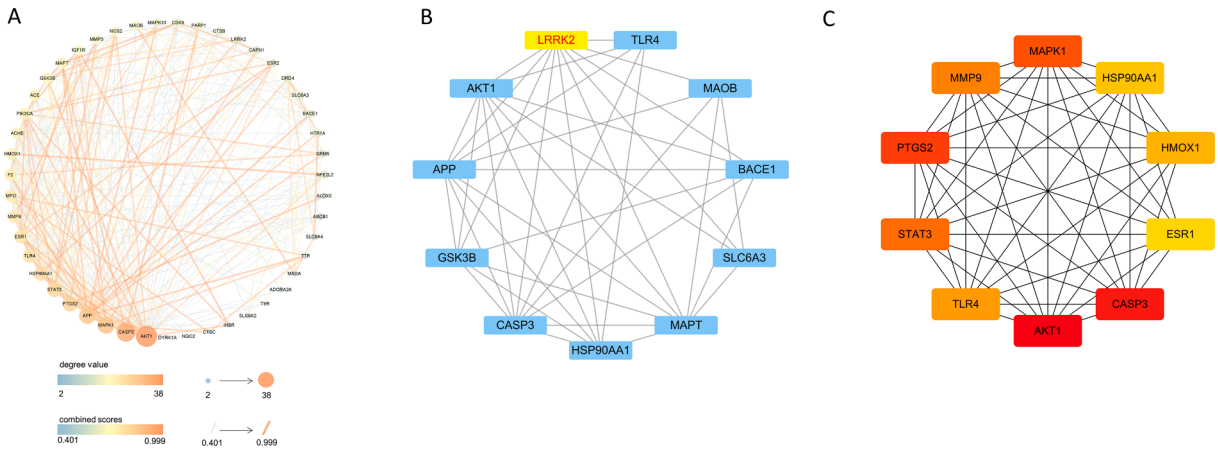

Fig. 6. (A) The PPI network of the key targets. (B) The PPI network centered on LRRK2. (C) Hub gene clusters generated by the MCC method.

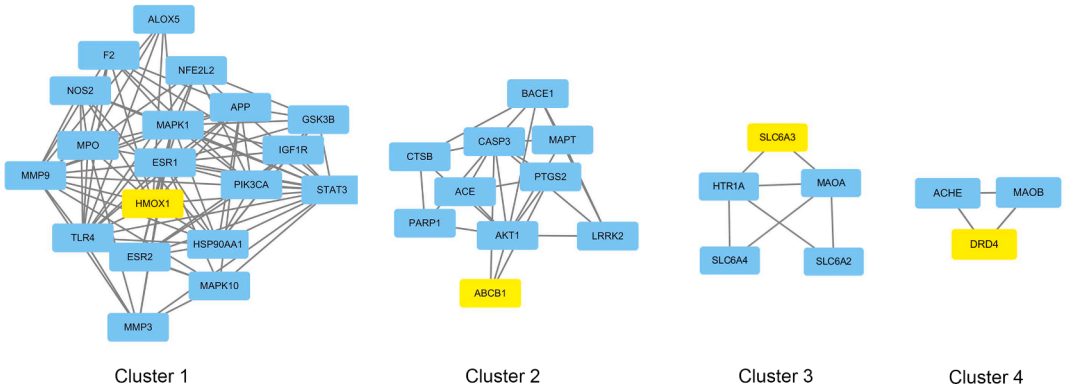

Fig. 7. Clusters of interacting proteins in coffee against PD identified using the MCODE algorithm. The seed nodes of each cluster are presented in yellow.

pathways. Experimental and clinical evidence has demonstrated that the activation of the HIF-1 signaling pathway in neurodegenerative disease conditions can upregulate specific HIF-regulated target genes, including neurotrophic factors and antioxidant enzymes, resulting in the induction of pro-survival cell signaling cascades (Z. Zhang et al., 2011). Another study found that a low concentration of coffee strongly induced vascular endothelial growth factor expression via the activation of HIF-1α, *in vitro*, which may promote the growth of dopaminergic neurons (Silverman et al., 1999). Increasing HIF-1 activity may be an important potential strategy for preventing the onset or ameliorating the pathogenesis of neurodegenerative diseases. The combined evidence from histopathologic, epidemiologic, and pharmacologic studies supports a role for TNF in eliciting dopaminergic neuron loss and nigrostriatal degeneration (Mccoy & Tansey, 2008). A recent clinical study showed that early exposure to anti-inflammatory anti-TNF therapy was associated with substantially reduced PD incidence in inflammatory bowel disease (Peter et al., 2018). A previous clinical trial showed that the long-term consumption of coffee could reduce fasting glucose levels and insulin resistance (Sarria et al., 2016). PD has a potential relationship with abnormal mitochondrial function, which could lead to the development of insulin resistance (Stark & Roden, 2007). Randomized trials have evaluated several established treatments for insulin resistance (pioglitazone and exenatide) as possible disease-modifying drugs in PD, which may be effective in both disease processes by acting on mitochondrial function (Aviles-Olmos et al., 2013). The dopaminergic synapse and serotonergic synapse are also important pathways targeted by coffee constituents in PD. In addition to the effects of the dopaminergic synapse on the motor system, the serotonergic nervous system has been associated with the pathophysiological basis of PD, especially in the modulation of extrapyramidal motor disorders, cognitive impairment, mood disorders, or psychosis, which are frequently observed symptoms in patients with PD. Clinical trials found a negative association between coffee consumption and the severity of the mood/cognition domain of non-motor symptoms in PD (Cho et al., 2018).

4.3. *Potential targets in coffee against PD*

Several potential core targets were identified by the PPI network and hub genes collected using the MCC algorithm, which were verified by molecular docking analysis. The majority of the identified targets showed great affinity with key active compounds found in coffee. The inflammatory response is generally associated with the activation of TLRs, which are key molecules in the regulation of the host immune response (Ahmad et al., 2013). The absence of TLR4 reduces the development of neuroinflammation through the nuclear factor (NF)-κB, activator protein (AP)-1, and inflammasome-mediated pathways in an *in vivo* PD model (Campolo et al., 2019). PTGS2, which is induced during the inflammatory response, was upregulated in the substantia nigra during a postmortem autopsy of PD patients (Knott et al., 2000). PTGS2 inhibitors can also reduce the destruction of dopaminergic neurons in animal models of PD (Teismann & Ferger, 2001). CASP3 plays a crucial function in the intrinsic and extrinsic pathways of programmed cell death and in cell proliferation (Louneva et al., 2008). CASP3-deficient dopaminergic neurons can be protected against cell death induced by oxidative stress (Kim et al., 2018). STAT3, when activated in inflamed microglia, drives dopaminergic neurons toward programmed cell death through the transcriptional activation of cell death-mediating genes (Tiwari & Pal, 2017). MMP9 knockout mice and mice treated with the MMP9 inhibitor GM6001 effectively maintained blood–brain barrier (BBB) integrity, promoted the elimination of damaged mitochondria via

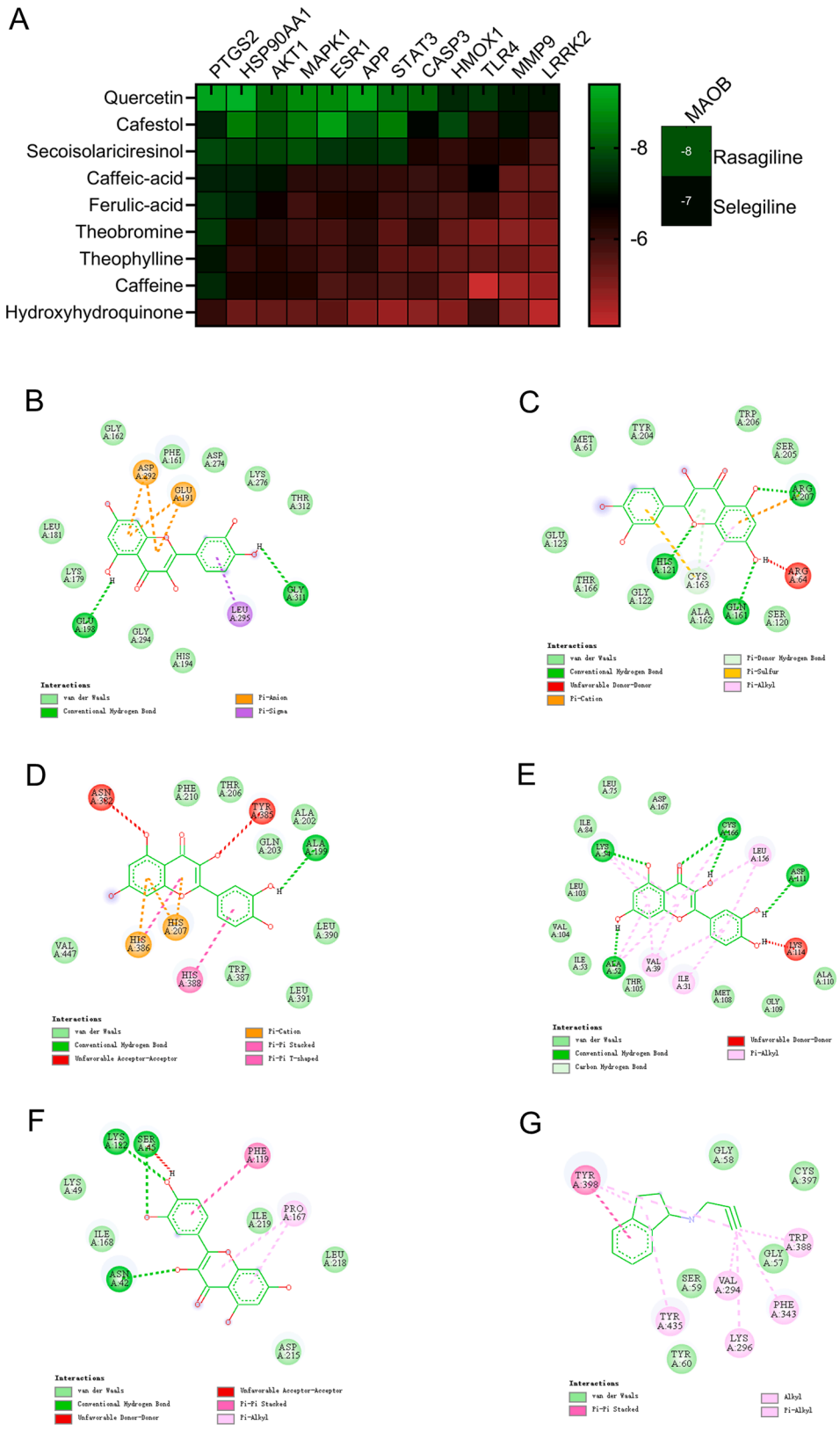

Fig. 8. (A) Volcano map of molecular docking scores. (B–F) Planar chart of molecular docking between quercetin and core genes. (B) AKT1–quercetin docking. (C) CASP3-quercetin docking. (D) PTGS2–quercetin docking. (E) MAPK1–quercetin docking. (F) LRRK2–quercetin. (G) Planar chart of molecular docking between MAOB and rasagiline.

mitophagy, and prevented the development of PD via the autophagy pathway (Lin et al., 2020). Another study found that the activity of robusta coffee could decrease the expression of C-reactive protein and MMP9 in response to hyperlipidemia (Prasetya et al., 2020). Combined with our study results, these findings suggested that coffee may protect the integrity of the BBB in PD. A recent study used a bioinformatics-based approach to identified the proteins that interact with α-synuclein. This study demonstrated that interactions between α-synuclein and Bcl-2-associated protein X (BAX) may play a crucial role in the cell death process of PD, where apoptosis and mitochondrial permeability transition-driven necrosis may coexist (Chakrabarti et al., 2020). LRRK2, α-synuclein, Parkin, PTEN-induced kinase 1 (PINK1), and protein deglycase DJ1 are the major drivers of PD. In our study, the core protein targets (such as AKT1, MAPK, CASP3, and STAT3) may play a role upstream or downstream of the major drivers, and participate in a variety of cellular reactions and pathological mechanisms, such as autophagy, apoptosis, oxidative stress, and inflammation. Furthermore, LRRK2 was revealed to be a direct interaction target of coffee against PD. This finding suggests that coffee may have a greater and more direct effect on PD among carriers of LRRK2 mutations.

4.4. The seeds of subnetworks identified through cluster analysis of coffee against PD

The results of the cluster analysis showed that HMOX1, ABCB1, DRD4, and SLC6A3 were the seeds of the significant modules identified by the PPI networks. Our previous study identified HMOX1 as a promising biomarker for PD (Hatano et al., 2016). HMOX1 transgenic mice develop a parkinsonian phenotype, characterized by neural oxidative stress, nigrostriatal hypodopaminergia associated with locomotor incoordination, and the overproduction of α-synuclein (Cressatti et al., 2019). Another study showed that kahweol could promote mitochondrial protection through the activation of the phosphoinositol 3-kinase (PI3K)/Akt and p38 MAPK/Nrf2/HMOX1 axis in H_2O_2-challenged SH-SY5Y cells (Fürstenau et al., 2019). Mitochondrial protection may play an important role in the molecular mechanism through which coffee protects against PD. DRD4 is involved in dopamine synthesis and release and neuronal firing. SLC6A3 encodes the transporter protein found within presynaptic neuronal membranes, which facilitates dopamine reuptake from the synaptic cleft (McGough, 2012). ABCB1 is an ATP-dependent, transmembrane efflux protein that is widely expressed on the luminal side of brain capillary endothelial cells in the BBB (Müller, 2018) and is involved in drug pharmacokinetics, affecting drug absorption, disposition, and elimination. Evidence suggests that coffee may interfere with dopaminergic transmission and amplify the effects of levodopa treatments (Tran et al., 2015). Our results indicated that coffee might regulate the transmission of dopamine through DRD4, SLC6A3, and ABCB1, to relieve motor symptoms.

The present study also has some limitations. First, the prediction results were obtained based on existing limited databases. Second, coffee compounds are very complex, and it is likely that some have not yet been identified, preventing their inclusion in the analysis. Further verification experiments remain necessary to explore the key mechanisms mediated by coffee.

5. Conclusion

On the basis of the principles of NP, this study systematically explored the synergistic effects and molecular mechanism exerted by coffee against PD. We found that coffee may play a beneficial role against PD through antioxidant, neuroprotective, and anti-inflammatory activity, mitochondrial protection, apoptosis inhibition, the regulation of multiple neurotransmitters, and the maintenance of BBB integrity. These results are consistent with the reported complexity of PD pathogenesis and involve many biological processes, providing a new perspective for scientific research on the multi-component and multi-target mechanisms through which coffee acts against PD, and providing new clues for experimental researchers to design new experiments and optimize resources.

CRediT authorship contribution statement

Fangjun Li: Methodology, Validation, Visualization, Writing – original draft. **Taku Hatano:** Investigation, Supervision, Software, Writing – review & editing. **Nobutaka Hattori:** .

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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