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Determining antihypertensive herbal formulas based on molecular mechanisms and protein-compound interactions of selected Indonesian medicinal plants using a network pharmacological approach

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ABSTRACT

In Indonesia, herbs are used to treat various diseases, including hypertension. A network pharmacology approach can be a breakthrough in research strategy by discovering a new drug using the multicomponent-multitarget concept. The protein-compound mechanism in plants is a potential target for creating new combination formulas that will be developed into new drug discoveries in hypertension therapy. This study aims to determine a new formula from Indonesian medicinal plants using a network pharmacological approach based on molecular mechanisms and protein-compound-plant interactions that have antihypertensive effects. We use graph mining and machine learning to explore compounds and plant-related hypertension target proteins and determine plant formulations based on the molecular mechanisms of hypertension-related target proteins. A search found 78 Indonesian medicinal plants that contain trans zeatin, seselin, sesamin, and other compounds effective as antihypertensive agents. The study obtained a formula with a combination of 2 plants, including 31 formulas bound to 5 target proteins, and a combination of 3 plants, with 85 formulas bound to 6 hypertension target proteins. The plant formula illustrates the involvement of several mechanisms in lowering blood pressure using protein-plant compounds. Some of the plant formula's constituents, such as antioxidants and anti-inflammatories, have blood pressure-lowering properties. Proteins mitogen-activated protein kinase 3, peroxisome proliferator-activated receptor alpha, estrogen receptor 1, endothelin 1, vascular endothelial growth factor A, and fos proto-oncogene are possible biomarkers that could be used to target hypertension medication in the coming years.

INTRODUCTION

Hypertension is a global health problem and is one of the causes of increased morbidity and mortality in Indonesia. The disease poses a public health threat because it can lead to complications such as stroke, coronary artery disease, and renal failure [1]. The prevalence of hypertension is projected to continue to increase, with an estimated 29% of adults worldwide having hypertension by 2025 [2].

Jamu is a traditional Indonesian medicine practiced in Indonesian society for centuries to maintain health and treat illnesses. Despite the increasing use of modern medicine, which has become a first-line medical treatment, herbal medicines still enjoy great popularity in both rural and urban areas [3]. Formulas or combinations of several medicinal plants have synergistic and complementary effects that can strengthen their therapeutic effect [4]. A combination of celery herb extract, cat's whisker leaf, and noni fruit lowers blood pressure in both normotensive and hypertensive rats [5]. The mechanism for reducing blood pressure is a synergistic combination of the active compounds contained in these three plants, where apigenin in celery herb is known to

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reduce blood pressure by blocking calcium channel influx [6]. The sinensetin content in cat's whisker leaves can lower blood pressure because of its diuretic properties, increasing the urine volume. The scopoletin content in a noni fruit is known to have acted as an inhibitor of the angiotensin-converting enzyme (ACE inhibitor), which reduces blood pressure [7].

Based on network topology analysis, Setiani *et al.* [8] anticipate that 35 proteins are related to hypertension, including mitogen-activated protein kinase 3 (MAPK3), peroxisome proliferator-activated receptor alpha (PPARA), estrogen receptor 1 (ESR1), endothelin 1 (EDN1), vascular endothelial growth factor A (VEGFA), and fos proto-oncogene (FOS). Molecular processes of related antihypertensive pathways include the MAPK signaling route, the TNF signaling pathway, the Wnt signaling pathway, and the ERK5, JNK, and p38 MAP kinase pathways [8]. Network pharmacology is a computational method for discovering and developing new drugs. Network pharmacology is a link that will provide new methodologies and opportunities to discover bioactive compounds and biomarkers by building "disease-target compound" networks and exploring scientific evidence of herbal formulas based on complex biological systems [9]. In recent years, an increasing number of people have taken advantage of network pharmacology technology to elucidate the biological basis of the use of herbal medicines with antihypertensive effects. Network pharmacology can also play an important role in new drug discovery, drug repurposing, and rational formulation discovery. Many combinations of bioactive targets have been investigated experimentally. Data synthesis using network pharmacology provides information on how conventional pharmaceutical formulations perform based on bioactive ingredients. This is an inverse approach that uses integrated techniques to infer the molecular mechanisms of action of formulations. Our current network analysis is based on the research conducted and available literature [10].

MATERIAL AND METHODS

Search for the interaction of compounds with target proteins

Data mining of herbal compounds

KNApSAcK (http://www.knapsackfamily.com) is a database of active chemicals and plant databases. Proteins associated with hypertension can be found at UniProt (http://www.uniprot.org/), OMIM (https://www.omim.org/), DrugBank (https://go.drugbank.com/), and PubMed (https://pubmed.ncbi.nlm.nih.gov/) database. The query keyword used is "hypertension."

Search for features of protein dipeptides

Searches for protein-dipeptide functions are available in the BindingDB database (https://www.bindingdb.org/bind/index.jsp) and DrugBank (https://go.drugbank.com/). The target chemical compound was obtained from the protein by dipeptide feature extraction. This result was used as training data, which were later combined with test data using the Python program**.**

Compound fingerprint search

Fingerprint search of chemical compounds and herbal compounds using PubChem fingerprints (https://pubchem.ncbi.nlm.nih.gov/) is carried out on compound data from crawling results and compound test data. Adjust the file name, ID column, and name column, and then run the Python code or getfp.ipynb. The results can be seen in fp_CIDS.csv as training data and fp_herbalDBnew.csv as test data. Then install or import the required packages and define the split function to split the fingerprint data. Define a function for feature extraction, first read Excel data, and then store ID and compound name data in different variables. Then, create a matrix of 0 measuring 881, the number of compounds, and make it a data frame. Repeat for all data, saving the compound ID in the first row and then taking the fingerprint with.cactvs_fingerprint. Separate the data and save it to the data frame that already has the compound ID, and then add the compound ID and save the results to an a.csv file.

Combination of a protein dipeptide and a compound fingerprint

The compound fingerprint file is adjusted, the compound name and International Union of Pure and Applied Chemistry name are deleted, and the PubChem compound identity number (CID) is moved to the first column. Next, after the compound and protein files are complete, join the two files with the combine.ipynb file. Then, install and import the required packages, read the compound fingerprints that were previously taken, and add the key = 1 column to combine the data. Then, from the protein data obtained by crawling the drug-target interaction, read the protein data for training data and separate the protein ID. Import the scaler package and define the scaler with the training protein data. After that, the test protein is read, which separates the ID, transforms the test protein data, and adjusts it. After merging the data with the ID, add key = 1, with merge on the key, and then delete the key and save the merged data to comma-separated value (CSV). Read the compound test data, add a key, join it with the test protein data, and save it to a CSV file.

Prediction of protein-compound interaction data using the Orange tool

The model used is random forest (RF) in the Orange application. At this stage, data input is carried out by creating data classes, dividing data, modeling, and evaluating data. Import the data in .csv format to begin the workflow. Next, click the file to import the compound–protein interaction data. In the last column 3, where the first column is the compound CID, the second column is the protein CID; the label column is to state the class [11].

Search for plant-compound-protein interactions using graph mining analysis

Search for plants that have protein-compound interactions

Indonesian plants that have antihypertensive activity and that contain interacting compounds were obtained through the results of the previous compound database through the KNApSAck website, HerbalDB (http://herbaldb.farmasi.ui.ac.id/v3/), and Google Scholar (https://scholar.google.com/).

Plant-compound-protein construction

Plant-protein compound tissue construction was carried out using the Cytoscape application by combining plant compounds and protein compounds.

Determination of antihypertensive plant formulas

Determination of plant reduction based on traditional Chinese medicine (TCM) molecular studies

Plant reduction is performed to narrow down the plants to be made into a formula. Plant reduction was carried out based on studies of molecular mechanisms found in several TCM formulas published in international journals. Search results of several TCM formulas used in China were collected and analyzed separately to determine the hypertensive protein targets of these formulas. In this process, Indonesian plants were obtained based on the similarity of protein targets and molecular mechanisms with the TCM formula.

Plant reduction based on native Indonesian plants

We then selected plants native to Indonesia and ran the plant reduction process again. The purpose of this process is to narrow down plant species that are widely cultivated by Indonesians.

Plant reduction based on availability at B2P2TOOT Ministry of Health

Indonesian native plants were then rescreened based on their availability. The Indonesian plants available at the Ministry of Health's Balai Besar Penelitian dan Pengembangan Tanaman Obat dan Obat Tradisional (B2P2TOOT) were then combined with plant formulas.

Determination of plant formula combinations based on the target protein and its mechanism

Selected and available plants were made into a formula, taking the target protein into account. The determination of this formula consists of two combinations and three combinations of plants, and the formula is based on the mechanisms of plants that can target proteins. The research flow is shown in Figure 1.

RESULT

Search for the interaction of compounds with target proteins

Data mining of herbal compounds

A significant protein related to hypertension was obtained from the results of the Setiani *et al.* [8] early-stage research screening. As shown in Table 1, 35 important hypertensive proteins are analyzed based on network topology. The results of herbal compound data mining yielded 833 herbal

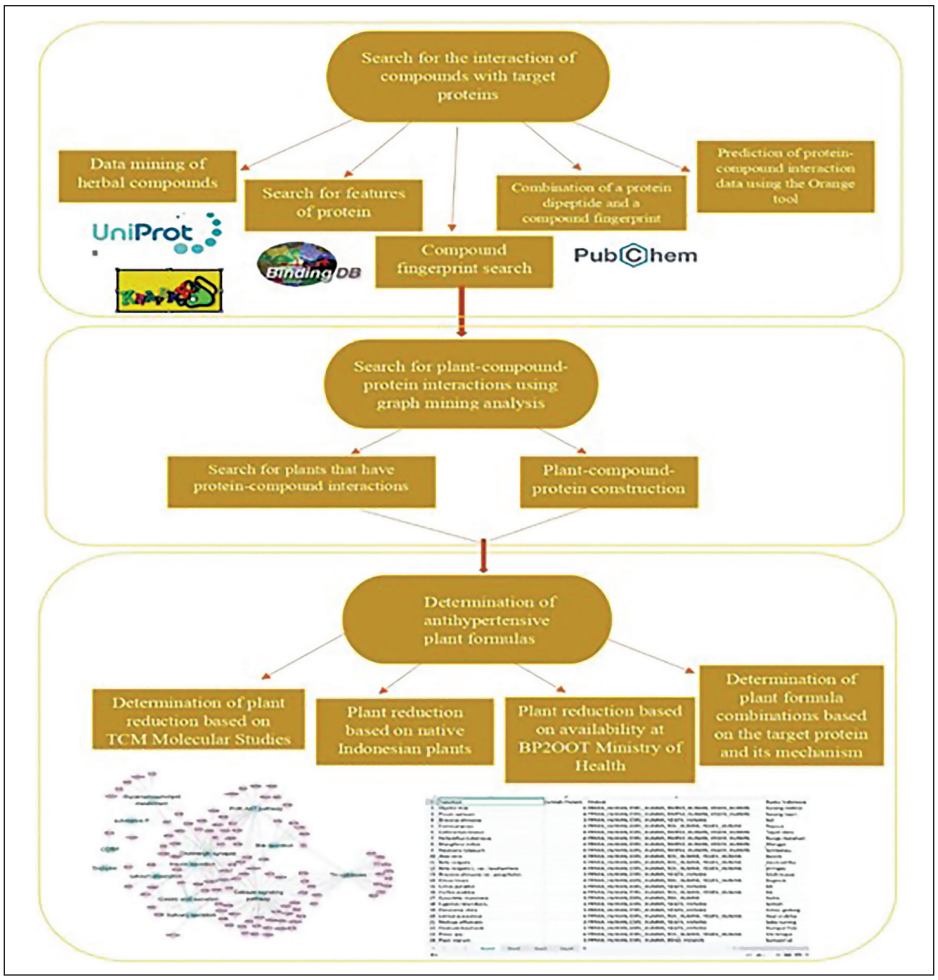

Figure 1. Research flow.

compounds with antihypertensive activity. This compound data will then be used to find fingerprints of compounds that will predict their interactions with significant hypertension proteins.

Search for features of protein dipeptides

This process obtained 400 features of the protein dipeptide. The search for protein dipeptide features represents protein features as amino acid sequences in numerical form so that they can be processed by a computer system and then processed to calculate the similarity between proteins.

Table 1. List of hypertension protein targets.

No	Protein	No	Protein
1	MAPK3	18	ESR1
2	MAPK1	19	PTK2
3	STAT3	20	MAPK14
4	PIK3R1	21	NFKB1
5	CTNNB1	22	GNAQ
6	TP53	23	FOXO1
7	JUN	24	FN1
8	AKT1	25	F2
9	RAC1	26	TNF
10	RHOA	27	FOXO3
11	FOS	28	EDN1
12	RXRA	29	GNAS
13	CAV1	30	INS
14	ITGB3	31	CYP1A1
15	KRAS	32	AGT
16	EGFR	33	ARRB1
17	VEGFA	34	PPARA
		35	PPARGC1A

Compound fingerprint search

The search for compound fingerprints was conducted on chemical compounds and was based on the PubChem fingerprint database. These results showed that the number of fingerprints on herbal compounds (test data) was 820, and for chemical compounds, the obtained 2,028 data points were in the form of numbers "1" and "0."

Combination of compound fingerprints and protein dipeptides

Combining composite fingerprints and protein dipeptides uses a Python program that groups the data into two types: training data and test data. The components of the training data are chemical compound fingerprints and protein dipeptides, and the components of the test data are herbal compound fingerprints and protein dipeptides. This merger produced up to 1,281 pieces of combined data that will be searched for interactions.

Table 2. Probability score.

Interaction	Compound	Protein	Probability score
1	Linoleic acid	PPARA_HUMAN	0,9960757788
1	Nervonic acid	PPARA_HUMAN	0,9960757788
1	Palmitic acid	PPARA_HUMAN	0.9809011756
1	Octadecanoic acid	PPARA_HUMAN	0.9809011756
1	Docosanoic acid	PPARA_HUMAN	0.9809011756
1	Lauric acid	PPARA_HUMAN	0.9789487947
1	Malvalic acid	PPARA_HUMAN	0.9719091121
1	Sterculic acid	PPARA_HUMAN	0.9719091121
1	Sebacic acid	PPARA_HUMAN	0.9431723972
1	Suberic acid	PPARA_HUMAN	0.9246009686

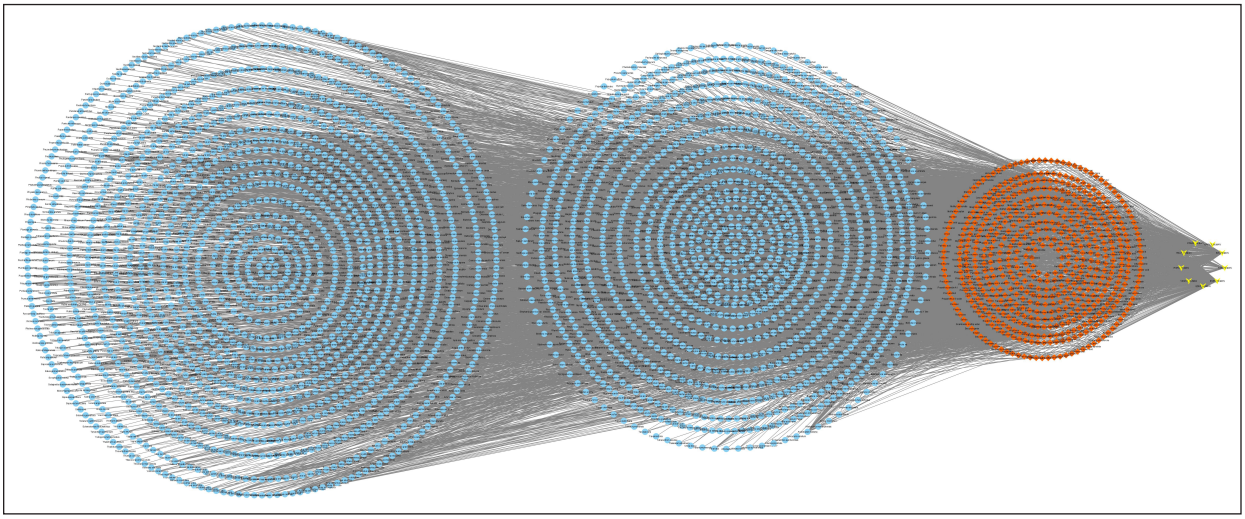

Figure 2. Interaction between protein-plant compounds. The blue circle represents plants; the diamond orange represents active plant compounds; and the yellow inverted triangle represents the target of the active compound.

Predicted protein-compound interaction data using Orange

In this process, the labeling results of the interactions of compounds and proteins are used. This prediction uses the RF method in Orange. The probability score calculation yielded 1,015 interactions of compounds and proteins with a probability score greater than 0.5. The following is the result of calculating the protein-compound probability score, as shown in Table 2.

Search for plant-compound-protein interactions using graph mining analysis

Search for plants that have protein-compound interactions

A search related to compounds and plants using graph mining obtained 562 compounds that interact with target proteins and 2,864 plants had related compounds.

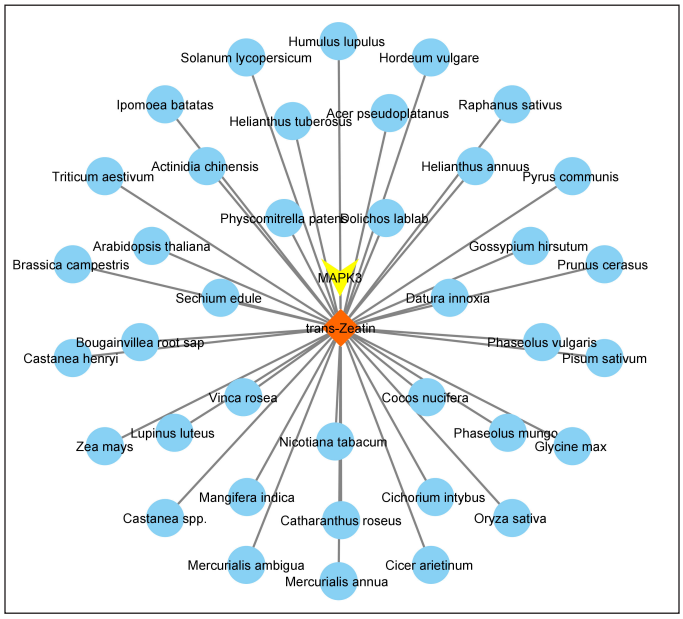

Figure 3. MAPK3 protein targets trans-zeatin compounds and some plants The blue circle represents plants; the diamond orange represents the active plant compound; and the yellow inverted triangle represents the target of the active compound trans-zeatin.

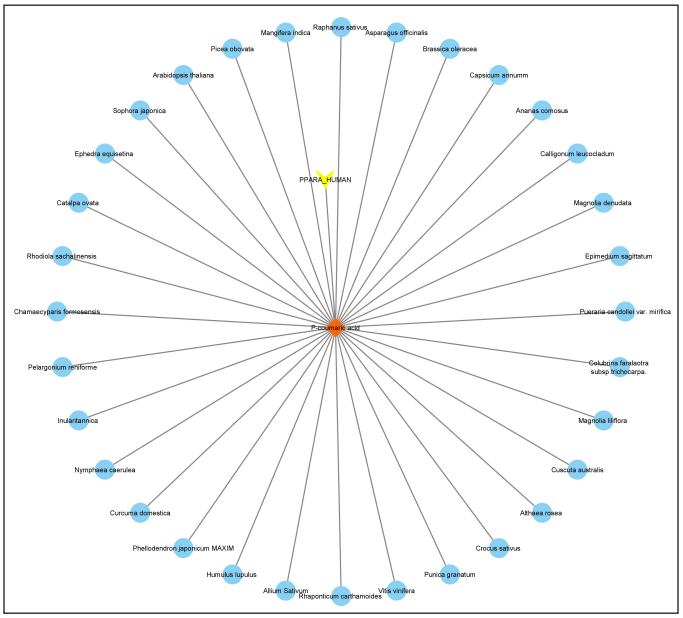

Figure 4. Compound-plant interaction in PPARA protein. The blue circle represents plants; the diamond orange represents the active plant compound; and the yellow inverted triangle represents the target of the active compound p-coumaric acid.

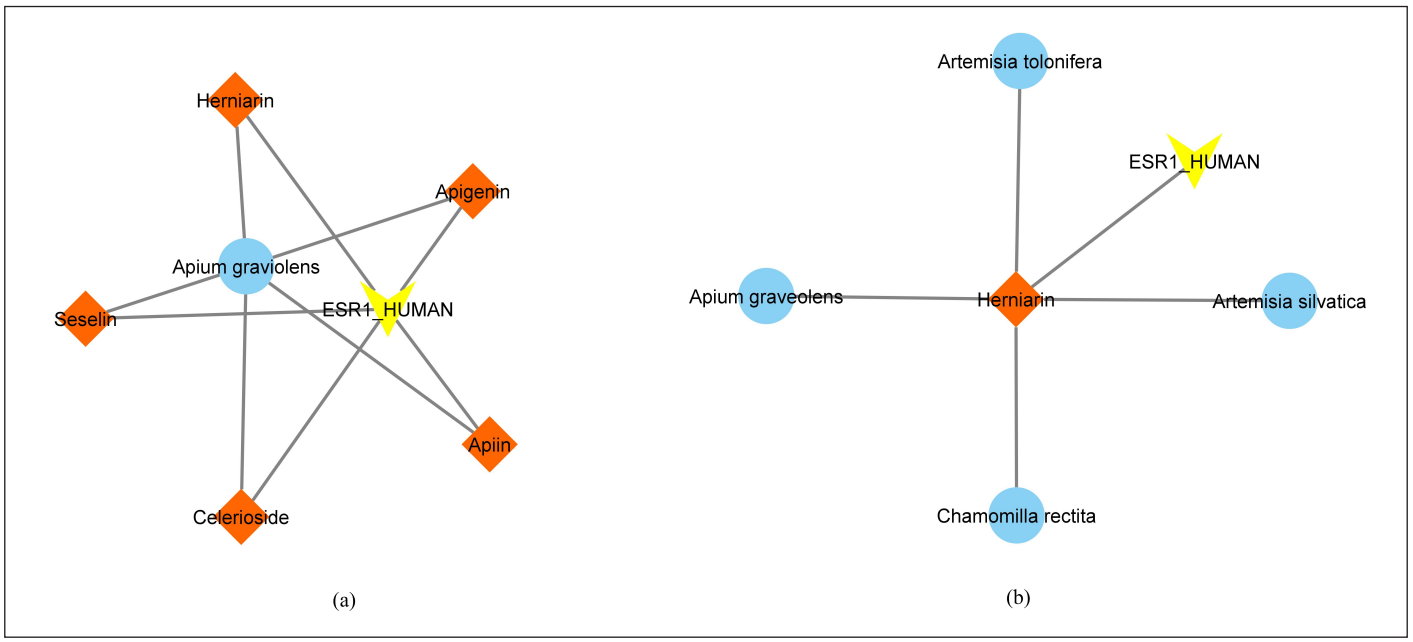

Figure 5. Compound-plan interaction in ESR1 proteins. (a) The *A. graveolens* plant contains herniarin, apiin, sesamin, apigenin, and celerioside compounds that have interactions with the ESRI protein. (b) Apart from being contained in the *A. graveolens* plant, hemiarin compounds are also contained in the plants *Artemisia tolonifera*, *Artemisia silvatica*, and *Chamomila rectita*.

Plant compound-protein construction

The protein-compound-plant interaction network was constructed using the Cytoscape application, which obtained 3,440 nodes and 6,865 edges. The results of this interaction search are shown in Figure 2.

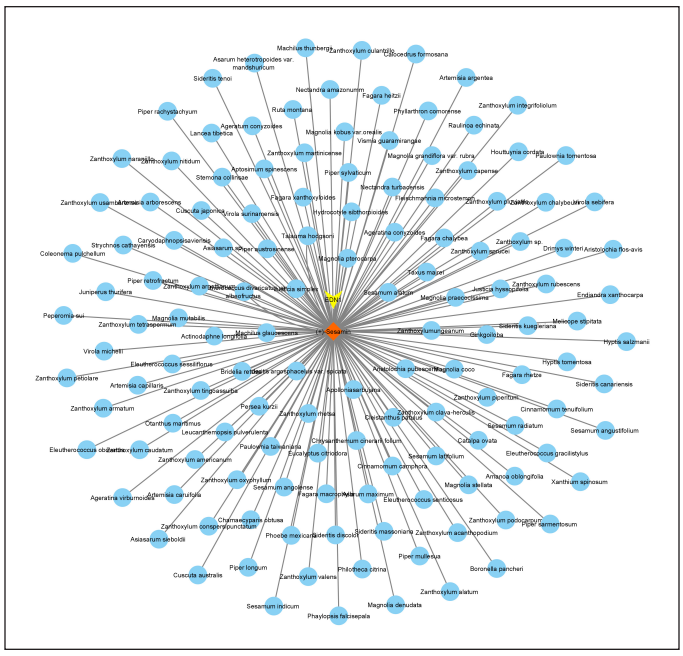

Figure 6. Compound-plant interaction in EDN1 protein. The blue circle represents plants; the diamond orange represents the active plant compound; and the yellow inverted triangle represents the target of the active compound (+)-sesamin.

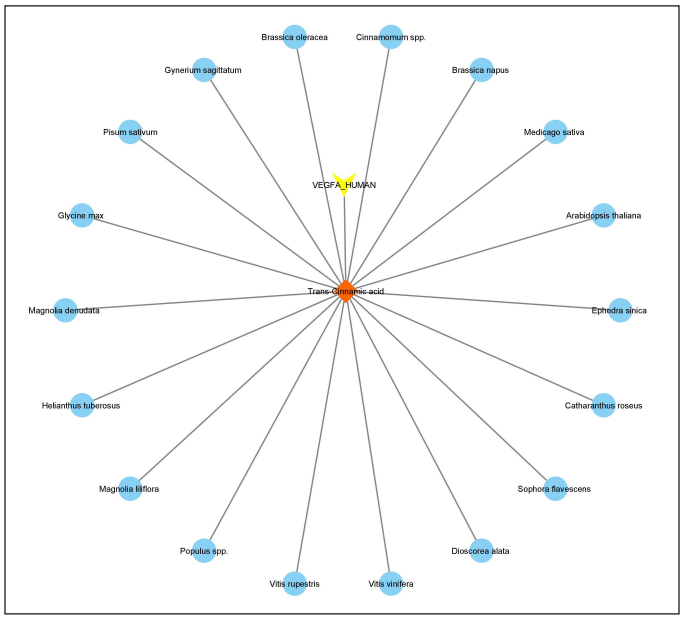

Figure 7. Compound-plant interaction in VEGFA protein. The blue circle represents plants; the diamond orange represents the active plant compound; and the yellow inverted triangle represents the target of the active compound trans-cinnamic acid.

Determination of hypertension plant combination formula

Plant reduction based on the mechanism of the TCM formula

The plants are then decreased depending on their interactions with TCM proteins. Six significant proteins, including MAPK3, PPARA, ESR1, EDN1, VEGFA, and FOS, were found based on references to 10 TCM formulae and 35 major proteins from Setiani *et al.*'s [8] research results. This approach produced 619 plants that worked molecularly to target the 6 proteins. Graph mining analysis was utilized to investigate plant-compound-protein interactions in a total of six significant proteins. Each of these proteins will bind compounds and spread to several plants.

The visualization of the plant compounds that target the MAPK3 protein is shown in Figure 3. Figure 3 shows the construction of plant-compound tissue that binds to the MAPK3 protein. Using the Cytoscape, an overview of the relationship between plants and compounds that bind to the MAPK3 protein can be shown. Trans-zeatin compounds and their derivatives can inhibit MAPK activation; this compound is found in 39 plants, including *Glycine max*, *Oryza sativa*, *Zea mays*, *Mangifera indica*, and *Solanum lycopersicum*. The plant-compound interactions in the PPARA protein are shown in Figure 4. P-coumaric acid compounds that bind to PPARA proteins can be found in several plants, including *M. indica*, *Allium sativum*, *Curcuma domestica*, and *Punica granatum*.

The ESR protein binds to several compounds in the *Apium graveolens* plant, including herniarin, apiin, and apigenin, as shown in Figure 5. The plant-compound interactions in EDN1 proteins are shown in Figure 6. Figure 6 shows the EDN1 protein attached to sesamin compounds found in various plants, including *Piper retrofractum*. The plant-compound

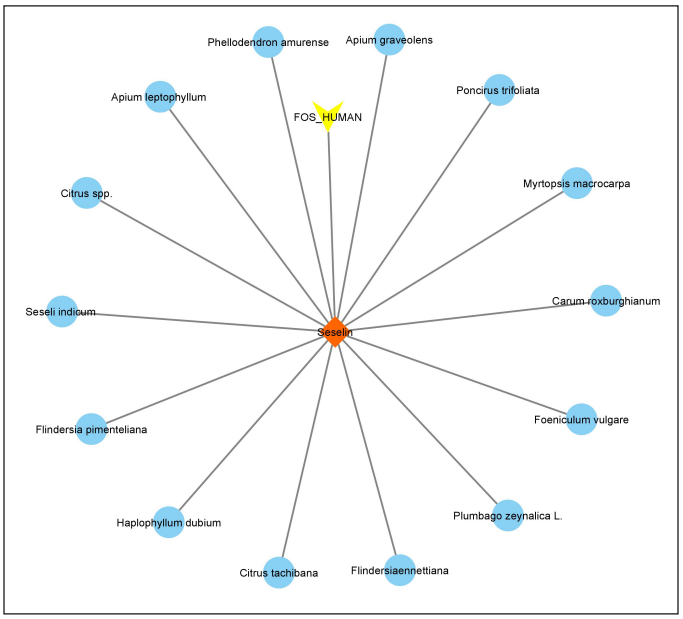

Figure 8. Compound-plant interaction in FOS protein. The blue circle represents plants; the diamond orange represents the active plant compound; and the yellow inverted triangle represents the target of the active compound seslin.

Table 3. Combination formula of two plants.

No.	Target proteins	Plant formulas
1	EDN1, PPARA, ESR1, VEGFA, MAPK3	*P. retrofractum, M. indica*
2	PPARA, ESR1, VEGFA, MAPK3, FOS	*P. retrofractum, C. roseus*
3		*P. retrofractum, G. max*
4		*P. retrofractum, P. vulgaris*
5		*P. retrofractum, P. sativum*
6		*P. retrofractum, R. sativus*
7		*P. retrofractum, Z. mays*
8		*P. retrofractum, C. papaya*
9		*P. retrofractum, C. limon*
10		*P. retrofractum, C. arabica*
11		*C. papaya, M. indica*
12		*C. papaya, C. roseus*
13		*C. papaya, G. max*
14		*C. papaya, P. vulgaris*
15		*C. papaya, P. sativum*
16		*C. papaya, R. sativus*
17		*C. papaya, Z. mays*
18		*C. limon, M. indica*
19		*C. limon, C. roseus*
20		*C. limon, G. max*
21		*C. limon, P. vulgaris*
22		*C. limon, P. sativum*
23		*C. limon, R. sativus*
24		*C. limon, Z. mays*
25		*C. arabica, M. indica*
26		*C. arabica, C. roseus*
27		*C. arabica, G. max*
28		*C. arabica, P. vulgaris*
29		*C. arabica, P. sativum*
30		*C. arabica, R. sativus*
31		*C. arabica, Z. mays*

interaction on the VEGFA protein can be seen in Figure 7. One of the trans-cinnamic acid compounds bound to this protein is spread in several plants, including *G. max*, *Cinnamomum* spp., and *Pisum sativum*. The seselin compound bound to the FOS protein in Figure 8 can be found in *Citrus* spp., *A. graveolens*, and *Foeniculum vulgare*.

Plant reduction based on native Indonesian plants

Plants that have been reduced based on molecular mechanisms are further reduced based on native Indonesian plants. There were 228 plants native to Indonesia.

Plant reduction based on availability at B2P2TOOT Ministry of Health

Plant reduction was carried out again based on plant availability in the Ministry of Health's B2P2TOOT. A total of 78 final plants were obtained, which would be used to make a combination plant formula.

Determination of the plant combination formula based on the target protein and its mechanism

The results of the plant reduction were then examined for its availability, and 78 plants were obtained, of which 21 plants targeting 6 proteins could be made into a formula. Plant formulas are created based on protein interactions, which are created in two and three plant combinations. The combination of three plants produced 85 formulas, and the combination of 2 plants produced 31 formulas. In the results of the combination formula with two plants, it was only able to bind five proteins (Table 3), and the three-plant combination formula resulted in the binding of all six hypertensive proteins (Table 4).

DISCUSSION

Hypertension is a global health problem and is one of the causes of increased morbidity and mortality in Indonesia. Jamu is widely used in Indonesia to treat many ailments, such as diarrhea, diabetes, heart disease, and hypertension. In this study, we used a network pharmacology approach to identify how a compound acts and its interaction with multiple targets and the molecular mechanism. First, we collect the database of herbal compounds and the key protein of hypertension based on Setiani *et al.* [8] and search features of dipeptide protein. The search for protein dipeptide features represents protein features as amino acid sequences in numerical form so that they can be processed by a computer system and then processed to calculate the similarity between proteins. The compounds that interact with 35 significant proteins for Binding DB and DrugBank binding were searched. Binding DB is a database used to find protein targets. The results of searching for protein targets yielded 2,036 chemical compounds that targeted 34 proteins, and 1 protein had no interaction data. The results of this data mining provided information on the names and IDs of compounds that had significant interactions with proteins. These data were used to compile training data that were used to compare the predicted interaction data of compounds that interact with proteins. Data on protein fasteners were obtained from UniProt, and 20 target protein fasteners were obtained to produce protein dipeptides. Then, the FASTA protein data were entered into the feature web to obtain the dipeptide. Then, we combined protein dipeptides with the compound's fingerprint to predict the interaction using Orange. This process aims to obtain the predictions of protein-compound interactions as indicated by the probability score. The closer the probability score is to one, the more likely it is that compounds and proteins will interact. The probability score calculation yielded 1,015 interactions of compounds and proteins with a probability score greater than 0.5, and we searched for plant-compound-protein interaction using graph mining analysis. Graph Mining, a technique used to analyze the properties of real-world graphs, predicts how the structure and properties of a given graph might affect some application and develop models that can generate realistic graphs that match the patterns found in real-world graphs of interest [12] and construct the plant-compound-protein interaction using Cytoscape.

Table 4. Combination formula of three plants.

No.	Target proteins	Plant formulas
1	PPARA, ESR1, VEGFA, MAPK3, EDN1, FOS	*P. retrofractum, M. indica, C. papaya*
2		*P. retrofractum, M. indica, C. limon*
3		*P. retrofractum, M. indica, C. arabica*
4		*P. retrofractum, M. indica, A. altilis*
5		*P. retrofractum, M. indica, A. graveolens*
6		*P. retrofractum, M. indica, C. sinensis*
7		*P. retrofractum, M. indica, C. aurantiifolia*
8		*P. retrofractum, M. indica, F. vulgare*
9		*P. retrofractum, M. indica, M. pudica*
10		*P. retrofractum, M. indica, P. pruatjan*
11		*P. retrofractum, M. indica, V. trifolia*
12		*P. retrofractum, M. indica, B. rotunda*
13		*P. retrofractum, C. roseus, C. papaya*
14		*P. retrofractum, C. roseus, C. limon*
15		*P. retrofractum, C. roseus, C. arabica*
16		*P. retrofractum, C. roseus, A. altilis*
17		*P. retrofractum, C. roseus, A. graveolens*
18		*P. retrofractum, C. roseus, C. sinensis*
19		*P. retrofractum, C. roseus, C. aurantiifolia*
20		*P. retrofractum, C. roseus, F. vulgare*
21		*P. retrofractum, C. roseus, M. pudica*
22		*P. retrofractum, C. roseus, P. pruatjan*
23		*P. retrofractum, C. roseus, V. trifolia*
24		*P. retrofractum, C. roseus, B. rotunda*
25		*P. retrofractum, G. max, C. papaya*
26		*P. retrofractum, G. max, C. limon*
27		*P. retrofractum, G. max, C. arabica*
28		*P. retrofractum, G. max, A. altilis*
29		*P. retrofractum, G. max, A. graveolens*
30		*P. retrofractum, G. max, C. sinensis*
31		*P. retrofractum, G. max, C. aurantiifolia*
32		*P. retrofractum, G. max, F. vulgare*
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51		*P. retrofractum, P. sativum, C. arabica*
52		*P. retrofractum, P. sativum, A. altilis*
53		*P. retrofractum, P. sativum, A. graveolens*
54		*P. retrofractum, P. sativum, C. sinensis*
55		*P. retrofractum, P. sativum, C. aurantiifolia*
56		*P. retrofractum, P. sativum, F. vulgare*
57		*P. retrofractum, P. sativum, M. pudica*
58		*P. retrofractum, P. sativum, P. pruatjan*
59		*P. retrofractum, P. sativum, V. trifolia*
60		*P. retrofractum, P. sativum, B. rotunda*
61		*P. retrofractum, R. sativus, C. papaya*
62		*P. retrofractum, R. sativus, C. limon*
63		*P. retrofractum, R. sativus, C. arabica*
64		*P. retrofractum, R. sativus, A. altilis*
65		*P. retrofractum, R. sativus, A. graveolens*
66		*P. retrofractum, R. sativus, C. sinensis*
67		*P. retrofractum, R. sativus, C. aurantiifolia*
68		*P. retrofractum, R. sativus, F. vulgare*
69		*P. retrofractum, R. sativus, M. pudica*
70		*P. retrofractum, R. sativus, P. pruatjan*
71		*P. retrofractum, R. sativus, V. trifolia*
72		*P. retrofractum, R. sativus, B. rotunda*
73		*P. retrofractum, Z. mays, C. papaya*
74		*P. retrofractum, Z. mays, C. limon*
75		*P. retrofractum, Z. mays, C. arabica*
76		*P. retrofractum, Z. mays, A. altilis*
77		*P. retrofractum, Z. mays, A. graveolens*
78		*P. retrofractum, Z. mays, C. sinensis*
79		*P. retrofractum, Z. mays, C. aurantiifolia*
80		*P. retrofractum, Z. mays, F. vulgare*
81		*P. retrofractum, Z. mays, M. pudica*
82		*P. retrofractum, Z. mays, P. pruatjan*
83		*P. retrofractum, Z. mays, V. trifolia*
84		*P. retrofractum, Z. mays, B. rotunda*
85		*P. retrofractum, tomato, C. papaya*

Six proteins linked to hypertension were discovered in Indonesian plants in this study. MAPK is a mitogen-activated protein kinase involved in smooth muscle cell proliferation and vasoconstriction. Therefore, MAPK is a potential target for hypertension therapy [13]. Trans-zeatin compounds and their derivatives can inhibit MAPK activation, reduce vasoconstriction, and dilate blood vessels [14]. Trans-zeatin, an important class of plant hormones, is a potential anti-aging agent. Studies show that zeatin may help protect against cognitive dysfunction, and antioxidants may play specific roles in various pathological conditions such as pain, inflammation,

cancer, and vascular dysfunction. It is known to fight free radicals [15].

Compound-plant interactions in PPARA proteins have multiple functions involved in regulating vascular tone, inflammation, and energy homeostasis, which are important targets of hypertension [16]. The compound p-coumaric acid is known to protect against the incidence of hypertension induced by a high-fructose diet [17].

Estrogen contributes to the development of hypertension associated with systemic disease, targets multiple organ damage, and has broad regulatory effects. Estrogen effects cause changes in the sympathetic nervous system, the renin-angiotensin-aldosterone system (RAAS), body weight, oxidative stress, endothelial function, salt sensitivity, and the regulatory mechanisms of all associated inflammatory conditions, ultimately resulting in changes in genetic factors. It affected heart, blood vessels, and kidney damage [18].

Indonesians have empirically used celery to treat high blood pressure. The results of this study show that consuming celery leaf juice or water lowers blood pressure in people with hypertension. The apigenin compound found in celery is known to act as a beta-blocker, slowing the heart rate, reducing the strength of heart contractions, reducing blood pumping, and lowering blood pressure. Apigenin, which is a natural flavonoid, affects the contractility of smooth muscles in blood vessels (a vasodilator); the mechanism of contraction occurs because of an increase in calcium in cells, which causes cytosolic calcium to increase and triggers the contraction of blood vessels, thereby increasing blood pressure [19]. When it occurs in myocardial cells, it increases the contraction of the heart muscle, making it harder for the heart to pump and increasing blood pressure. Apiin is thought to have diuretic properties, which help the kidneys remove excess fluid and salt from the body, resulting in reduced fluid in the blood, which will cause decreased blood pressure. Potassium in celery increases intracellular fluid by attracting extracellular fluid, altering the sodium-potassium pump balance, and lowering blood pressure. One strategy for treating hypertension is to alter the Na^+ balance, which is normally achieved by the oral administration of diuretics [20].

EDN1 is an amino acid produced by endothelial cells within blood vessels. This protein exerts an autocrine effect on endothelial cells via the endothelin b receptor, induces nitric oxide (NO) and prostacyclin attack, and exerts vasodilatory effects [21]. One compound that binds to the EDN1 target protein is sesamin, found in several Indonesian plants.

Sesamin compounds are regarded to have antioxidant and anti-inflammatory properties. Several studies have shown that oxidative stress and inflammation play a major role in various cardiovascular diseases. The data suggest that RAS/MAPK, PI3K/AKT, ERK1/2, p38, p53, IL-6, TNFa, and NF-κB signaling are all involved in regulating the effects of sesamin on cardiovascular disease. It shows that those risk factors are involved. Experimental trials show that sesamin can reduce cardiovascular risk. Sesamin has the potential as an adjunctive therapy to treat cardiovascular disease and many other risk factors [22].

VEGF is a potent angiogenic factor that affects blood pressure and causes NO-mediated vasodilation. ACE inhibitors regulate VEGF expression. The results of this study suggest that genetic polymorphisms in the VEGFA gene may influence the antihypertensive response to enalapril, a drug belonging to the ACE inhibitor class [23]. Transcoumaric acid compounds have been shown to promote angiogenesis by increasing the expression of VEGFA and Flk-1/KDR, thereby ameliorating vascular microcirculatory dysfunction [24].

FOS proteins are associated with the pathophysiology of hypertension. The results of this study suggest that FOS is associated with blood pressure response to thiazide diuretic treatment and may be a determinant of the molecular mechanisms of hypotension, but the underlying FOS regulation and response are unclear. Further research is needed to understand the underlying mechanisms in hypertension which affect blood pressure [25]. Seselin showed anti-inflammatory effects through its action on Jak2. These results demonstrate the potential application of seselin to treat inflammatory diseases by blocking the development of the inflammatory phenotype in macrophages [26].

We reduced the plant based on TCM, native Indonesian plants, B2P2TOOT Ministry of Health, and we have 78 plants, of which 21 plants targeting 6 proteins could be made into a formula. The combination of 3 plants produced 85 formulas, and the combination of 2 plants produced 31 formulas. An example of the combination of two plants that bind five hypertension target proteins can be given, including the combination of *P. retrofractum* and *M. indica*, which binds to the EDN1, PPARA, ESR1, VEGFA, and MAPK3 proteins. *Piper retrofractum* is a tropical plant native to Indonesia and has been used as a traditional medicine for generations. Traditionally, *P. retrofractum* can be mixed in concoctions to treat several problems, such as colds, fever, headaches, high blood pressure, and shortness of breath. The main compounds in *P. retrofractum* are piperine, piperonaline, guinein, and essential oils. Piperine compounds have aphrodisiac, anti-inflammatory, analgesic, antioxidant, and immunomodulatory properties [27].

According to the data mining extraction results, the compounds found in *P. retrofractum* include sesamin, which has antioxidant and anti-inflammatory properties. Several studies have shown that oxidative stress and inflammation play important roles in various cardiovascular diseases, and experimental results show that sesamin can reduce the risk of cardiovascular disease [22]. *Mangifera indica* plants grow a lot in Indonesia. *Mangifera indica*, which consists of leaves, fruit, fruit skin, and stems, is a type of plant used to treat hypertension because of its flavonoid content, which has a role in preventing oxidative stress. As shown in several research results, the content of the mangiferin compound in *M. indica* leaves is known to reduce blood pressure in rats with hypertension [28].

According to the data mining extraction results, various chemicals in the *M. indica* plant have a mechanism for lowering blood pressure. The results of this study demonstrate that caffeic acid content can modulate the RAAS inhibitory pathway with lower toxicity symptoms compared to conventional antihypertensive drugs, indicating new potential as an antihypertensive drug [29]. Ferulic acid shows very promising activity on blood pressure regulation and modulates

left ventricular dysfunction and cardiac hypertrophy in both human and animal studies, as well as in decreasing plasma lipids in experimental animals [30]. The antihypertensive effect of p-coumaric acid is closely related to various mechanisms. The antioxidant properties of phenolic acids can restore endothelial function in rats suffering from spontaneous hypertension by increasing the bioavailability of NO [17].

The combination of three plants that bind six protein targets for hypertension (PPARA, ESR1, VEGFA, MAPK3, EDN1, and FOS) illustrates the existence of several mechanisms involved in reducing blood pressure between protein-plant compounds. In this case, protein is a potential biomarker that can be a therapeutic target in the next few years. The protein-compound mechanism represented in plants is a potential target for creating a new combination of plant formulas, which will then be developed into discoveries in antihypertensive therapy. From the results of this study, one formula can be taken with a combination of two or three plants, which is then formulated for doses to test the effect and safety. An alternative to selecting plant formulas can be based on convenience and availability because each formula targets several pathways of the antihypertensive mechanism. However, what needs to be taken into consideration is that this study did not see how many compounds were contained in each plant, which might affect the antihypertensive effect of each formula.

CONCLUSION

In this study, we obtained six hypertension target proteins (MAPK3, PPARA, ESR1, EDN1, VEGFA, and FOS) found in 78 Indonesian plants, which were found in a combination of two and three plants. The combination of three plants that bind six hypertension-related protein targets demonstrates the existence of various processes implicated in blood pressure reduction between protein-plant combinations. Antioxidants and anti-inflammatories are among the blood pressure-lowering mechanisms found in several of these plants. Plant compounds can modify the mechanism of inhibition of the RAAS and restore endothelial function, which plays a role in blood pressure regulation.

LIST OF ABBREVIATIONS

ACE: angiotensin-converting enzyme; B2P2TOOT: Balai Besar Penelitian dan Pengembangan Tanaman Obat dan Obat Tradisional; CID: compound identity number; CSV: comma separated value; EDN1: endothelin 1; ESR1: estrogen receptor 1; FOS: fos proto-oncogene; MAPK3: mitogen-activated protein kinase 3; PPARA: peroxisome proliferator-activated receptor alpha; TCM: traditional Chinese medicine; VEGFA: vascular endothelial growth factor A.

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AUTHOR CONTRIBUTIONS

All authors made substantial contributions to the conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

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