ACTA SCIENTIFIC NEUROLOGY (ISSN: 2582-1121)

Volume 7 Issue 12 December 2024

Research Article

Possible Mechanism of Antidepressant Action from *Phyllanthus emblica* Fruit using Pharmacological Network Analysis and Molecular Docking

Novi Ayuwardani[1,6], Zullies Ikawati[2]*, Nanang Fakhrudin[3,5] and Zaenal Muttaqien Sofro[4]

[1]*Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia*

[2]*Departement of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Gadjah Mada, Sekip Utara, Yogyakarta, Indonesia*

[3]*Departement of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Sekip Utara, Yogyakarta, Indonesia*

[4]*Departement of Physiology, Faculty of Medicine, Nursing, and Public Health, Univeristas Gadjah Mada, Sekip Utara, Yogyakarta, Indonesia*

[5]*Medicinal Plants and Natural Products Research Center, Faculty of Pharmacy, Universitas Gadjah Mada, Sekip Utara, Yogyakarta, Indonesia*

[6]*Departement of Pharmacy, STIKES Bhakti Husada Mulia, Madiun, Indonesia*

***Corresponding Author:** Zullies Ikawati, Departement of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Gadjah Mada, Sekip Utara, Yogyakarta, Indonesia.

DOI: 10.31080/ASNE.2024.07.0790

Received: November 04, 2024

Published: November 29, 2024

Abstract

The fruit of *Phyllanthus emblica*, also known as amla fruit, has been reported to have potential an antidepressant effects due to its high content of phenolic compounds. However, the mechanism underlying the antidepressant effect remains unclear. Therefore, this study aims to explore the mechanism of action of *P. emblica* fruit extract for antidepressant activity using network pharmacology and molecular docking. The compound components of the extract were obtained from untargeted metabolomic LC-HRMS, *P. emblica*-related targets, and depression therapeutic targets from an extensive database. Protein-protein interaction networks were constructed to screen the core targets using the STRING database and Cytoscape software. Metascape database was used for GO enrichment and KEGG analysis. Then, the top ten pathways were selected to construct a network analysis of compound components, disease proteins, and signaling pathways. In addition, molecular docking was used to explore and verify the interaction of compound components with core targets. Our study identified 9 compound components and 94 potential targets from *P. emblica* as antidepressants. The PPI network identified MAOA, COMT, SLC6A3, MAOB, DRD2, SLC6A4, and SNCA as possible targets, while GO enrichment and KEGG analysis showed that the relevant biological processes involved in the treatment of depression by *P. emblica* included synaptic signaling and behavior, and the molecular function included neurotransmitter receptor activity. The results of the KEGG pathway analysis identified serotonergic synapse and dopaminergic synapse signaling pathways associated with core targets. Gallic acid and ellagic acid components were selected to view the binding interaction with core targets related to molecular docking, which resulted in ellagic acid with strong binding affinity. Our study provides insight into the potential of *P. emblica* fruit for treating depression as a basis for further validation and exploration of experimental research.

Keywords: *Phyllanthus Emblica*; Depression; Network Pharmacology; Molecular Docking; Neurotransmitter Receptor Activity

Introduction

Depression is a condition of impaired emotional response of moderate to severe intensity that results in loss of pleasure, sadness, depressed mood, and loss of desire for activity for most of the day for at least two weeks [1]. Other symptoms of depression include poor concentration, excessive feelings of guilt or low self-esteem, hopelessness about the future, sleep disturbances, changes in appetite or weight, feeling very tired, and thoughts of death or suicide [1,2]. Depression affects nearly 5% of the adult population worldwide, and the number may be higher as not all people are diagnosed with depression. Depression is more common in women than in men. According to the severity of depressive symptoms experienced in the previous two weeks, depression is most common in people aged 18 to 29 (21%), then in people aged 45 to 64 (18.4%), people aged 65 and older (18.4%), and people aged 30 to 44 (16.8%) [1,3]. Adolescents with depression have more severe impairments in social and educational functioning and have an increased risk of smoking, substance abuse, obesity, and suicide compared to adults with depression [4,5].

The pathophysiology of depression is complex, including the presence of genetic factors and environmental factors, and involves different biological systems, namely the disruption of the monoamine system as the main hypothesis in the pathophysiology of depression, characterized by deficiencies in biogenic amine systems (especially serotonin and norepinephrine), neuroplasticity processes, and neurodegenerative processes, primarily through hypothalamic-pituitary-adrenal (HPA) alterations, inflammatory processes [6,7]. Selective serotonin reuptake inhibitors (SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), serotonin modulators, and atypical antidepressants are the second class of antidepressants used as first-line therapy. Antidepressants are the most commonly prescribed medications for adults between the ages of 18 and 60 [8]. In addition to antidepressants, psychotherapies such as behavioral therapy, cognitive therapy, cognitive-behavioral therapy, interpersonal psychotherapy, psychodynamic therapy, and supportive therapy are used to treat depression. The combination of antidepressants and psychotherapy is commonly used in severe depression and reduces the risk of relapse [9,10].

Increased research is needed on using herbal plants as an alternative treatment because herbal plants contain many phytochemical compounds that have pharmacological effects on treating various disease conditions. One of the herbal plants that has antidepressant potential is *P. emblica*. The fruits showed behavioral improvement, as indicated by a decreased in immobility in the FST (forced swim test) and TST (tail suspension tests) and monoamine oxidase (MAO) levels in mice [11,12]. *P. emblica* is a tree growing in India, Southeast Asia, China, Iran, and Pakistan. It has many medicinal benefits as it is rich in bioactive compounds [13,14]. Scientific evidence shows that ascorbic acid, tannins, polyphenols, fiber, minerals, proteins, and amino acids are compounds contained in *P. emblica* fruit, which are essential components in promoting health and are used as medicinal components [15,16].

The development of plant-based drugs as antidepressants can be done using a computational approach. It is of great interest because it uses an online database displaying compound components and different targets to obtain pharmacological networks [17,18]. Pharmacological networking is a new field of research that includes pharmacology and pharmacodynamics, and it allows us to elucidate the synergistic effects and underlying mechanisms of various compounds by evaluating the underlying multi-level interactions [19]. This study aimed to determine the therapeutic targets and investigate the potential mechanism of action of *P. emblica* fruit as a possible antidepressant herbal medicine, using multimodal computational analysis. Pharmacological networks were used to identify the major bioactive compounds of the amla plant, and the targets involved in depression. In addition, molecular docking analysis was used to explore the binding interactions between the bioactive compounds and the identified target proteins.

Materials and Methods

Preparation of *P. emblica* fruit extract

We obtained *P. emblica* fruit from the forest area of the Giringan hydroelectric power plant, Madiun district, Indonesia. The plant was identified by a botanist from the Department of Biology, Faculty of Pharmacy, Gadjah Mada University. The collected fruits were washed and cut into smaller parts to facilitate the drying process at room temperature until dried simplisia of *P. emblica* fruit was obtained. In addition, the dried simplisia was pulverized using a grinder until the powder was obtained. The *P. emblica* fruit powder was extracted using two different solvents, namely 90% ethanol solvent and water solvent. The fruit powder was macerated using 90% ethanol solvent until it was completely submerged for 24 hours. The maceration process was carried out for 2 x 24 hours.

The filtrate was extracted with 90% ethanol and evaporated in a rotary evaporator and water bath at 50 °C until thick. The aqueous extract of *P. emblica* fruit was obtained by boiling the powder with boiling water for 30 minutes. The obtained hot decoction was allowed to cool at room temperature and filtered twice with cloth and filter paper. The filtrate obtained was then evaporated, dried in a water bath at 50 °C, and lyophilized. Both extracts obtained were stored in a desiccator to avoid easy damage to the extracts.

Analysis and identification of phytochemical compound components with untargeted metabolomic profiles

Analysis and identification of phytochemical compound components with untargeted metabolomic profiles were performed by liquid chromatography-high resolution mass spectrometry (LC-HRMS) analysis, using analytical procedures previously performed to obtain more detailed metabolite characterization (20). Analyses were performed using liquid chromatography (Thermo Scientific™ Vanquish™ UHPLC Binary Pump) and Orbitrap high-resolution mass spectrometry (Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap™ High-Resolution Mass Spectrometer). Liquid chromatography was performed using a Thermo Scientific™ Accucore™ Phenyl-Hexyl analytical column 100 mm × 2.1 mm ID × 2.6 µm. The mobile phases used were water (LC-MS grade) containing 0.1% formic acid (A) and methanol (LC-MS grade) containing 0.1% formic acid (B) using the gradient technique at a flow rate of 0.3 mL/min. Metabolite analysis was performed using Compound Discoverer software (Thermo Scientific, Rockford, IL, USA) in total ion chromatograms (TIC) to identify the metabolite composition. The metabolite compounds were selected from both extracts by filtering from all compounds found with the most suitable compounds with MzCloud data, ChemSpider, MS2 for DDA for preferred ions, and matching mzCloud Best Match values.

Identification and analysis of protein targets related to compounds

Compounds identified in ethanol and aqueous extracts of *P. emblica* fruit using LC-HRMS were then searched for Simplified Molecular-Input Line-Entry System (SMILES) profiles using PubChem database(https://pubchem.ncbi.nlm.nih.gov/, accessed on July 19, 2024). The candidate compounds were screened for gastrointestinal (GI) absorption index and drug-like properties using Swiss ADME (http://www.swissadme.ch/, accessed on July 19, 2024). The GI absorption index screening is essential to determine the ability of molecules to be well-digested and absorbed in the stomach after oral administration. In contrast, the drug-like index is the probability of a compound molecule becoming an oral drug. Compounds with a probability greater than 0 were selected for evaluation of potential drug toxicity using the ProTox 3.0 database (https://tox.charite.de/protox3/?site=compound_input, accessed on July 20, 2024) and further analyzed using Swiss Target Prediction (http://www.swisstargetprediction.ch/, accessed on July 20, 2024) to predict compound interactions with the proteins targeted.

Identification and analysis of depression-related protein targets

Depression-related target analysis was performed using two databases, namely the GeneCards database (https://www.genecards.org/, accessed on July 19, 2024), which provides comprehensive information on human genes and is easy to use for predicting the relationship between human genes, diseases, variants, proteins, cells, and biological pathways [21]. For the second target analysis, we used the OMIM database (https://omim.org/, accessed on July 19, 2024), a comprehensive and reliable compendium of human gene and trait information that is openly accessible and regularly updated. In addition, Venn diagrams were used to intersect the plant compound and depression-associated proteins to predict the proteins associated with each other.

Protein-protein interaction (PPI) network analysis

The relationships between *P. emblica*-derived target proteins and depression were analyzed using the STRING (Search Tool for Retrieval of Interacting Genes/Proteins) database (https://string-db.org/, accessed July 21, 2024). STRING is a database that can integrate all known and predicted relationships between proteins, including physical and functional interactions [19]. Homo sapiens was used for organism selection in the STRING database study, and a high confidence score criterion of 0.70 was applied to ensure strong interactions. Next, the PPI network was analyzed using Cytoscape v3.10.2 software to obtain degree, betweenness centrality, and closeness centrality values. The degree value indicates the number of nodes in the network that directly interact with the node and is usually used to evaluate the importance of nodes in the network. Meanwhile, betweenness centrality and closeness centrality values are measures of the importance of a node in the network based on its ability to interact with other nodes or serve as a bridge to

connect information between different nodes [19,22]. The PPI network is selected based on boundary criteria through network topology analysis. The proteins that have a median value more significant than the specified degree value [5], BC value (0.01353), and CC value (0.321569) are selected. The clustering algorithm uses the ClusterOne plug-in with a p-value <0.05 rule to cluster proteins in the PPI network and the CytoHubba plug-in to search for protein core targets.

Data enrichment and pathway analysis

The potential protein targets were subjected to Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis using the Meta scape database (https://metascape.org/, accessed on July 29, 2024). The target data obtained from PPI were entered into the Meta scape database by restricting the species to "Homo sapiens" and setting a p cut-off value of 0.01 and a minimum overlap of three for enrichment analysis, then selecting GO Molecular Functions (MF), GO Biological Processes (BP), GO Cellular Components (CC), and KEGG pathway analysis. The results were then analyzed using bioinformatics software (https://www.bioinformatics.com.cn/en). The previous active compound potential target signaling pathway network was visualized using Cytoscape v3.10.2 software to establish the relationship between these elements and build a regulatory network.

Molecular docking

Molecular docking was used to analyze the relationship between the bioactive compound components of *P. emblica* and the core therapeutic targets in depression. The chemical structures of the compound components were obtained from PubChem and then imported into Marvin Sketch 5.2.5.1 software to get the PDB files. The chemical structures of the core therapeutic targets were obtained from the PDB database (http://www.rcsb.org/, accessed on August 22, 2024) based on the following criteria: the scientific name of the source organism was Homo sapiens; the refined resolution was more significant than 2.5 A, and there were no mutations. Discovery Studio 2021 software was used to remove water molecules and separate ligands from proteins. Auto Dock Tool 1.5.7 software was used to hydrogenate, add charge, and incorporate non-polar hydrogen and parameter grid box settings on the protein and ligand, then saved as PDBQT files. Auto Dock Vina v1.2.5 was used to determine molecular docking simulations on proteins and bioactive compounds as ligands using Windows Terminal.

Results and Discussion

Phytochemical compounds of *P. emblica* fruit extract with untargeted metabolomic profile

The flowchart for this investigation is displayed in figure 1. The phytochemical compounds from the ethanol and water extracts of *P. emblica* fruit, obtained through LC-HRMS analysis with an untargeted metabolomic profile, were found to be 144 in the ethanol extract and 40 in the water extract. Filtering the compounds found with the most suitable compounds with MzCloud data, ChemSpider, MS2 for DDA for preferred ions, and mzCloud Best Match values >90, then confirmed on PubChem and ChemSpider. Thus, nine compounds in ethanol extracts and six compounds in water extracts were obtained, as presented in table 1.

Identifying with untargeted metabolomic profiles using LC-HRMS analysis, 12 compounds that meet the predetermined criteria were obtained, including gallic acid, ascorbic acid, d-glucosamine, 2-furoic acid, 3-indoleacrylic acid, ellagic acid, hexadecanamide, quercetin, myricitrin, ar-turmerone, myricetin, and dl-tryptophan. Gallic acid, ellagic acid, quercetin, and ascorbic acid are the secondary metabolites found in *P. emblica* fruit in both ethanol and aqueous extracts, so the identification of compound components by LC-HRMS is based on previous studies [16,23-27]. *P. emblica* fruit's high antioxidant content is one of its finest features. Compared to flavonoids and tannins, it contains more polyphenolic chemicals and vitamin C [13,16,27,28].

Screening of compound-associated protein targets

The compound-associated protein target screening was performed using the Swiss ADME tool to filter compounds based on high gastrointestinal (GI) absorption and drug-like properties. GI absorption and bioavailability criteria are critical because they ensure efficient absorption and delivery in the body for effective oral therapy. Drug-likeness indicates that the compound

No	Compound	Compound Chemical Formula	*Molecular Weight* (MV)	Retention Times (RT) (min)	Peak Area
Ethanol 90% extract					
1	Gallic acid	C7 H6 O5	170.020	1.268	562083848.217
2	D-Glucosamine	C6 H13 N O5	179.079	0.84	111996444.437
3	2-Furoic acid	C5 H4 O3	112.016	1.654	106855234.389
4	Hexadecanamide	C16 H33 N O	255.256	14.477	74467480.285
5	Ellagic acid	C14 H6 O8	302.006	4.448	65723218.775
6	Quercetin	C15 H10 O7	302.043	7.64	49124774.944
7	Myricitrin	C21 H20 O12	464.096	5.706	46441097.049
8	Ar-Turmerone	C15 H20 O	216.151	13.013	42841562.845
9	Myricetin	C15 H10 O8	318.038	6.635	7619813.315
Water extract					
1	Gallic acid	C7 H6 O5	170.022	0.901	844991145.139
2	Ascorbic acid	C6 H8 O6	176.032	0.896	587281098.169
3	3-Indoleacrylic acid	C11 H9 N O2	187.063	2.371	102543719.575
4	Ellagic acid	C14 H6 O8	302.006	4.483	84303599.417
5	2-Furoic acid	C5 H4 O3	112.0163	1.632	13247134.26
6	DL-Tryptophan	C11 H12 N2 O2	204.089	2.372	6419228.728

Table 1: Compounds identified in the *P. emblica* extract using LC-HRMS method.

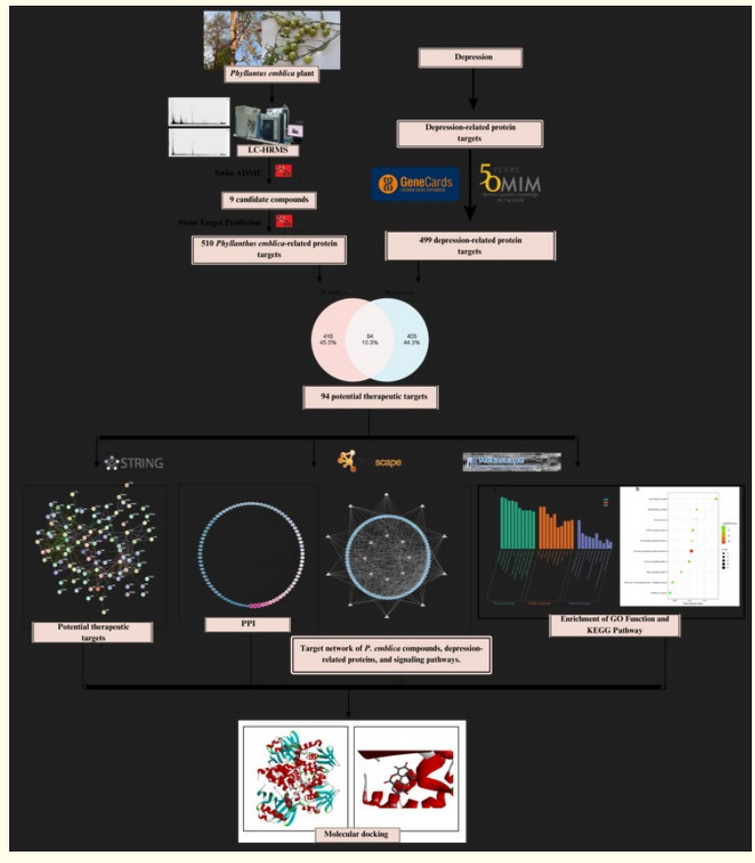

Figure 1: A flowchart of network pharmacology is employed to investigate the probable molecular mechanisms of antidepressant from *P. emblica* fruit.

has properties and potency similar to drugs. Toxicity prediction uses the ProTox 3.0 database to determine each compound's predicted LD50 and toxicity class; the lower the value, the more toxic the compound. We excluded d-glucosamine, myricitrin, and myricetin because they have GI absorption and low bioavailability, and d-glucosamine has a class 2 toxicity, indicating it could be fatal if swallowed [29], presented in table 2. We identified nine potential bioactive compounds for further protein target analysis using Swiss Target Prediction. The protein targets obtained were 785 protein targets from related compounds, then we removed duplicates and recombined, resulting in 510 protein targets related to the compound.

Compound	GI Absorption	Druglikeness	BBB Permeant	Water solubility	Computational Prediction of Toxicity	
					Predicted LD50 (mg/kg)	Toxicity Class
Gallic acid	High	Yes	No	Very soluble	2000	4
Ascorbic acid	High	Yes	No	Highly soluble	3367	5
D-Glucosamine	Low	Yes	No	Highly soluble	10000	2
2-Furoic acid	High	Yes	Yes	Very soluble	592	3
3-Indoleacrylic acid	High	Yes	Yes	Soluble	2500	5
Ellagic acid	High	Yes	No	Soluble	2991	4
Hexadecanamide	High	Yes	Yes	Moderately soluble	1000	4
Quercetin	High	Yes	No	Soluble	159	3
Myricitrin	Low	Yes	No	Soluble	5000	5
ar-Turmerone	High	Yes	Yes	Soluble	2000	4
Myricetin	Low	Yes	No	Soluble	159	3
DL-Tryptophan	High	Yes	No	Very soluble	80	3

Table 2: ADME and toxicity information in of *P. emblica* compound.

Screening of depression-related protein targets

The protein target search used the GeneCards and OMIM databases, with "depression" as the search keyword. A total of 548 protein targets were obtained, consisting of 358 protein targets from the Gene Cards database and 190 protein targets from the OMIM database. Next, we removed duplicate protein targets from both search tools, resulting in 499 proteins related to depression. Based on the protein targets associated with *P. emblica* and depression, 94 targets were identified and considered as potential therapeutic targets of *P. emblica* used in depression therapy, as shown in the Venn diagram.

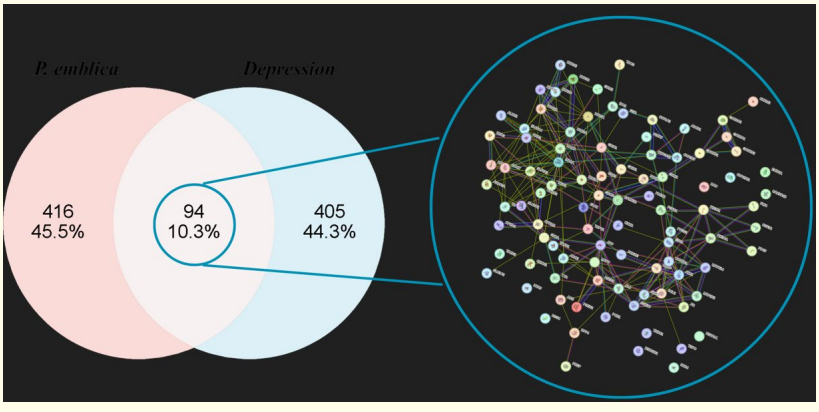

Figure 2: Venn diagram of protein targets related to *P. emblica* and depression.

PPI network construction

Potential therapeutic protein targets were visualized using the STRING database to analyze the relationship between 94 protein targets and PPI network analysis using Cytoscape v3.10.2 software. The results of PPI network visualization obtained 94 nodes, 250 edges, and an average node degree of 5.32, presented in Figure 2. Nodes are single proteins and edges are interactions between proteins. PPI network analysis obtained degree, betweenness centrality, and closeness centrality values. The greater the degree, betweenness centrality, and closeness centrality values, the more significant the role of a protein in a network. Based on the network topology results, we got 33 protein target proteins and we used the Cluster One plugin, which generated 4 clusters with different biological functions from the protein target interaction. We selected clusters (p-value = 0.001) that have a close biological function between *P. emblica* and depression. Additionally, we used the CytoHubba plug-in to identify the core proteins that have the highest ranking score on maximal clique centrality (MCC), resulting in seven core protein targets from the built pharmacology network and the network topology analysis results. These targets are MAOA, COMT, MAOB, SLC6A3, SLC6A4, DRD2, and SNCA, which are considered essential targets of *P. emblica* against depression presented in figure 3.

These targets are associated with multiple components in *P. emblica*, describing traditional herbal medicine's multi-target and multi-component characteristics. The identification of MAOA and MAOB indicates that monoamine oxidase (MAO) is a potential biomarker in depression, contributing to the metabolism of monoamine transmitters (serotonin, norepinephrine, and dopamine). A special enzyme called flavin A-containing amine oxidase helps break down biogenic amines and xenobiotics. It is very important for the metabolism of biogenic amines like 5-hydroxytryptamine (5-HT), norepinephrine, and epinephrine in the brain and other tissues, and these amines are linked to depression [30-32]. In contrast, MAOB preferentially metabolizes phenylethylene and benzylamine [32]. The enzyme catechol-O-methyltransferase (COMT) breaks down about 60% of dopamine. It does this in the prefrontal cortex (PFC) by turning dopamine into metabolites that are not active in the body, and it is one of the gene variants linked to depression [33,34]. Sodium-dependent serotonin transporter (SLC6A4) plays a role in maintaining serotonin levels in the presynaptic region; sodium-dependent dopamine transporter (SLC6A3); D (2) dopamine receptor (DRD2); and alpha-synuclein (SNCA) play a role in the severity of depressive symptoms and towards suicidal behavior. Thus, all are associated with depression shown by increased gene expression [35-38].

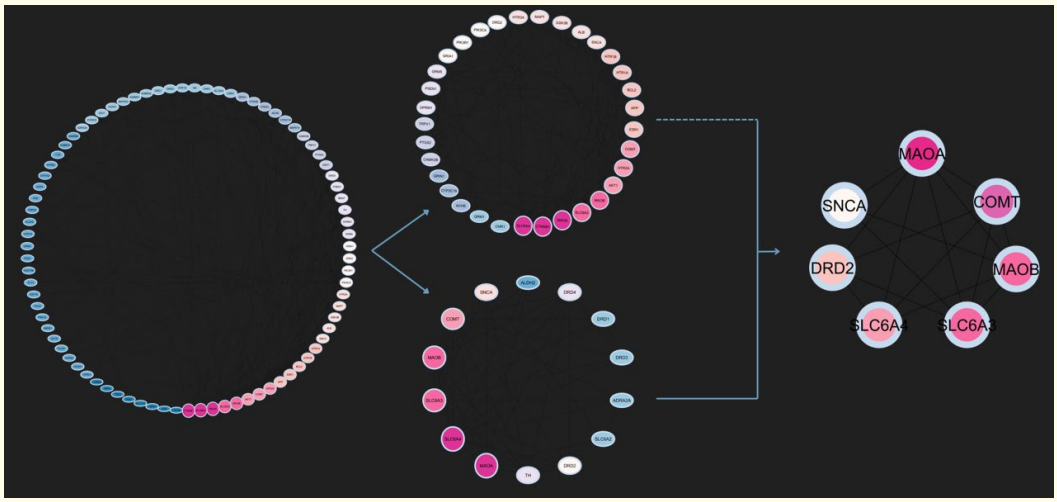

Figure 3: PPI Network Visualization with network topology analysis, ClusterOne plug-in, and CytoHubba plug-in.

GO function and KEGG pathway enrichment

GO function enrichment was obtained from the Metascape database, a total of 1457 with details of 1202 BP, 97 CC, and 158 MF. The GO function enrichment of the active ingredient components of *P. emblica* in depression, the results of BP items include synaptic signaling, trans-synaptic signaling, chemical synaptic transmission, anterograde trans-synaptic signaling, behavior, cellular response to nitrogen compounds, modulation of chemical synaptic transmission, regulation of trans-synaptic signaling, cellular response to organonitrogen compounds, and regulation of monoatomic ion transport. The enrichment results of CC items include dendrite, dendritic tree, presynapse, synaptic membrane, postsynapse, presynaptic membrane, postsynaptic membrane, receptor complex, cell body, and axon. The enrichment results of MF items include neurotransmitter receptor activity, G protein-coupled amine receptor activity, G protein-coupled serotonin receptor activity, amine binding, serotonin binding, postsynaptic neurotransmitter receptor activity, catecholamine binding, dopamine binding, extracellular ligand-gated monoatomic ion channel activity, and G protein alpha subunit binding. The KEGG pathway enrichment results showed 153 signaling pathways, then we took the top 10 signaling pathways visualized in the bubble plot, and the color depth indicated the p-value. The size of the bubble plot indicated the number of genes involved in each pathway. The obtained signaling pathways associated with depression include serotonergic synapses, cAMP signaling pathway, calcium signaling pathway, neuroactive ligand-receptor interaction in dopaminergic synapses, neurotrophin signaling pathway, and RAP1 signaling pathway. In contrast, the signaling pathways in the pathways of neurodegeneration-multiple diseases, cancer, and prostate cancer are not associated with depression.

A target network of compound components, disease proteins, and signaling pathways from KEGG was previously created to show the complex relationship between *P. emblica* and depression. The network visualization results obtained 97 nodes and 549 edges, presented in figure 4. Blue round shapes indicate protein targets, pink diamond shapes indicate *P. emblica* compound components and yellow triangular shapes indicate signaling pathways. Ar-Turmerone has one possible target related to depression (DRD2), DL-tryptophan has three possible targets related (SLC6A3, SLC6A4, COMT), hexadecanamide has three possible targets related (DRD2, SLC6A4, SNCA), ellagic acid has four possible targets related (MAOA, SNCA, MAOB, SLC6A3), gallic acid has four potential targets related (COMT, MAOB, MAOA, DRD2), quercetin has one possible target related (MAOA), and ascorbic acid and 3-indoleacrylic acid do not. The target network of compound components, disease proteins, and signaling pathways revealed that *P. emblica* considers ellagic and gallic acid compounds important due to their availability in 90% ethanol and aqueous extracts. These compounds have four potential depression-related targets: MAOA, MAOB, SNCA, SLC6A3, COMT, and DRD2. In this study, the neuroactive ligand-receptor interaction signaling pathway is essential because it has the highest degree value [32]. It includes the serotonergic synapse and dopaminergic synapse signaling pathways, which are specific pathways that interact with depression-related protein targets. Both pathways are part of how depression works in the body [39,40].

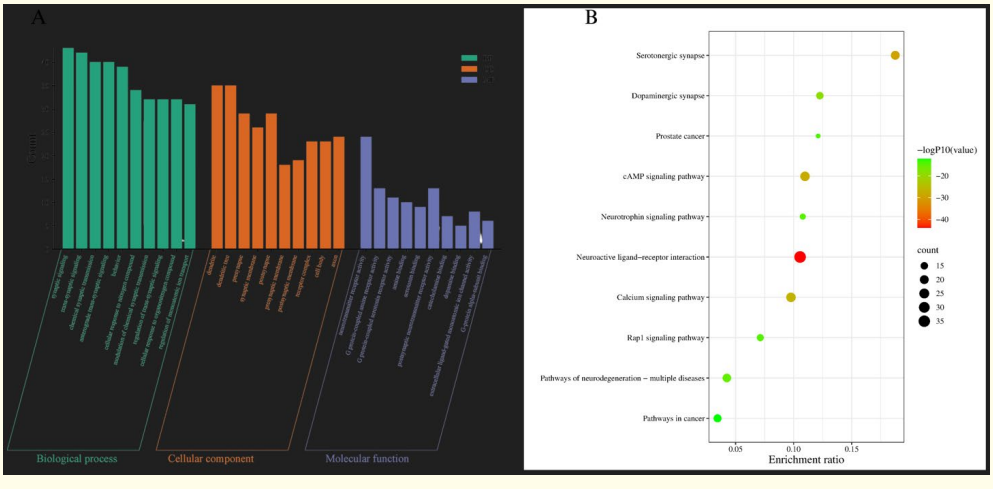

Figure 4: GO function enrichment pathway analysis of *P. emblica* in the depression. (A) GO function enrichment analysis, including biological process (BP), cellular component (CC), and molecular function (MF). (B) Bubble plot of KEGG pathway enrichment.

Based on the results of the target network of compound components, disease proteins and pathways from KEGG, gallic acid and ellagic acid were found to be the most crucial compound components as antidepressants. Previous experimental studies have indicated that gallic acid is the primary component in *P. emblica* fruit extract [26,41]. Many researchers have previously reported the neuroprotective role of phenolic acid compounds, such as gallic acid and ellagic acid [42,43]. Previous studies have reported that gallic acid exhibits antidepressant activity by reducing MAOA, reducing corticosterone, reducing oxidative stress, increasing neurotransmitters, and improving depressive-like behavior in experimental animals [42,44,45]. Researchers have reported that ellagic acid exhibits antidepressant effects through antioxidant and anti-inflammatory activities, which reduce neuroinflammation in the hippocampus and enhance depressive-like behavior in experimental animals [48]. The KEGG pathway contains multiple protein targets, including the "dopaminergic synapse" pathway, which targets MAOA, MAOB, SLC6A3, and DRD2, and the "serotonergic synapse" pathway, which targets MAOA, MAOB, and SLC6A4. Therefore, the path represents a potential mechanism of action for *P. emblica* extract as a antidepression activity, targeting proteins such as MAOA, MAOB, SLC6A3, SLC6A4, COMT, and DRD2. The pathology of depression, which involves neurotransmitter imbalances in the modulation of emotions, motor activity, and cognitive function, closely links to MAO. Toledo-Lozano et al.'s research also shows that MAOA and MAOB are linked to more severe depression, either on their own or because of bad experiences in childhood and genetic predisposition to certain genes. SLC6A3 and DRD2 are also associated with depressive symptoms at low dopamine levels [47,48].

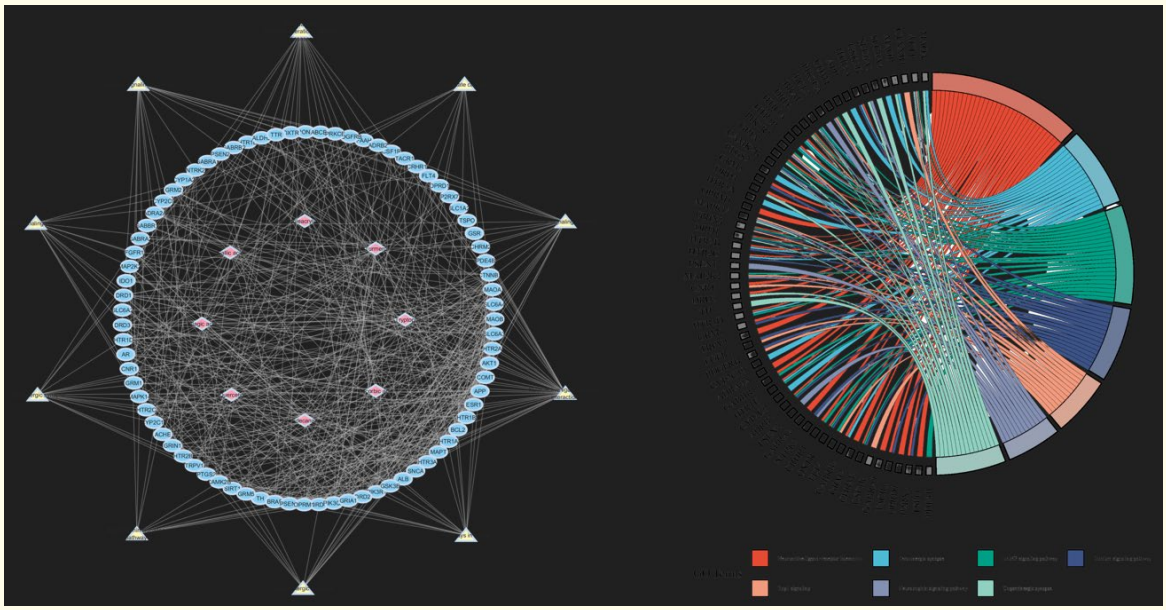

Figure 5: KEGG pathway enrichment analysis of *P. emblica* in the depression. (A) Target network of P. emblica compound components, depression-related proteins, and signaling pathways. (B) Gene ontology cord of potential targets and depression-related signaling pathways.

Molecular docking

Molecular docking was used to determine the binding probability between the bioactive compound components of *P. emblica* and the core target of depression therapy, using AutoDock Vina. Previous literature proved that binding affinity values < -4.25 kcal/mol indicate that both molecules have standard binding ability, < -5.0 kcal/mol means good binding, while < -7.0 kcal/mol indicates strong binding activity [49,50]. This study paired six core targets in serotonergic synapse and dopaminergic synapse signaling pathways (MAO, MAOB, DRD2, SLC6A3, SLC6A4, and COMT) with two components of *P. Emblica* bioactive compounds in table 3. The binding affinity showed the best strong binding

between ellagic acid and MAOA, MAOB, and DRD2 (< -9.0 kcal/mol). The docking results of ellagic acid and three core targets were refined by exploring the specific binding and spatial distance using Discovery Studio 2021 software. The results showed that in MAOA, ellagic acid had a hydrogen bond with amino acid residue THR204; the distance was 4.25 Å. In MAOB, ellagic acid and amino acid residue SER59 (4.86 Å) had a hydrogen bond. In DRD2, ellagic acid has two hydrogen bonds with amino acid residues SER193 (4.20 Å) and TYR416 (4.48 Å), available in figure 6.

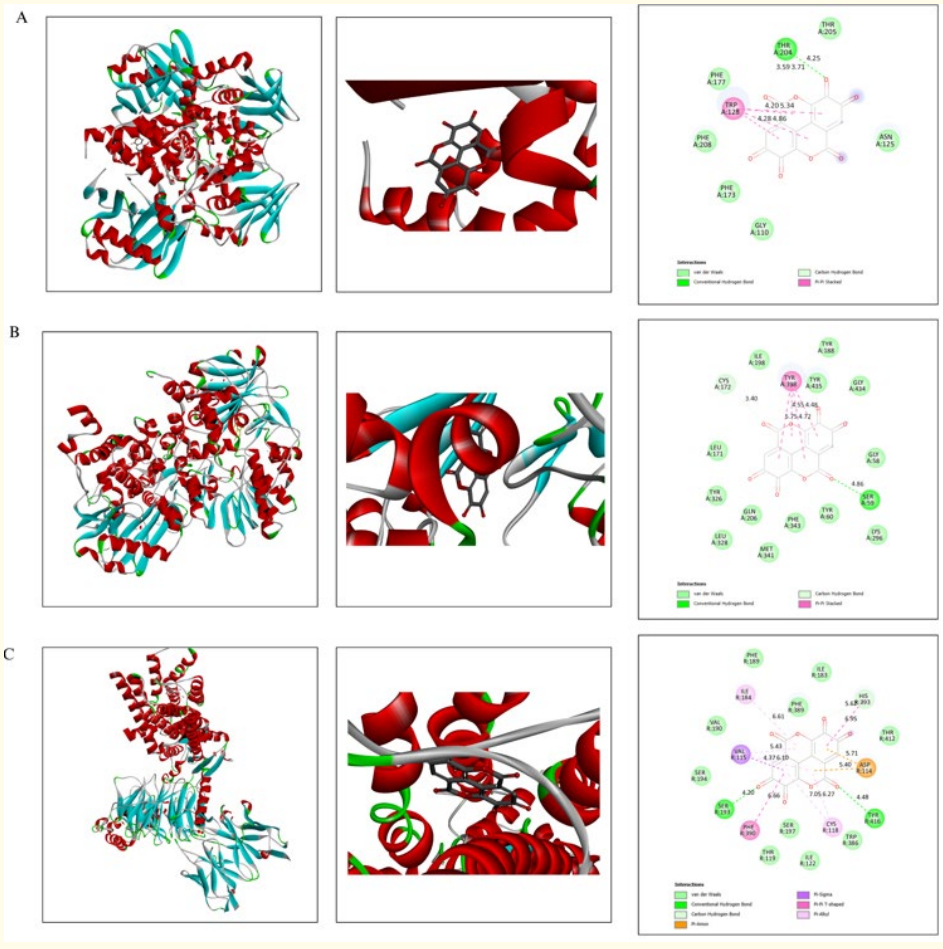

Figure 6: The molecular docking of ellagic acid and core targets. (A) ellagic acid docking with MAOA. (B) ellagic acid docking with MAOB. (C) ellagic acid docking with DRD2.

Target	PDB ID	Original ligand	Binding affinity with active compound (kcal/mol)		
			Original ligand	Gallic acid	Ellagic acid
MAOA	2BXR	FAD, MLG	-8.796	-6.818	-9.109
MAOB	1GOS	FAD, NYP	-8.818	-7.303	-9.619
DRD2	8IRS	R5F	-8.446	-6.631	-10.02
SLC6A3	8Y2C	NAG	-4.349	-4.73	-6.433
SLC6A4	7TXT	KWC	-8.903	-6.616	-8.917
COMT	3A7E	DNC, MG, SAM	-6.419	-7.628	-7.629

Table 3: Results of molecular docking between bioactive compounds and core targets.

Conclusion

Based on the network pharmacology and molecular docking results in this study, it provides essential insights into the potential antidepressant mechanism of *P. emblica*, particularly its ability to interact with several core targets through dopaminergic and serotonergic synapse pathways that affect neurotransmitters. However, this study still faces a limitation: the disease progression is influenced by pathological processes. The antidepressant activity of *P. emblica* fruit extract needs to be considered in the multiple doses tested in experimental animal studies. In addition, since this study was based on database analysis and computational approaches, there were no experimental findings to validate our results. Therefore, we need to conduct experimental studies in vitro and in vivo to determine the involvement of neurotransmitter activity (serotonin, dopamine, and norepinephrine) and the impact on depressive-like behavior to validate the mechanism of action.

Acknowledgements

The author would like to thank the Faculty of Pharmacy, Gadjah Mada University, Yogyakarta, for providing facilities and support.

Conflict of interest

The authors declare no conflict of interest.

Bibliography

1. WHO. World Health Organization. Depressive Disorder (Depression) (2023).
2. Brigitta B. "Pathophysiology of depression and mechanisms of treatment". *Dialogues in Clinical Neuroscience* 4.1 (2002): 7-20.
3. Villarroel MA and Terlizzi EP. "Symptoms of Depression Among Adults: United States, 2019". *NCHS Data Brief* 379 (2020): 1-8.
4. Zhou X., *et al*. "Comparative efficacy and acceptability of antidepressants, psychotherapies, and their combination for acute treatment of children and adolescents with depressive disorder: a systematic review and network meta-analysis". *The Lancet Psychiatry* 7.7 (2020): 581-601.
5. Thapar A., *et al*. "Depression in adolescence". *Lancet* 379.9820 (2012): 1056-1067.
6. Ramaholimihaso T., *et al*. "Curcumin in Depression: Potential Mechanisms of Action and Current Evidence-A Narrative Review". *Frontiers in Psychiatry* 11 (2020): 572533.
7. Zunszain PA., *et al*. "Glucocorticoids, cytokines and brain abnormalities in depression". *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 35.3 (2011): 722.
8. Brody DJ and Gu Q. "Antidepressant Use Among Adults: United States, 2015-2018". *NCHS Data Brief* 377 (2020): 1-8.
9. Kovich H., *et al*. "Pharmacologic Treatment of Depression". *American Family Physician* 107.2 (2023): 173-181.
10. Qaseem A., *et al*. "Nonpharmacologic versus pharmacologic treatment of adult patients with major depressive disorder: A clinical practice guideline from the American College of Physicians". *Annals of Internal Medicine* 164.5 (2016): 350-359.
11. Dhingra D., *et al*. "Possible Involvement of Monoaminergic Neurotransmission in Antidepressant-like activity of *Emblica officinalis* Fruits in Mice". *CNS Neuroscience and Therapeutics* 18.5 (2012): 419-425.
12. B.Dattatray P., *et al*. "Antidepressant Activity Of Aqueous Extracts Of Fruits Of *Terminalia Chebula* and *Phyllanthus Emblica* In Behavioural Models Of Depression: Involvement Of Monoaminergic System". *International Journal of Pharmacy and Pharmaceutical Sciences*.8 (2014): 615-620.
13. Gul M., *et al*. "Functional and Nutraceutical Significance of Amla (*Phyllanthus emblica* L.): A Review". *Antioxidants (Basel)* 11.5 (2022): 816.
14. Ikram A., *et al*. "Nutritional and Biochemical Composition of Amla (*Emblica officinalis*) and its Therapeutic Impact: A Review". *Acta Scientific Nutritional Health* 5.2 (2021): 153-160.
15. Kapoor MP., *et al*. "Clinical evaluation of *Emblica officinalis* Gatertn (Amla) in healthy human subjects: Health benefits and safety results from a randomized, double-blind, crossover placebo-controlled study". *Contemporary Clinical Trials Communications* 17 (2020): 100499.
16. Yan X., *et al*. "Current advances on the phytochemical composition, pharmacologic effects, toxicology, and product development of *Phyllanthi Fructus"*. *Frontiers in Pharmacology* 13 (2022): 1017268.
17. Tallei TE., *et al*. "Molecular insights into the anti-inflammatory activity of fermented pineapple juice using multimodal computational studies". *Archiv der Pharmazie (Weinheim)* 357.1 (2024).

18. Ali A., *et al*. "Biochemical and Computational Assessment of Acute Phase Proteins in Dairy Cows Affected with Subclinical Mastitis". *Current Issues in Molecular Biology* 45.7 (2023): 5317.

19. Hu M., *et al*. "Use of network pharmacology and molecular docking to explore the mechanism of action of curcuma in the treatment of osteosarcoma". *Scientific Reports* 13.1 (2023): 1-13.

20. Windarsih A., *et al*. "Untargeted metabolomics and proteomics approach using liquid chromatography-Orbitrap high resolution mass spectrometry to detect pork adulteration in Pangasius hypopthalmus meat". *Food Chemistry* 386 (2021): 132856.

21. Stelzer G., *et al*. "The GeneCards Suite: From Gene Data Mining to Disease Genome Sequence Analyses". *Current Protocols in Bioinformatics* 54 (2016): 1.30.1-1.30.33.

22. Permatasari HK., *et al*. "Novel Functional Food Properties of Forest Onion (*Eleutherine bulbosa* Merr.) Phytochemicals for Treating Metabolic Syndrome: New Insights from a Combined Computational and In Vitro Approach". *Nutrients* 16.10 (2024): 1441.

23. Saini R., *et al*. "Traditional uses, bioactive composition, pharmacology, and toxicology of *Phyllanthus emblica* fruits: A comprehensive review". *Journal of Ethnopharmacology* 282 (2022): 114570.

24. Sapkota BK., *et al*. "Phytochemical Analysis and Antioxidant and Antidiabetic Activities of Extracts from *Bergenia ciliata*, *Mimosa pudica*, and *Phyllanthus emblica"*. *Advances in Pharmacological and Pharmaceutical Sciences* (2022): 4929824.

25. Rose K., *et al*. "Phenolic compounds isolated and identified from amla (*Phyllanthus emblica*) juice powder and their antioxidant and neuroprotective activities". *Natural Product Communications* 13.10 (2018): 1309-1311.

26. Li W., *et al*. "HPLC fingerprint analysis of *Phyllanthus emblica* ethanol extract and their antioxidant and anti-inflammatory properties". *Journal of Ethnopharmacology* 254 (2020): 112740.

27. Jhaumeer Laulloo S., *et al*. "Phytochemical Screening and Antioxidant Properties of *Phyllanthus emblica* from Mauritius". *Chemistry of Natural Compounds* 54.1 (2018): 50-55.

28. Chahal AK., *et al*. "Bioactive constituents of *Emblica officinalis* overcome oxidative stress in mammalian cells by inhibiting hyperoxidation of peroxiredoxins". *Journal of Food Biochemistry* 44.2 (2020): e13115.

29. Banerjee P., *et al*. "ProTox-II: A webserver for the prediction of toxicity of chemicals". *Nucleic Acids Research* 46.W1 (2018): W257-W263.

30. Sun X., et al. "The MAOA Gene Influences the Neural Response to Psychosocial Stress in the Human Brain". *Frontiers in Behavioral Neuroscience* (2010): 1-10.

31. Chappell K., *et al*. "The MAOA rs979605 Genetic Polymorphism Is Differentially Associated with Clinical Improvement Following Antidepressant Treatment between Male and Female Depressed Patients". *International Journal of Molecular Sciences* 24.1 (2023): 497.

32. Uzbekov MG. "Monoamine Oxidase as a Potential Biomarker of the Efficacy of Treatment of Mental Disorders". *Biochemstry* 86.6 (2021): 773-783.

33. Tosato S., *et al*. "COMT but Not 5HTTLPR Gene Is Associated with Depression in First-Episode Psychosis: The Role of Stressful Life Events". *Genes (Basel)* 14.2 (2023).

34. Karoum F., *et al*. "3-Methoxytyramine Is the Major Metabolite of Released Dopamine in the Rat Frontal Cortex: Reassessment of the Effects of Antipsychotics on the Dynamics of Dopamine Release and Metabolism in the Frontal Cortex, Nucleus Accumbens, and Striatum by a Simple T". *Journal of Neurochemistry* 63.3 (1994): 972-979.

35. Rafikova E., *et al*. "SLC6A3 (DAT1) as a Novel Candidate Biomarker Gene for Suicidal Behavior". *Genes (Basel)* 12.6 (2021): 861.

36. Shadrina M., *et al*. "Genetics factors in major depression disease". *Frontiers in Psychiatry* 9 (2018): 382604.

37. Cabana-Domínguez J., *et al*. "Comprehensive exploration of the genetic contribution of the dopaminergic and serotonergic pathways to psychiatric disorders". *Translational Psychiatry* 12.1 (2021): 1-10.

38. Gibbons A., *et al*. "An investigation into nicotinic receptor involvement in mood disorders uncovers novel depression candidate genes". *Journal of Affective Disorders* 288 (2021): 154-160.

39. Sun Y., *et al*. "Dissecting the molecular mechanisms underlying the antidepressant activities of herbal medicines through the comprehensive review of the recent literatures". *Frontiers in Psychiatry* 13 (2022): 2924.

40. Galts CPC., *et al*. "Depression in neurodegenerative diseases: Common mechanisms and current treatment options". *Neuroscience and Biobehavioral Reviews* 102 (2019): 56-84.

41. Yang F., *et al*. "Chemical constituents from the fruits of *Phyllanthus emblica* L". *Biochemical Systematics and Ecology* 92 (2020): 104122.

42. Cordeiro ML da S., *et al*. "Phenolic Acids as Antidepressant Agents". *Nutrients* 14.20 (2022): 4309.

43. Szwajgier D., *et al*. "The Neuroprotective Effects of Phenolic Acids : Molecular Mechanism of Action". *Nutrients* 9.5 (2017): 1-21.

44. Nabavi SF., *et al*. "Post-Stroke Depression Modulation and *in Vivo* Antioxidant Activity of Gallic Acid and Its Synthetic Derivatives in a Murine Model System". *Nutrients* 8.5 (2016): 248.

45. Moghadas M., *et al*. "Histopathological Analysis from Gallic Acid Administration on Hippocampal Cell Density , Depression , and Anxiety Related Behaviors in A Trimethyltin Intoxication Model". *Cell Journal* 17.4 (2016): 659-667.

46. Mazrooei Z., *et al*. "Ellagic acid through attenuation of neuro-inflammatory response exerted antidepressant-like effects in socially isolated mice". *Heliyon* 9.4 (2023): e15550.

47. Ludmer JA., *et al*. "DRD2 and SLC6A3 moderate impact of maternal depressive symptoms on infant cortisol". *Psychoneuroendocrinology* 62 (2015): 243-251.

48. Toledo-Lozano CG., *et al*. "Individual and Combined Effect of MAO-A/MAO-B Gene Variants and Adverse Childhood Experiences on the Severity of Major Depressive Disorder". *Behaviour Science (Basel)* 13.10 (2023): 1-12.

49. Saikia S and Bordoloi M. "Molecular Docking: Challenges, Advances and its Use in Drug Discovery Perspective". *Current Drug Targets* 20.5 (2018): 501-521.

50. Xiang C., *et al*. "Network Pharmacology and Molecular Docking to Elucidate the Potential Mechanism of Ligusticum Chuanxiong Against Osteoarthritis". *Frontiers in Pharmacology* 13 (2022): 1-13.