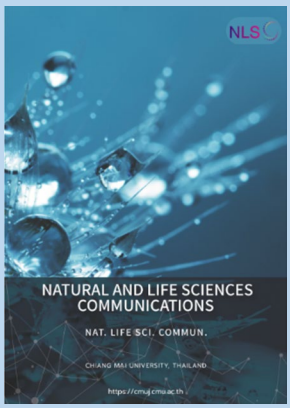
Research article



Active Compounds and Potential Actions of Anti-aging Remedy in Mild Cognitive Impairment Based on Network Pharmacology

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ABSTRACT

Mild cognitive impairment (MCI) may occurs during the aging process and often progress to dementia. This study investigates an anti-aging remedy (AAR) from Thai traditional medicine, which is known for promoting lifespan, by exploring its interactions with biological systems using network pharmacology. Natural compounds related to AAR were retrieved from databases (NPASS, Duke's, and literatures) and assessed for bioactivity based on pharmacokinetic properties and other criteria. Protein targets linked to MCI were identified using SwissTargetPrediction and DisGeNET databases, and the network was constructed with Cytoscape software and its plugins (MCODE, BinGo, JEPETTO). A total of 178 bioactive compounds and 105 MCI-related protein targets were identified. The top 10 protein targets in the AAR network include GRB2, SRC, TP53, MAPK1, ESR1, PRKCA, STAT3, PIK3R1, FYN, and AKT1, which play important roles in cell processes associated with aging-related cognitive dysfunction. KEGG pathway analysis revealed 37 significant pathways, such as neuroactive ligand-receptor interaction, long-term potentiation, long-term depression and neurotrophin signaling pathways, providing insights into the molecular mechanisms involved in biological processes in cognitive impairment. Gene ontology (GO) analysis identified five modules related to cellular functions potentially contributing to MCI, including metabolic process, and multicellular organismal process. This study enhances the understanding of AAR's protective effects on cognitive function and could inform improvements in the quality and efficacy of AAR treatments for age-related cognitive decline.

Keywords: Network pharmacology, Thai traditional medicine, Anti-aging remedy

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INTRODUCTION

In the elderly population, changes in learning and memory are common due to neurodegenerative. Some cases may change from a little abnormal to dementia, known as a transition phase, called Mild Cognitive Impairment (MCI). Recent studies indicated that MCI is related to the loss of volume in brain such as hippocampal and temporal lobe. There is evidence that MCI is associated with inflammatory and oxidative stress, but the mechanisms underlying MCI are complex and not fully understood. The researchers will focus on protecting the brain from neuroinflammations and oxidative stress by reducing both external factors and internal factors. Therefore, it is important to develop drug for protect aging to MCI and change to Alzheimer's disease.

In Thai traditional medicine has identified several longevities herbal formulars that believe slow down the aging process. These formulars explain a longer and healthier lifespan. It is a research question that how these traditional remedies might contribute to longevity and contribute to learning in aging. The one of formular consisted of six herbs, *Albizia procera* (Roxb.) Benth. (AP), *Diospyros rhodcoalyx* Kurz (DR), *Tinospora crispa* (L.) Hook. f. and Thomson (TC), *Cyperus rotundus* L. (CR), *Streblus asper* Lour. (SA) and *Piper nigrum* L. (PN). Several herbs in this formula have demonstrated effects on learning and memory.

Piper nigrum can improve learning behavior by enhancing nicotinic acetylcholine receptors in mouse brain (Chaiwiang et al., 2016). *Streblus asper* can protect against cognitive impairment induced by scopolamine in rat. *Cyperus rotundus* can improve expression of Brain-Derived Nerve growth factor (BDNF), a protein that controls neurogenesis and synaptic plasticity in the brain. Additionally, it is related to Na^+/K^+ ATPase activity in mice brain (Ngamrojanavanich et al., 2006.), and protects against neuronal death by restoring SOD and CAT enzyme levels that are induced by SIN-1 (Kumar et al., 2013). Furthermore, these herbs have all demonstrated AChE inhibitory activity (Yusoff et al., 2014)

The interactions between herbs in this remedy have not been studied for their effects on longevity, and their combined effects on learning ability have not been established. Some individual herbs in the remedy show potential for improving cognitive function in aging. Researchers are interested in understanding the biological mechanisms of remedy interact with the body and whether they have an impact on learning and memory in older adults. Natural products play a role in medicine due to abilities in interacting and modulating with target disease. In herbal medicines have several biological structures for treat multi-symptoms. They have lower side effects, cheaper, and easy to access. These characteristics underscore their potential in traditional medicine, as they can target pharmacological pathways through bioactive compounds (Noor et al., 2022). Future advancements, such as the application of nanotechnology, can enhance the stability and efficacy of these compounds. For instance, technologies like niosomes could be utilized to stabilize bioactive compounds, which are critical in addressing aging-related cognitive decline. (Doungsaard, P. et al, 2024)

Network pharmacology plays a vital role in advancing the drug development process by employing network-based approaches to anticipate therapeutic targets. This is achieved through the integration of public databases and up-to-date research findings. Additionally, it examines the mechanisms of drugs in maintaining system equilibrium and comprehending pharmacological pathways (Ke et al., 2016). After obtaining promising compounds through screening, it is necessary to examine their properties before conducting animal testing. In recent years, there has been a growing emphasis on the utilization of multitarget and combination medicines in the treatment of complicated diseases, as these approaches have shown greater efficacy (Muhammad et al., 2018).

MATERIAL AND METHODS

Data preparation

The natural compounds of the Anti-aging remedy (AAR) from six herbs were obtained from phytochemical databases, namely the Natural Product Activity and Species Source Database; NPASS (http://bidd.group/NPASS/index.php) (Zeng et al., 2018), Dr. Duke's Phytochemical and Ethnobotanical Databases (https://phytochem.nal.usda.gov) and published literatures. A keyword search was conducted using the scientific name of herbs consisting of *Albizia procera* (AP), *Diospyros rhodcoalyx* (DR), *Tinospora crispa* (TC), *Cyperus rotundus* (CR), *Streblus asper* (SA), and *Piper nigrum* (PN).

The natural compounds were evaluated as bioactive compounds based on their pharmacokinetic properties (Huang et al., 2017) including absorption, distribution, metabolism, and excretion (ADME). At least two criteria were chosen: oral bioavailability (OB), intestinal epithelial permeability (Caco-2 cells), drug-likeness (DL) and Lipinski's rule (LR) of five. The information of pharmacokinetic properties was retrieved from NPASS.

Oral bioavailability (OB) refers to the rate at which a drug is absorbed after oral administration. Intestinal epithelial permeability (Caco-2 cells) showed the ability of a drug or compounds to pass through the intestinal epithelial layer, thereby providing insights into their absorption potential and mechanisms of transport and metabolism within the gastrointestinal tract. Drug-likeness (DL) assesses the similarity of specific functional groups within a compound, that helps in assessing the potential of a compound to be developed as a drug. Lipinski's rule (LR) of five is used to identify compounds that are considered druggable, and it consists of 4 conditions.

Protein targets associated with bioactive compounds and diseases were identify by searching the SwissTargetPrediction database (http://www.swisstargetprediction.ch/) and the DisGeNET database (https://www.disgenet.org/), respectively. The search was conducted using SMILES notation for bioactive compounds and the terms "Mild cognitive impairment", "Cognitive deterioration", and "Age-related cognitive decline" for the disease. The dataset was collected in an Excel program, which presented both the nodes (bioactive compound and genes) and the targets (protein target and disease).

Network construction and analysis

The data from the Excel program was utilized to analyze the interactions using the Advanced network merge and analyze network functions in Cytoscape version 3.9.1 (http://www.cytoscape.org/). These features allowed for the examination of the network connections between bioactive compounds, protein targets, and diseases. The results were then visualized to present the outcomes in the form of biological networks. This protocol is shown in figure 1.

Identification of significant pathways

For pathway enrichment analysis, the JEPETTO version 1.31 (Java Enrichment of Pathways Extended to Topology), was utilized in Cytoscape. It analyzes significant pathways using the human gene set from the KEGG database, considering a confident score of ≥ 0.5. The results presented the XD-score, which shows the average distance of interactions within the network. The XD-score was calculated from network properties (degree centrality, betweenness centrality, and clustering coefficient) to evaluate the druggability of protein targets. This score provides insights into the level of interaction within the analyzed pathways. Overlap/Size showed relation between interested gene set with gene in particular pathway, that the higher number of overlaps, the stronger the association between the pathway and the gene set of interest.

Modular analysis

The biological network was analyzed using the MCODE version 2.0.0, plugin in Cytoscape. This plugin helps identify important sub-networks within a biological network based on density of protein targets. The analysis was performed using the default settings to detect data. Once the modules were identified, they were subjected to Gene Ontology (GO) analysis using the BinGO version 3.0.4 plugin. A significance level of 0.05 was used to identify enriched GO terms within the modules. The results reveal the biological processes that are significantly associated with each module within the biological network.

The GO and the KEGG pathway enrichment analysis results were compared with biological process using The Database for Annotation, Visualization, and Integrated Discovery; DAVID (https://david.ncifcrf.gov). DAVID database provided a platform for interpreting and visualizing the functional annotation results, thereby enabling a comprehensive understanding of the identified pathways and processes.

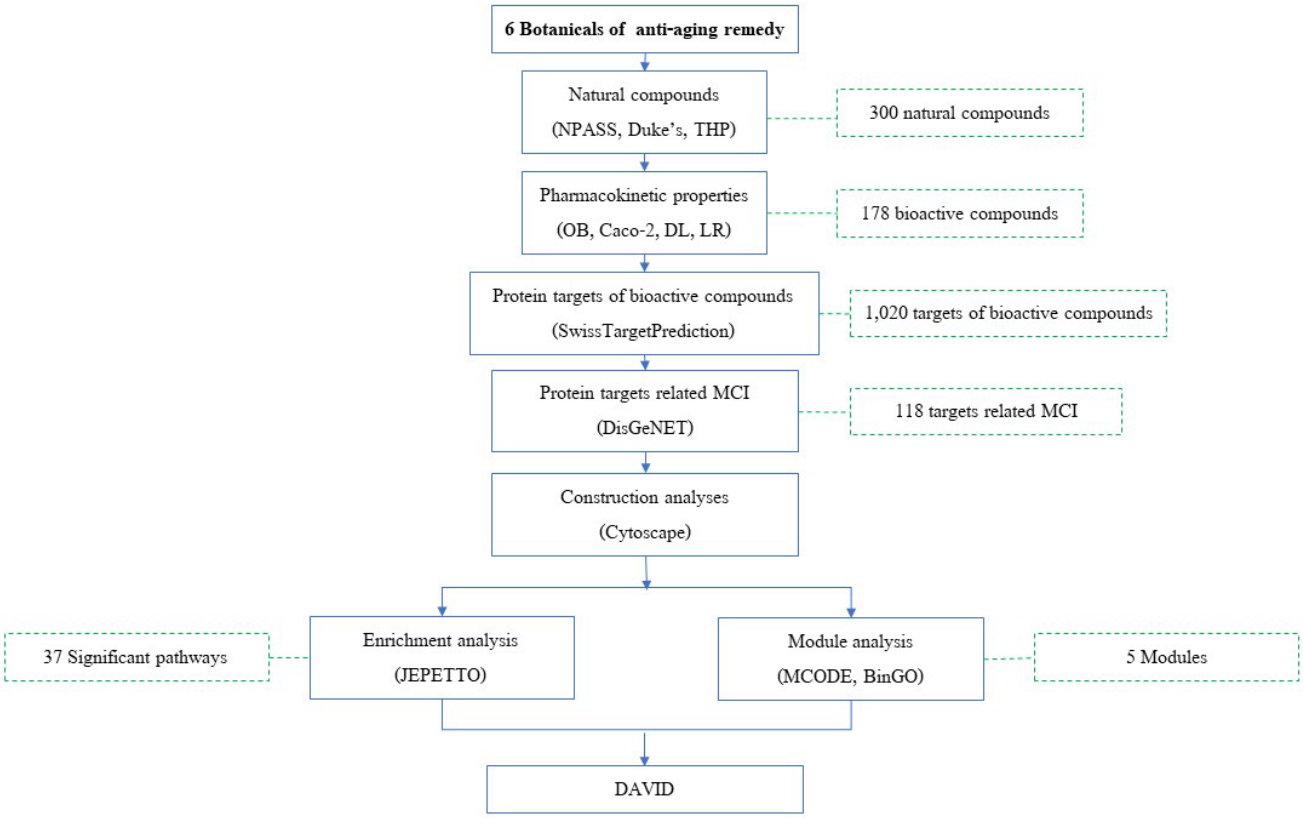


Figure 1. Summary protocol in the network pharmacology of AAR.

RESULTS

A total of 300 compounds were collected from six herbs, consisting of 148 compounds from *Piper nigrum* (PN), 77 compounds from *Cyperus rotundus* (CR), 26 compounds from *Albizia procera* (AP), 19 compounds from *Streblus asper* (SA) and *Tinospora crispa* (TC), and 11 compounds from *Diospyros rhodcoalyx* (DR). All bioactive compounds were identified using pharmacokinetic properties obtained from the NPASS database, that it was using ADMETlab to verified and predicted (Xiong et al., 2021; Zhao et al., 2023).

The evaluation of the oral bioavailability (OB) in humans indicated that drugs with an F30% value ranging from 0.0 to 0.7 were classified as having excellent to medium bioavailability. Permeability values of Caco-2 cell membranes that above -5.15 were deemed to be of exceptional quality. The QED value (Quantitative Estimate of Drug-likeness) was employed to assess the drug-likeness (DL) characteristics of the compounds. This value is calculated based on molecular chemical properties, with values exceeding 0.67 were considered to be of exceptional quality. All of the compounds that were evaluated according to Lipinski's rule were found to be compliant. Overall, a total of 178 bioactive compounds were identified in the Anti-aging remedy (AAR) after removing duplicate compounds using a Venn Diagram tools (https://bioinformatics.psb.ugent.be). From 202 bioactive compounds, it was found that 24 bioactive compounds were duplicated, specifically within the compounds from CR and PN, a total of 21 duplicate compounds, 2 compounds between CR and DR, and 1 compound between AP and SA.

The protein targets of the 178 bioactive compounds were retrieved from the SwissTargetPrediction database. The targets were selected based on a probability value greater than zero. In total, 1,020 protein targets were identified. There were 10 protein targets that were duplicated across the six herbs. These duplicated protein targets include ALOX12, BACE1, CA4, CA2, ALOX15, CYP19A1, ESR1, CA6, AR, and ESR2.

The protein targets associated with diseases related to Mild Cognitive Impairment (MCI) were retrieved from the DisGeNET database, focusing on human genes. A total of 105 protein targets were identified. The 82 targets were specifically related to MCI, while 5 targets each were associated with cognitive deterioration (CD) and age-related cognitive decline (ARCD). There are 13 targets that were shared between MCI and/or other diseases, including APP, ACE, ALB, ACHE, BCHE, VCAM1, IL6, PSEN1, COMT, ESR2, SIRT1, ESR1, and HDAC3.

Network construction and analysis

The network of AAR was constructed with a total of 1,208 nodes and 7,203 edges. The network diameter, which represents the longest shortest path between any two nodes, is 6. The network radius, which is the minimum eccentricity of any node, is 4. The characteristic path length, or the average shortest path length of the network, which indicates the average distance between nodes, is equal to 3.407. The degree interaction of protein target shown by degree, that refers to the number of connections (or edges) that a particular protein node has within a network. It measures the relationships between protein and other nodes (e.g., other proteins, compounds, or genes) in the network. The interaction is shown in figure 2.

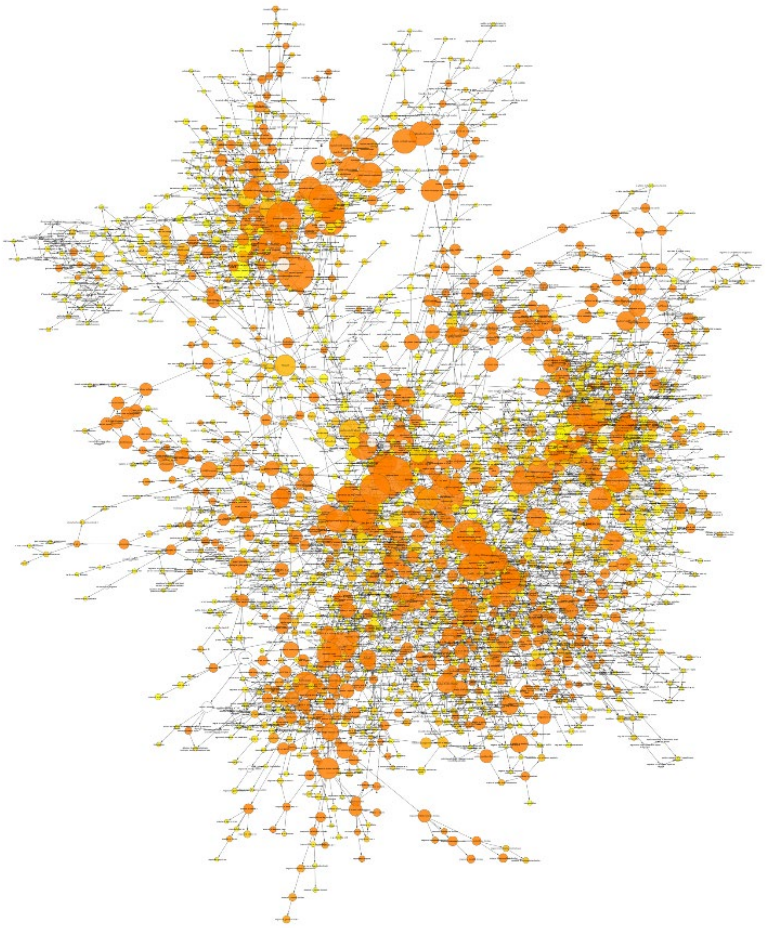


Figure 2. Network pharmacology of Anti-aging remedy (AAR).

When considering the degree of protein target in the AAR network, the top 10 targets based on their degree are as follows: GRB2 (Growth factor receptor-bound protein 2) with a degree of 66.0, SRC (SRC proto-oncogene, non-receptor tyrosine kinase) with a degree of 64.0, TP53 (tumor protein p53) with a degree of 59.0, MAPK1 (mitogen-activated protein kinase 1) with a degree of 51.0, ESR1 (estrogen receptor 1), PRKCA (protein kinase C alpha), STAT3 (signal transducer and activator of transcription 3) with a degree of 48.0, PIK3R1 (phosphoinositide-3-kinase regulatory subunit 1) with a degree of 46.0, FYN (FYN proto-oncogene, Src family tyrosine kinase) with a degree of 44.0 and AKT1(AKT serine/threonine kinase 1) with a degree of 42.0. These proteins exhibit high connectivity and play important roles in the AAR network.

The top 10 compounds based their degree in the AAR network are as follows: (2R)-1-(Propan-2-Ylamino)-3-(2-Prop-2-Enylphenoxy)Propan-2-Ol and Alprenolol with a degree of 112, Linoleic Acid with a degree of 109, (S)-Verapamil with a degree of 108 this equal as (2Z,4Z)-5-(1,3-Benzodioxol-5-Yl)-1-Piperidin-1-Ylpenta-2,4-Dien-1-One, (R)-Verapamil, (S)-Alprenolol, Piplartine with a degree of 107, Magnolol and Obovatol with a degree of 106. These compounds have high degrees of connectivity in the AAR network.

Identification of significant pathways

The pathway enrichment was analyzed via KEGG results. The maximum XD-score, which represents the average distance to all pathways, was 2.058. This

positive score indicates a strong interaction of pathways in the network. The q-value, which indicates the significance of the overlap between pathways using Fisher's exact test, was found to be less than 0.05. In this study, the threshold value of the XD-score was 0.67. As a result, a total of 37 pathways were identified as significantly enriched in the AAR network. These findings provide valuable insights into the functional relationships and interactions within the network at the pathway level (Table 1).

Table 1. The significant pathways of the AAR network.

Pathway or Process	XD-score	q-value	Overlap/ Size
Linoleic acid metabolism	2.058	0.000	9/11
Steroid hormone biosynthesis	1.858	0.000	10/15
Arachidonic acid metabolism	1.828	0.000	17/26
Neuroactive ligand-receptor interaction	1.480	0.000	125/214
Renin-angiotensin system	1.385	0.000	10/16
Acute myeloid leukemia	1.319	0.000	28/52
Long-term potentiation	1.319	0.000	34/63
Non-small cell lung cancer	1.303	0.000	27/51
ErbB signaling pathway	1.256	0.000	39/84
Glioma	1.250	0.000	32/60
Adipocytokine signaling pathway	1.230	0.000	24/57
Bladder cancer	1.216	0.000	21/38
GnRH signaling pathway	1.211	0.000	42/83
Thyroid cancer	1.188	0.001	12/25
Pancreatic cancer	1.172	0.000	34/70
Type II diabetes mellitus	1.149	0.000	20/43
VEGF signaling pathway	1.141	0.000	32/62
Fc epsilon RI signaling pathway	1.126	0.000	34/65
Calcium signaling pathway	1.100	0.000	74/152
PPAR signaling pathway	1.098	0.001	16/39
Retinol metabolism	1.040	0.004	7/12
Prostate cancer	1.013	0.000	43/84
Epithelial cell signaling in Helicobacter pylori infection	1.013	0.000	24/59
Metabolism of xenobiotics by cytochrome P450	0.963	0.000	11/20
Long-term depression	0.911	0.000	23/57
Neurotrophin signaling pathway	0.871	0.000	52/121
Chronic myeloid leukemia	0.842	0.000	29/69
Insulin signaling pathway	0.838	0.000	50/123
Progesterone-mediated oocyte maturation	0.811	0.000	36/79
T cell receptor signaling pathway	0.786	0.000	41/102
Leishmaniasis	0.767	0.002	21/62
Shigellosis	0.759	0.008	18/56
Vascular smooth muscle contraction	0.758	0.000	39/89
Endometrial cancer	0.748	0.000	22/50
Aldosterone-regulated sodium reabsorption	0.747	0.002	15/38
Ether lipid metabolism	0.725	0.006	8/16
B cell receptor signaling pathway	0.708	0.000	25/69

There are a total of 37 significant pathways in the AAR network that were identified, classified into four main categories. The first category, Environmental Information Processing, included four pathways: the ErbB signaling pathway, VEGF signaling pathway, calcium signaling pathway, and neuroactive ligand-receptor interaction. In the second category, Human Diseases, most of the results showed in cancers such as acute myeloid leukemia, non-small cell lung cancer, glioma, bladder cancer, thyroid cancer, pancreatic cancer, prostate cancer, chronic myeloid leukemia, and endometrial cancer. In addition, this group is related to Infectious disease and Endocrine and metabolic disease such as epithelial cell signaling in Helicobacter pylori infection, Shigellosis, Leishmaniasis, and Type II diabetes mellitus. The third category, Metabolism, plays a role in various metabolic processes including lipid metabolism pathways such as linoleic acid metabolism, steroid hormone biosynthesis, arachidonic acid metabolism, and ether lipid metabolism. Additionally, it includes retinol metabolism, and metabolism of xenobiotics by cytochrome P450 pathways.

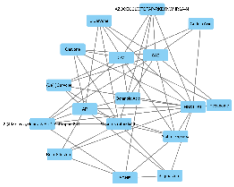
The last category, Organismal Systems, encompassed many components of the endocrine system, including the renin-angiotensin system, adipocytokine signaling pathway, GnRH signaling pathway, PPAR signaling pathway, insulin signaling pathway, and progesterone-mediated oocyte maturation. The category of signaling pathways encompasses various components of the immune system, including the Fc epsilon RI signaling route, as well as the signaling pathways associated with the T-cell receptor and B-cell receptor. Furthermore, it is crucial to note that these phenomena are intricately connected to the functioning of the neurological system. Specifically, they encompass long-term potentiation, long-term depression, and the neurotrophin signaling pathway. In addition, the circulatory system encompasses the process of vascular smooth muscle contraction, whereas the excretory system entails the pathway of sodium reabsorption mediated by aldosterone.

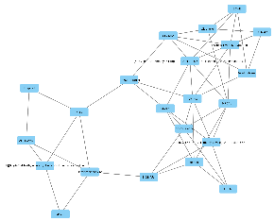
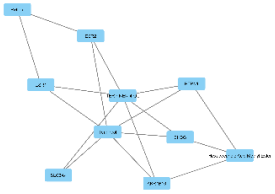
All the significant pathways in the AAR network provide insights into the molecular mechanisms underlying various biological processes and diseases, contributing to a comprehensive understanding of the anti-aging remedy.

Module analysis

The functional modules of AAR were analyzed using the MCODE, plugin in Cytoscape. Gene ontology (GO) analysis was performed on all modules using BinGO, with a significance threshold of $P < 0.05$. A total of 5 modules were identified, containing 632 pathways associated with proteins. After removing the overlapped information, 66 unique pathways remained (Table 2).

Table 2. The functional modules in the AAR network.

No.	Module	MCODE scores	Nodes	Edges	Number of pathways	Genes
1		6.375	17	51	91	ACHE AR CA1 CA2 HSD11B1

No.	Module	MCODE scores	Nodes	Edges	Number of pathways	Genes
2		4.476	22	47	366	ADORA2A DRD2 SLC6A2 KDR NPC1L1 SIGMAR1 NR1H3 BCHE MAOB NR3C1 CHRM2
3		3.778	10	17	123	AKR1B10 SHBG ESR1 ESR2 SLC6A4 PTPN1
7		2.667	4	4	1	AKR1C3
9		2.4	6	6	108	PSEN1 PTPN2 TRPV1

Abbreviations: ACHE, acetylcholinesterase; ADORA2A, aldo-keto reductase family 1 member B10; AKR1C3, aldo-keto reductase family 1 member C3; AR, androgen receptor; BCHE, butyrylcholinesterase; CA1, carbonic anhydrase 1; CA2, carbonic anhydrase 2; CHRM2, cholinergic receptor muscarinic 2; DRD2, dopamine receptor D2; ESR1, estrogen receptor 1; ESR2, estrogen receptor 2; HSD11B1, hydroxysteroid 11-beta dehydrogenase 1; KDR, kinase insert domain receptor; MAOB, monoamine oxidase B; NPC1L1, NPC1 like intracellular cholesterol transporter 1; NR1H3, nuclear receptor subfamily 1 group H member 3; NR3C1, nuclear receptor subfamily 3 group C member 1; PSEN1, presenilin 1; PTPN1, protein tyrosine phosphatase non-receptor type 1; PTPN2, protein tyrosine phosphatase non-receptor type 2; SHBG, sex hormone binding globulin; SIGMAR1, sigma non-opioid intracellular receptor 1; SLC6A2, solute carrier family 6 member 2; SLC6A4, solute carrier family 6 member 4; TRPV1, transient receptor potential cation channel subfamily V member 1

DISCUSSION

This study has shown the top 10 degrees of protein targets in the AAR network, which are GRB2, SRC, TP53, MAPK1, ESR1, PRKCA, STAT, PIK3R1, FYN, and AKT1. It's associated with cognitive declines such as cell process, cell signaling, neuronal survival, and synaptic plasticity that link to dysregulation or altered activity in aging-related cognitive dysfunction. In a previous study by Yao et al. (2023), they

investigated the effect of berberine on improving cognitive function. These results showed similar top protein targets in the AAR network, including MAPK1, SRC, CTNNB1, AKT1, PIK3CA, TP53, JUN, and HSP90AA1, are involved in the PI3K-AKT and MAPK pathways. Moreover, the study's results in mice confirmed the protective role of the AKT1, CTNNB1, TP53, and JUN genes in neuronal cells (Yao et al., 2023).

Growth factor receptor-bound protein 2 (GRB2) forms a complex with GRB2-NOX4, which leads to the inhibition of nitrogen oxide activity and decreases slingshot homolog 1 (SSH-1) protein expression (Majumder et al., 2017). The concentration of GRB2 in neuronal cell bodies has been found to increase in Alzheimer's patients (Raychaudhuri and Mukhopadhyay, 2010). In the context of ischemic brain conditions, studies have shown an increase in the phosphorylation activity of the SRC proto-oncogene, non-receptor tyrosine kinase (SRC), in microglia cells, which stimulates the hippocampus and is associated with microglial cell activation (Choi et al., 2005).

In patients with MCI and AD, high levels of Tumor protein p53 (TP53) have been found to be predictive of disease progression from long-term MCI to AD (Stanga et al., 2012). Alterations of TP53 in the cerebral cortex result in the induction of pro-apoptotic proteins and neuronal cell death (Cenini et al., 2008). In the rat model of Alzheimer's disease (AD), inhibition of Mitogen-activated protein kinase 1 (MAPK1) was found to improve cognitive activity and cellular apoptosis rates by decreasing the levels of acetylcholinesterase (AChE), inducible nitric oxide synthase (iNOS), reactive oxygen species (ROS), malondialdehyde (MDA), MAPK1, and phosphorylated MAPK1 (p-MAPK1). Additionally, the levels of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and miR-132 were increased (Qi et al., 2020; Zhu et al., 2002).

In the central nervous system, Estrogen receptor 1 (ESR1) plays role in protecting the central nervous system through mechanisms mediated by estrogen receptors (ERs). Estrogen bind with ESR1, can modulate neuronal function, synaptic plasticity, and neuroinflammatory responses, providing neuroprotection and promoting overall brain health (Elcoroaristizabal Martín et al., 2012). And in the central nervous system, Protein kinase C alpha (PRKCA) has been implicated in numerous neuronal functions, including synaptic plasticity, neuronal survival, and neuronal excitability, that are associated with neurodegenerative diseases and psychiatric disorders (Zhang et al., 2023).

The dysregulation of the FYN proto-oncogene, Src family tyrosine kinase (FYN), is related to demyelination, protein aggregation, neuroinflammation, and cognitive dysfunction (Guglietti et al., 2021), in addition to synaptic plasticity loss (Mahaman et al., 2021). The protein signal transducer and activator of transcription 3 (STAT3) acts as a mediator in the production of oxidative stress, which affects both parenchymal and vascular amyloid pathology in the brain (Mehla et al., 2021). In ischemic mice treated with salvianolic acid, expression of STAT3, VEGF, and VEGF receptor 2 was found, which had an impact on cognitive deficits and angiogenesis (Wang et al., 2018).

Phosphoinositide-3-kinase regulatory subunit 1 (PIK3R1) is particularly involved in PTEN, PIK3R1, and mTOR in the mTOR signaling pathway, which is important in insulin resistance and the development of insulin-related diseases. An increase in the phosphorylation of PTEN proteins was shown in obese mice due to high-fat diet intake, which may affect brain function (Chen et al., 2023). AKT serine/threonine kinase 1 (AKT1) is involved in the endothelial PTEN/AKT pathway, which is related to serving as an energy source and signaling molecule within the brain from enhanced lactate transport across the brain endothelium (J. Wang et al., 2019). The viability of cells in the hippocampus increases rapidly upon modulation with Puerarin, as observed by elevated levels of phosphorylated AKT1, GSK-3β, and MCL-1 through the activation of the PI3K/Akt1/GSK-3β/MCL-1 signaling pathway (Tao et al., 2017).

However, the proteins mentioned above, including GRB2, SRC, TP53, MAPK1, ESR1, PRKCA, STAT3, PIK3R1, FYN, and AKT1, have been found to interact with

various AAR compounds. Including, (2R)-1-(Propan-2-Ylamino)-3-(2-Prop-2-Enylphenoxy)Propan-2-Ol, Alprenolol, Linoleic Acid, (S)-Verapamil, (2Z,4Z)-5-(1,3-Benzodioxol-5-Yl)-1-Piperidin-1-Ylpenta-2,4-Dien-1-One, (R)-Verapamil, (S)-Alprenolol, Piplartine, Magnolol, and Obovatol. The proteins were associated with flavonoids and terpenes groups, which play a role in antioxidant and anti-inflammatory activities. This occurs through multiple pathways involved in the inhibition of oxidative stress, enhancement of antioxidant activity, reduction of lipid peroxidation, suppression of inflammatory mediators, reduction of synaptic dysfunction, protection of acetylcholinesterase activity, and prevention of neuron cell death (Tsai et al., 2022).

These findings provide valuable insights into the network's key regulatory nodes and may serve as a foundation for future research aimed at developing targeted therapeutic interventions or understanding the molecular mechanisms underlying associated diseases.

Significant pathways

From the results, the 37 significant pathways of AAR compounds were predicted to several biological pathways in MCI, classified into 4 parts. Environmental information processing involves signal transduction, which sends signals from external stimuli to molecule functions internally, and cells response. This process is related to intracellular signaling pathways, including the ErbB signaling pathway, the VEGF signaling pathway, and the calcium signaling pathway. Additionally, neuroactive ligand-receptor interaction plays a crucial role in neuron cells; binding to intracellular receptors, a binding factor in translation and gene expression, leads to a decline in memory function.

In human disease, it is closely linked with several cancers, such as acute and chronic myeloid leukemia, non-small cell lung cancer, Glioma, Bladder cancer, Thyroid cancer, Pancreatic cancer, Prostate cancer, and Endometrial cancer. Cancer occurs from internal and external factors including infection diseases. Both acute and chronic infectious diseases may affect cognitive function through mechanisms that, when combined with degeneration in aging, lead to a risk of cognitive decline becoming dementia. For example, a helicobacter pylori infection will stimulate neutrophil release of ROS, contribute to DNA damage, and contribute to MCI from chronic atrophic gastritis (Kountouras et al., 2007). Additionally, Type II diabetes mellitus, classified as an endocrine and metabolic disease, showed long-term metabolism dysfunction leading to chronic illness. Uncontrollable levels of blood sugar and high levels of neutrophil-lymphocyte ratio (NLR) in DM patients correlate with cognitive decline and periods of disease (Alkethiri et al., 2021). These factors are possible predictors for poorer cognitive performance outcomes in Metabolic syndrome (Pipatpiboon et al., 2022).

Metabolism is an essential process that makes energy for cell activities, such as lipid metabolism, linoleic acid metabolism, and arachidonic acid metabolism, that maintain functions in the brain. In aging and AD patients, the middle frontal gyrus of AD involves changes in linoleic acid, linolenic acid, arachidonic acid, and docosahexanoic acid levels (Snowden et al., 2017). Arachidonic acid metabolism is associated with inflammation, which converts into mediators that stimulate inflammatory responses in various processes.

Organismal systems describe several systems that are related to cognitive decline. Such as the plasticity of vascular smooth muscle cells (VSMCs) in the circulatory system, controls cerebrovascular dynamics for response to oxygen and nutrition demand, which plays a role in complex processes in neurodegeneration (Hayes et al., 2022). Aldosterone-regulated sodium reabsorption in the excretory system controls physiologic reflexes involved in oxidative stress, inflammation, fluid disruption, and abnormal tension in organs and the central nervous system (Tsilosani et al., 2022). Renin-angiotensin system, Adipocytokine signaling pathway, GnRH signaling pathway, PPAR signaling pathway, Insulin signaling pathway, and

Progesterone-mediated oocyte maturation show in the endocrine system that it is related to control and connects with other systems. Including T cell and B cell receptor signaling pathways plays a main role in an effective immune system.

Especially the nervous system, which directly influences cognitive function, such as long-term potentiation (LTP) for improving long-term synaptic, and conversely, long-term depression (LTD) can decrease synaptic (Hirano, 2013). The neurotrophin signaling pathway plays a role in the release of neurotrophin proteins, which support the survival of synaptic plasticity and neurogenesis within the peripheral and central nervous system. Alterations in the signaling of brain-derived neurotrophic factor (BDNF) and TrkB receptor are mechanisms implicated in cognitive decline in the elderly (Numakawa and Odaka, 2022).

The anti-aging remedy demonstrated potential in multiple biological pathways associated with enhancing longevity and was also linked to pathways involved in cognitive function. Future laboratory studies should focus on validating the specific mechanisms within each identified pathway. This validation could include detailed investigation of pathway activation, protein interactions, and cellular responses. Additionally, research should examine the safety and efficacy of individual compounds and their combinations, potentially leading to the development of evidence-based food supplements targeting age-related decline.

Module in the network

In this result, five modules showed important sub-networks of AAR within a biological network. Module 1 showed significant associations with ACHE, AR, CA1, CA2, and HSD11B1 genes, which are involved in the metabolic process (P = 0.0125). These processes encompass chemical reactions and pathways in living organisms, including both anabolism and catabolism for chemical conversions. Module 2 included genes ADORA2A, DRD2, SLC6A2, KDR, NPC1L1, SIGMAR1, NR1H3, BCHE, MAOB, NR3C1, CHRM2, which are involved in multicellular organismal processes (P = 0.0048). These processes represent complex biological activities occurring at the organism level. Module 3 contained genes AKR1B10, SHBG, ESR1, ESR2, SLC6A4, PTPN1, also involved in multicellular organismal processes (P = 0.0119). These genes contribute to cellular signaling, tissue differentiation, hormone regulation, neurotransmission, and overall organismal development and homeostasis. Module 7 identified the AKR1C3 gene's involvement in organic acid metabolic processes (P = 0.040), which regulate the conversion, synthesis, and breakdown of specific organic acids within cells. Module 9 was associated with PSEN1, PTPN2, and TRPV1 genes, which connect extracellular molecules through receptor activation (P = 0.0048). This module also relates to chemical stimulus response processes (P = 0.0010), spanning from initial signal activation to cellular response.

The findings revealed that the AAR remedy exhibits a complex mechanism of action through multiple biological pathways. Each module related to cellular functions is associated with deterioration that may lead to cognitive impairment. The results suggest that AAR remedy may regulate age-related metabolic changes and multicellular organismal processes, particularly through its effects on neurotransmission and hormone regulation, indicating a possible effects on impact on systemic aging processes.

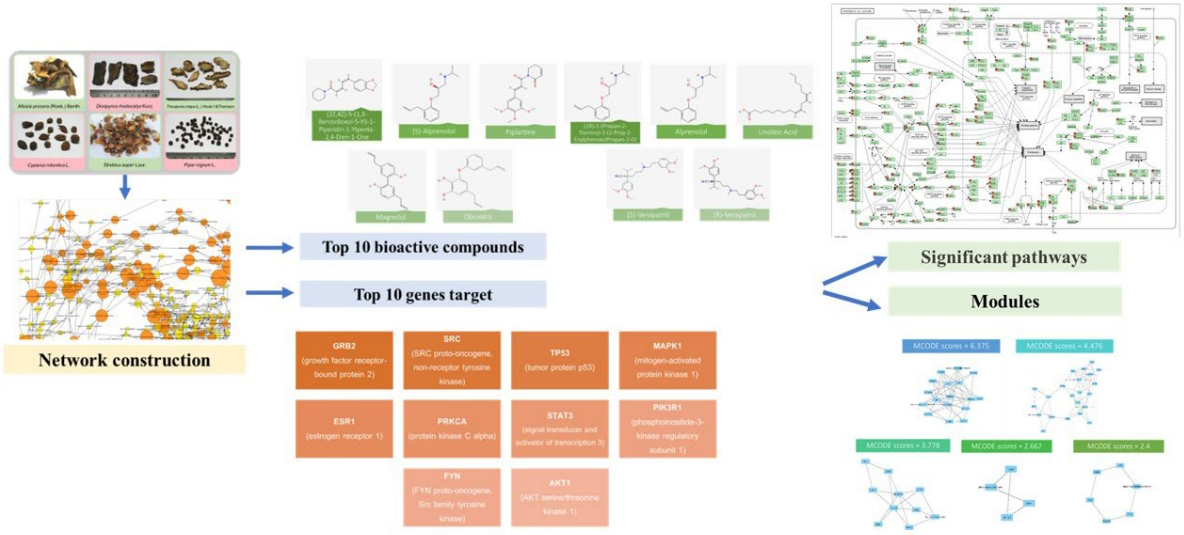


Figure 3. Construction of network pharmacology in anti-aging remedy.

This study employed computational prediction and network analysis to explore the mechanisms of action of AAR in aging-related processes. It identified significant pathways, gene modules, and key protein targets associated with AAR's therapeutic effects (Figure 3). While the findings highlight the complexity of herbal medicines, they rely on computational data and existing databases, which may not capture all compound-protein interactions or newly discovered pathways. The study also cannot address potential *in vivo* metabolic modifications, optimal dosages, or side effects, emphasizing the need for experimental validation and further research to confirm these results.

Future studies should focus on validating the pathway interactions through both in vitro and *in vivo* experiments, with an emphasis on the specific interactions between identified protein targets and compounds, as well as their underlying mechanisms and potential therapeutic targets. Furthermore, efforts should be developed to AAR-based supplements with optimized bioavailability. Clinical trials are necessary to confirm the safety and efficacy of the AAR remedy in promoting longevity and aging. Long-term studies are also needed to evaluate the remedy's impact on aging and cognitive function comprehensively.

CONCLUSION

This study applies network pharmacology to analyze the mechanisms of anti-aging remedy, that used in Thai traditional medicine. The identified pathways and protein targets provide valuable insights into how AAR may impact aging-related processes and cognitive function. The results suggest that AAR remedy has therapeutic potential due to its ability to modulate multiple biological pathways simultaneously, offering a comprehensive approach to addressing age-related conditions. This research establishes a foundation for future laboratory studies and clinical trials, which will be crucial for validating these findings and translating them into practical therapeutic applications.

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AUTHOR CONTRIBUTIONS

Suthita Obhasi is responsible for conceptualization, methodology, validation, formal analysis, investigation, resources, data curation, and writing the original draft. Santi Phosri is responsible for validation and formal analysis. Autaiphon Kaikaew is involved in conceptualization, methodology, validation, and formal analysis. Pratchaya Kaewkaen is in charge of validation, formal analysis, supervision, and project administration.

CONFLICT OF INTEREST

The authors declare that they hold no competing interests.

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