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# Pharmacological and molecular mechanism of *Syzygium polyanthum* leaves as antihypertensive with network pharmacology approach

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**Abstract**

**Background:** In Central Sulawesi, Indonesia, *Syzygium polyanthum* leaves (SPL) have been traditionally used by society to reduce high blood pressure. However, the molecular mechanism behind this effect still remains unclear.

**Objective:** This study aims to explore the antihypertensive molecular mechanisms of SPL using a network pharmacology approach.

**Method:** Previous studies have investigated some bioactive compounds contained in SPL. Those studies identified 57 bioactive compounds, of which 31 were filtered for further analysis. Target proteins from SPL and disease were identified using GeneCards. Then, 62 selected target proteins which overlapped through Venn diagrams would be analyzed using Cytoscape software to produce compounds-target proteins' network. Protein-protein interactions were assessed using STRING and visualized by Cytoscape. GO Function and KEGG Pathways were obtained using ShinyGo.

**Results:** The results of this study revealed pathways associated with hypertension through the cellular senescence pathway with target proteins mTOR, ERK (also known as MAPK), p53, CycD, and IL6. The chemical constituents associated with these target proteins are hydroxychavicol, farnesol, naphthalene, phloretin, pyrogallol, and decanal, which were identified as compounds closely related to the target protein of hypertension.

**Conclusion:** The bioactive compounds in SPL play a significant role in regulating hypertension by influencing various target genes, particularly those associated with cellular senescence.

**Keywords:** Antihypertensive, bay leaf, cellular senescence, network pharmacology, *Syzygium polyanthum*

## 1. Introduction

Hypertension is a disease with a high prevalence rate, not only in Indonesia but also all over the world. The number of hypertensive patients is increasing every year and is estimated to reach 1.56 billion in 2025 worldwide (Kearney *et al*., 2005; Ventura & Lavie, 2019). Hypertension can also be a comorbid disease of other diseases such as heart disease, stroke, chronic kidney disease, central organ damage, and various other secondary diseases (Di Palo & Barone, 2020; Fan *et al*., 2019; Pistoia *et al*., 2016; Wenzel *et al*., 2017), which can cause damage and affect the patient's life.

Currently, antihypertensive drugs are divided into five categories based on their pharmacological mechanisms. These categories include β-blockers, diuretics, angiotensin-converting enzyme inhibitors, angiotensin II receptor antagonists, and calcium channel blockers. Unfortunately, while these drugs offer beneficial effects, they can also cause side

effects. These side effects such as for diuretic category drugs can result uremia and hyperglycemia (Blowey, 2016). Other side effects such as brain freeze and risk of Alzheimer's disease (Rouzaud-Laborde *et al.*, 2018). Therefore, there is a need to develop a new and safe antihypertensive drug.

On the other hand, traditional antihypertensive treatment has been used for many years in Indonesia. One of those traditional formula is infusion of *Syzygium polyanthum* leaves (SPL) which has been recorded in the Original Indonesian Herbal Medicine Formulary (Formularium Obat Herbal Asli Indonesia Volume III, 2013). This formula comes from Central Sulawesi Province. Traditionally, people with hypertension boil leaves of SPL and drink it twice a day, morning and night. On the other hand, from the previous literature, SPL extract contains the bioactive compounds squalene, hexadecenoic acid, methyl ester, 9,12-Octadecadienoic acid, vitamin E, stigmasterol (Widyawati *et al*., 2022). An in vivo study revealed that SPL can reduce blood pressure (Ismail & Wan Ahmad, 2017).

Network pharmacology is a tool developed based on biological and pharmacological systems from various directions. This means that the exploration of mechanisms will focus on many proteins/genes that are targeted by a drug/compound, not just on one specific target (Luo *et al.*, 2020). This study aims to utilize the network pharmacology method associated with SPL for treating hypertension, particularly focusing on the active compounds of SPL against targets related to hypertension. The general workflow of this study presents in Figure 1. Hopefully, the results of this study can provide a reference for further pre-clinical and clinical research.

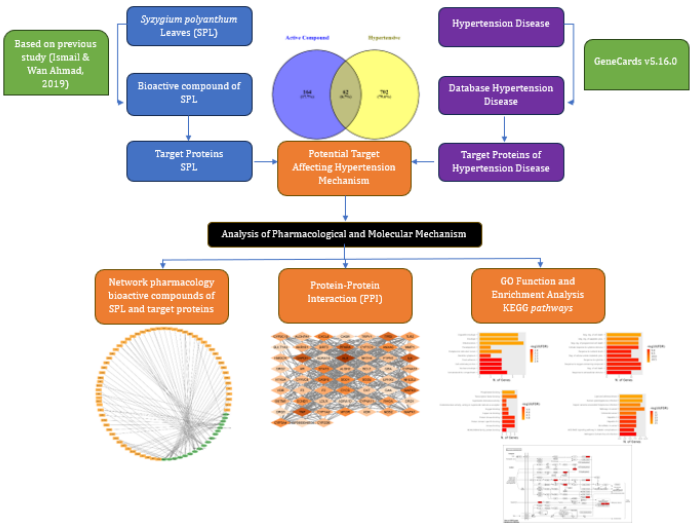


**Figure 1.** General workflow network pharmacology SPL against hypertension

## 2. Methodology

*2.1 Bioactive compounds in Syzygium polyanthum*

Bioactive compounds contained in SPL were obtained from previous studies (Ismail & Wan Ahmad, 2019). The simplified-molecular-input-line-entry-specification (SMILES) of all the compounds collected was traced via PubChem (https://pubchem.ncbi.nlm.nih.gov/) and the pharmacokinetic data of all the compounds was obtained through pkCSM (https://biosig.lab.uq.edu.au/pkcsm/prediction). While toxicity data profile was obtained through Tox-Prediction (https://tox-new.charite.de/protox_II/index.php?site=compound_input). After all these data were collected, data was filtered based on pharmacokinetic principles and the Lipinsky rules of five such as molecular weight (< 500 Dalton), LogP (< 5), hydrogen bond acceptors (< 10), hydrogen bond donors (< 5), intestinal absorption (human) (≥ 80), VDss (human) (> -0.15), CYP2D6 substrate (No), renal OCT2 substrate (No), and toxicity prediction category (> 3). All databases were accessed on June 10, 2023.

*2.2 Collection of protein target from bioactive compound*

After obtaining the bioactive compounds that have been filtered based on the previous method described, then the proteins target of bioactive compounds was also obtained. Protein target from bioactive compound SPL were collected using GeneCards v5.16.0https://www.genecards.org/ (accessed on June, 10 2023). Top 25 target proteins were selected from among the many target proteins displayed.

*2.3 Collection of protein target database related to hypertension disease*

Protein target related to hypertension disease were obtained through GeneCards v5.16.0 (https://www.genecards.org/) by entering the keywords “hypertensive” and “high blood pressure” in the target protein search field. The target proteins associated with the two keywords were collected for further analysis. This involved compiling data from both sets and removing any duplicates. The results of that compilation were then intersected with the target protein from the bioactive compound SPL which had been obtained in the previous stage. The intersection was performed using Venny 2.1.0 (https://bioinfogp.cnb.csic.es/tools/venny/). All databases were accessed on June, 10 2023.

*2.4 Compound and protein target construction network*

To understand the molecular mechanism of the active compound in SPL, an analysis of the connection between the active compound and the target protein was carried out using software Cytoscape v3.10.0. Later, the results of network interactions between the active compounds and the target protein will be obtained. The existence of this interaction is indicated by a line connecting the two (active compound and target protein).

*2.5 Protein-protein interaction network*

At this stage, the results of interactions between proteins will be obtained, named as Protein-Protein Interaction (PPI). The interactions were obtained using STRING v11.5 (https://string-db.org/) (accessed on June, 10 2023). The PPI network is obtained by selecting the minimum required interaction score ≥ 0.4. The PPI results will provide visual data along with scores for Degree Centrality, Closeness Centrality, and Betweenness Centrality. For each protein, the scores from these three categories are combined. Proteins with a total score of ≥ 3 are selected, resulting in the top 20 target proteins.

*2.6 Enrichment analysis through GO functions and KEGG pathways*

Functional enrichment analysis of gene ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG pathways) were collected using ShinyGo v0.77 (http://bioinformatics.sdstate.edu/go/) (accessed on June, 10 2023). This method was done to determine the potential targets that should be used in the treatment of hypertension. Enter the names of the selected target genes (those with the top 20 highest scores from the previous PPI analysis) in the search field and select the species "human". After that, it was analyzed based on Biological Process (BP), Cellular Component (CC), Molecular Function (MF), and KEGG. From KEGG pathways, we can find out the potential bioactive compounds that have significant role in molecular pathway of this disease. These compounds were collected by tracing the protein target of bioactive compound that related to protein target showed in KEGG pathways with red color.

## 3. Results and discussion

The Indonesian people, especially those from Poso Regency, Central Sulawesi Province, used the infusion of SPL to reduce high blood pressure for many years. The SPL formula contains

multiple components and multiple targets that align with hypertension disease. However, investigating the pharmacological and molecular mechanism have its own challenges. Network pharmacology, as a tool for exploring this mechanism, could potentially be an effective method for unveiling the mechanisms of SPL against hypertension. In this study, a network pharmacology approach was used to identify the bioactive compounds in SPL as well as potential targets, and to explore the potential mechanisms of SPL in the treatment of hypertension.

Previous study revealed that infusion of SPL given orally to Wistar strain rats with hypertension models at doses of 0.01, 0.1, 1 and 10 mg/mL gave the greatest aortic vasorelaxation effect at a dose of 10 mg/mL with a relaxation percentage of 72.58% ($p < 0.001$) (Ismail & Wan Ahmad, 2016). Similarly, other research related to toxicity SPL, methanol extract of SPL showed liver damage in male rats at dose 2000 mg/kg (Harun *et al*., 2021). However, the mechanism that plays a role in reducing high blood pressure from the bioactive compound SPL is still unknown (Ismail & Wan Ahmad, 2016).

*3.1 Bioactive compounds in Syzygium polyanthum*

There are 57 bioactive compounds contained in SPL according to previous studies (Ismail & Wan Ahmad, 2019). The prediction of physicochemical and pharmacokinetic properties was carried out through pkCSM database while the prediction of toxicological properties was carried out through Tox-prediction database. Among the 57 bioactive compounds, molecular weight range from 76.095 to 436.853 g/mol. Propylene glycol has the lowest molecular weight, while hentriacontane boasts the highest. Furthermore, 41 compounds exhibited LogP values below 5, 56 compounds had HBA values below 10, and all 57 compounds had HBD values less than 5. The results of the bioactive compounds that had been filtered for several parameters are 38 compounds and shown in Table 1. Meanwhile, the number of bioactive compounds that met the pharmacokinetic and toxicological screening criteria were 31 compounds and shown in Table 2. Out of 31 compounds, 12 of them belong to the terpenoid group. Terpenoid compounds which can also be found from other plant essential oils have been shown to have an activity on reducing blood pressure in rats (Menezes *et al*., 2010).

**Table 1.** Physicochemical properties of bioactive compounds that meets screening criteria

| No | Compound | PubChem ID | BMt | logP | Rotatable Bonds | HBA | HBD | Surface Area |
|---|---|---|---|---|---|---|---|---|
| 1 | 1-(2,3,5-trihydroxy methylphenyl)octane-1-one | 132281267 | 294,391 | 2.7067 | 10 | 4 | 3 | 125.99 |
| 2 | 1-(2,3,5-trihydroxy-4-methylphenyl)decan-1-one | 129862762 | 294,391 | 4.43522 | 9 | 4 | 3 | 125.99 |
| 3 | 1-(2,3,5-trihydroxy-4-methylphenyl)hexane-1-one | 132275589 | 238,283 | 2.87482 | 5 | 4 | 3 | 100.53 |
| 4 | 1H cyclopropa[a]naphthalene | 12343165 | 140,185 | 2,744 | 0 | 0 | 0 | 65,472 |
| 5 | 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one | 119838 | 144,126 | -0.2639 | 0 | 4 | 2 | 57,732 |
| 6 | 4-allyl-1,2-dihydroxybenzene (hydroxychavicol) | 70775 | 150,177 | 1.8263 | 2 | 2 | 2 | 65,425 |
| 7 | 4-hydroxy-3,5-dimethoxy benzoic acid | 10947890 | 198,174 | 1.1076 | 3 | 4 | 2 | 80503 |
| 8 | 4-hydroxy-3-methoxy benzoic acid | 8468 | 168,148 | 1,099 | 2 | 3 | 2 | 69,025 |
| 9 | Azulene | 9231 | 128,174 | 2.7914 | 0 | 0 | 0 | 60,468 |
| 10 | Caffeic acid | 689043 | 180,159 | 1.1956 | 2 | 3 | 3 | 74,381 |
| 11 | Caryophyllene oxide | 1742210 | 220,356 | 3.9364 | 0 | 1 | 0 | 99,255 |
| 12 | Decanal | 8175 | 156,269 | 3,326 | 8 | 1 | 0 | 70,185 |
| 13 | Farnesol | 445070 | 222,372 | 4.3979 | 7 | 1 | 1 | 100,574 |
| 14 | gallic acid | 370 | 170.12 | 0.5016 | 1 | 4 | 4 | 67,135 |
| 15 | Heptane | 8900 | 100,205 | 2.9767 | 4 | 0 | 0 | 46,929 |
| 16 | Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) (palmitin) | 129853056 | 330,509 | 4.3641 | 17 | 4 | 2 | 142,172 |
| 17 | Humulene epoxide II | 10704181 | 220,356 | 4.2466 | 0 | 1 | 0 | 99,571 |
| 18 | Linalool | 6549 | 154,253 | 2.6698 | 4 | 1 | 1 | 69,439 |
| 19 | Nerolidol | 5284507 | 222,372 | 4.3963 | 7 | 1 | 1 | 100,574 |
| 20 | n-heptanal | 8130 | 114,188 | 2.1557 | 5 | 1 | 0 | 51.09 |
| 21 | Octanal | 454 | 128,215 | 2.5458 | 6 | 1 | 0 | 57,455 |
| 22 | Phloretin | 4788 | 274,272 | 2.3245 | 4 | 5 | 4 | 114,922 |
| 23 | Propylene glycol | 1030 | 76,095 | -0.6405 | 1 | 2 | 2 | 31058 |
| 24 | Pyrogallol | 1057 | 126,111 | 0.8034 | 0 | 3 | 3 | 51,814 |
| 25 | Selina-4,11-diene (naphthalene) | 931 | 128,174 | 2.8398 | 0 | 0 | 0 | 60,113 |
| 26 | Valencia | 9855795 | 204,357 | 4.7252 | 1 | 0 | 0 | 94,458 |
| 27 | α-caryophyllene | 5281520 | 204,357 | 5.0354 | 0 | 0 | 0 | 94,774 |
| 28 | α-copaene | 19725 | 204,357 | 4.2709 | 1 | 0 | 0 | 94,141 |
| 29 | α-cubebene | 442359 | 204,357 | 4.2709 | 1 | 0 | 0 | 94,141 |
| 30 | α-paninsense | 578929 | 204,357 | 4.5591 | 0 | 0 | 0 | 94,141 |
| 31 | α-pinene | 6654 | 136,238 | 2.9987 | 0 | 0 | 0 | 63,322 |
| 32 | α-selinene | 10856614 | 204,357 | 4.7252 | 1 | 0 | 0 | 94,458 |
| 33 | α-zingiberene | 11127403 | 204,357 | 4.8913 | 4 | 0 | 0 | 94,774 |
| 34 | β-caryophyllene | 5281515 | 204,357 | 4.7252 | 0 | 0 | 0 | 94,458 |
| 35 | β-panasinsene | 595133 | 204,357 | 4.5591 | 0 | 0 | 0 | 94,141 |
| 36 | β-selinene | 442393 | 204,357 | 4.7252 | 1 | 0 | 0 | 94,458 |
| 37 | δ-cadinene | 441005 | 204,357 | 4.7252 | 1 | 0 | 0 | 94,458 |

**Table 2**. Pharmacokinetic properties of bioactive compounds that meet screening criteria

| Compound | Water solubility | GI absorption | VDSs | BBB permeability | CYP2D6 inhibitors | Skin Permeability | Total Clearances | Renal OCT2 substrates | Predicted LD50 (mg/kg) | Predicted Toxicity Class |
|---|---|---|---|---|---|---|---|---|---|---|
| 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one | -0.699 | 83,475 | 0.017 | -0.3 | No | -3,722 | 0.505 | No | 595 | 4 |
| n-heptanal | -2,406 | 95,432 | 0.115 | 0.546 | No | -1,571 | 0.464 | No | 5000 | 5 |
| Pyrogallol | -1,408 | 83,549 | 0.13 | -0.441 | No | -2,751 | 0.104 | No | 300 | 3 |
| Linalool | -2,612 | 93,163 | 0.152 | -0.598 | No | -1,737 | 0.446 | No | 2200 | 5 |
| Octanal | -3,059 | 95,081 | 0.18 | 0.668 | No | -1,391 | 1.53 | No | 5000 | 5 |
| Decanal | -4,337 | 94,394 | 0.288 | 0.705 | No | -1,319 | 1,608 | No | 5000 | 5 |
| Heptane | -3,639 | 94,145 | 0.291 | 0.77 | No | -1,169 | 1,483 | No | 750 | 3 |
| Farnesol | -5,393 | 91,531 | 0.36 | 0.66 | No | -1,514 | 1,754 | No | 5000 | 5 |
| Nerolidol | -5,176 | 91,887 | 0.37 | 0.652 | No | -1,477 | 1,739 | No | 5000 | 5 |
| Humulene epoxide II | -4,162 | 95,737 | 0.46 | 0.669 | No | -2,988 | 1,065 | No | 5000 | 5 |
| 4-allyl-1,2-dihydroxybenzene (hydroxychavicol) | -1,379 | 92.09 | 0.477 | 0.361 | No | -2,523 | 0.206 | No | 1930 | 4 |
| Selina-4,11-diene (naphthalene) | -3,496 | 95157 | 0.488 | 0.434 | No | -1,433 | 0.198 | No | 316 | 4 |
| α-caryophyllene | -5,191 | 94,682 | 0.505 | 0.663 | No | -1,739 | 1,282 | No | 3650 | 5 |
| Azulene | -3,654 | 95,451 | 0.519 | 0.77 | No | -1,649 | 0.208 | No | 300 | 5 |
| Caryophyllene oxide | -4,433 | 95,421 | 0.586 | 0.654 | No | -3,076 | 0.905 | No | 5000 | 5 |
| 1-(2,3,5-trihydroxy-4-methylphenyl)hexane-1-one | -2.4 | 91,194 | 0.594 | -0.934 | No | -2,777 | 0.334 | No | 3320 | 5 |
| α-zingiberene | -5,967 | **95561** | 0.629 | **0.796** | No | -1,273 | 1,441 | No | 1680 | 4 |
| β-selinene | -6,439 | 85,574 | 0.639 | 0.816 | No | -1,702 | 1,174 | No | 5000 | 5 |
| 1-(2,3,5-trihydroxy-4-methylphenyl)decan-1-one | -3,032 | 89.8 | 0.646 | -1,051 | No | -2,756 | 1021 | No | 3320 | 5 |
| β-caryophyllene | -5,555 | **94,845** | 0.652 | 0.733 | No | -1.58 | 1,088 | No | 5300 | 5 |
| α-pinene | -3,733 | 96041 | 0.667 | 0.791 | No | -1,827 | 0.043 | No | 3700 | 5 |
| β-panasinsene | -6.103 | 94,914 | 0.682 | 0.825 | No | -2,086 | 0.866 | No | 5000 | 5 |
| α-selinene | -6,074 | **94,127** | **0.686** | **0.776** | No | -1,461 | 1.172 | No | 5000 | 5 |
| Valencia | -6,074 | 94,127 | 0.686 | 0.776 | No | -1,461 | 1.205 | No | 5000 | 5 |
| δ-cadinene | -5,915 | 96128 | 0.689 | 0.773 | No | -1,462 | 1,182 | No | 4390 | 5 |
| α-cubebene | -5,968 | 95,964 | 0.717 | 0.86 | No | -1,997 | 0.98 | No | 5000 | 5 |
| 1H cyclopropa[a]naphthalene | -3,825 | 96028 | 0.719 | 0.463 | No | -1,736 | 0.13 | No | 2250 | 5 |
| Phloretin | -3,077 | 60.5 | 0.765 | -0.927 | No | -2,735 | 0.213 | No | 500 | 4 |
| α-paninsense | -5,774 | 94,759 | 0.78 | 0.792 | No | -1,743 | 0867 | No | 5000 | 5 |
| α-copaene | -5,705 | **96,221** | 0.806 | 0887 | No | -2,225 | 0.95 | No | 3700 | 5 |
| 1-(2,3,5-trihydroxy methylphenyl)octane-1-one | -2,972 | 93,636 | 0.825 | -0.829 | No | -2,775 | 1,427 | No | 2220 | 5 |

*3.2 Collection of bioactive compounds dan disease target protein*

There are 226 target proteins from bioactive compounds contained in SPL that obtained from GeneCards. Meanwhile, the target protein for hypertension was also obtained using

GeneCards with the 2 keywords "hypertensive" and "high blood pressure". Each of these keywords is taken from 1000 target protein data with the highest score. 2000 target proteins from each keyword were then remove duplicate data and 764 target proteins were collected. The 764 target proteins were then intersected with 226 target proteins belong to the bioactive compound SPL, resulting overlapped 62 target proteins were obtained (Figure 2).

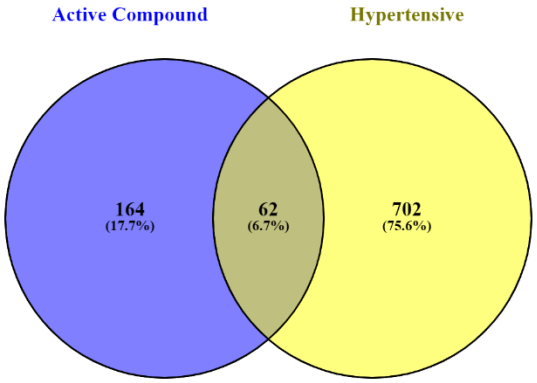


**Figure 2.** Venn diagram of slices between target proteins for hypertension disease and target proteins of bioactive compounds

*3.3 Construction of compound-target network*

The intersection between the bioactive compound (which already met the previous requirements criteria) to the target protein (venn diagram intersection results) was then analyzed using Cytoscape software to construct the network. The results of this network of bioactive compounds with target proteins are shown in Figure 3. There are 80 nodes and 87 edges, where each oval shape and green color represents bioactive compounds. While the square shaped and orange color represents the target protein. From the network results, there are 87 interactions between bioactive compounds and target proteins which are indicated by straight lines in gray. This shows that the bioactive compounds contained in SPL may work synergistically on several targets so that they can produce pharmacological effects on hypertension. From the network construction results, it was found that naphthalene compounds have interactions with the highest number of target proteins, which is 10 interactions. This is followed by phloretin, which has 9 interactions. Decanal and farnesol have 8 interactions each. Then, pyrogallol and octanal have 7 and 6 interactions, respectively. From the target proteins perspective, it was found that target protein with the highest interactions were CYP3A4, HMGCR, SOD1, ALB, CYP21A2, XDH and ANXA5, they have 3 interactions in each.

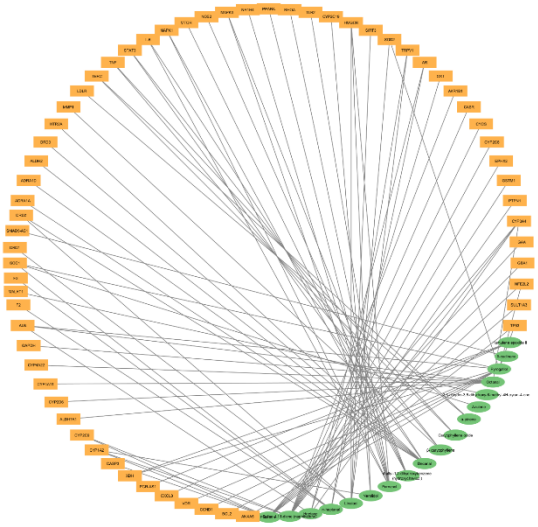

**Figure 3.** Cytoscape visualization of the relationship between bioactive compounds and target proteins for hypertension disease

*3.4 Protein-protein interaction network*

The 62 target proteins resulting from the Venn intersection (Figure 2) were further analyzed using STRING to see the interactions between these target proteins. From the results of the STRING, tsv format file have been downloaded, which will then be further analyzed and visualized using Cytoscape software. There are 59 nodes and 430 edges, where each node represents the target protein and the edges represent the number of interactions between the target proteins. The PPI results are presented in Figure 4. The tsv format file download from STRING contains information about the degree, betweenness centrality, and closeness centrality scores. The sum of those three categories was done. Visualization from Cytoscape shows the different colors of each node, the darker the node color, the higher the total degree, betweenness centrality and closeness centrality scores. On the other hand, the lighter the color shown at the node, the lower the value of degree, betweenness centrality, and closeness centrality. The details of the scores along with the number of degrees, betweenness centrality, and closeness centrality of each target protein are shown in Table 3. The highest number of scores is in the albumin target protein (ALB) with a total score of 45.033, while the Alpha Glucosidase (GAA) target protein has the lowest value in 1.348.

**Table 3.** Value result of degree, betweenness centrality, and closeness centrality protein targets

| Number | Protein name | Betweenness Centrality | Closeness Centrality | Degrees | Total |
|---|---|---|---|---|---|
| 1 | ALB | 0.209149459 | 0.823529412 | 44 | 45.0327 |
| 2 | GAPDH | 0.066575313 | 0.7 | 35 | 35.7666 |
| 3 | IL6 | 0.05618581 | 0.708860759 | 35 | 35,765 |
| 4 | TNF | 0.048932441 | 0.7 | 35 | 35.7489 |
| 5 | PPARA | 0.076618359 | 0.674698795 | 33 | 33.7513 |
| 6 | TP53 | 0.041986125 | 0.651162791 | 31 | 31.6931 |
| 7 | MAPK3 | 0.021296963 | 0.636363636 | 28 | 28.6577 |
| 8 | CASP3 | 0.008709343 | 0.615384615 | 26 | 26.6241 |
| 9 | STAT3 | 0.009466062 | 0.608695652 | 25 | 25.6182 |
| 10 | CXCL8 | 0.009371602 | 0.608695652 | 24 | 24.6181 |
| 11 | CYCS | 0.008527892 | 0.595744681 | 23 | 23.6043 |
| 12 | SOD1 | 0.01744979 | 0.602150538 | 22 | 22.6196 |
| 13 | MTOR | 0.002989225 | 0.589473684 | 22 | 22.5925 |
| 14 | ANXA5 | 0.007723095 | 0.577319588 | 22 | 22,585 |
| 15 | CYP3A4 | 0.034169902 | 0.602150538 | 21 | 21.6363 |
| 16 | SOD2 | 0.008633341 | 0.589473684 | 21 | 21.5981 |
| 17 | CCND1 | 0.003818946 | 0.577319588 | 20 | 20.5811 |
| 18 | MAPK1 | 0.00411499 | 0.571428571 | 18 | 18.5755 |
| 19 | NFE2L2 | 0.001956391 | 0.565656566 | 18 | 18.5676 |
| 20 | RHOA | 0.003655302 | 0.554455446 | 18 | 18.5581 |

Based on the topological analysis of the PPI network, the results revealed several target proteins associated with bioactive compounds for hypertension. Notably, proteins like ALB (albumin), IL6 (Interleukin 6), GAPDH (Glyceraldehyde-3-Phosphate Dehydrogenase), and TNF (Tumor Necrosis Factor) exhibited the highest degree scores. Among these target proteins, ALB in urine can be used as a marker to predict the development of high blood pressure and an increase in high blood pressure in patients (Takase *et al*., 2015). Meanwhile, the GAPDH target protein has an important role in [$Ca^{(2+)}$]i signaling in cases of pulmonary arterial hypertension (Tan *et al.*, 2013). In TNF target proteins, especially TNF-α, which is a pleiotropic cytokine that can increase in chronic inflammatory conditions such as hypertension and can play a role in mediating the increase and decrease in blood pressure (Ramseyer & Garvin, 2013).

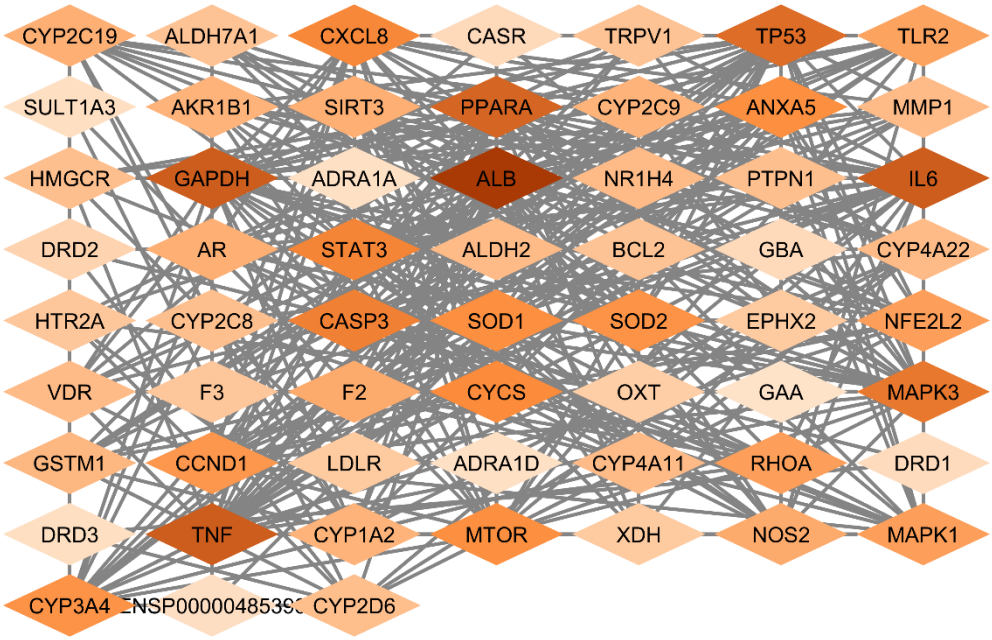


**Figure 4.** PPI network between protein targets of bioactive compounds and hypertension analyzed using STRING and visualized using Cytoscape

*3.5 GO Functions and KEGG pathways*

Enrichment analyzes GO and KEGG were conducted on the top 20 target proteins, identified by their highest scores in Degree, Betweenness Centrality and Closeness Centrality (Table 3), with a confidence level ($P < 0.05$). This analysis was performed using ShinyGo and the results of 20 types of gene action were graphically displayed in a bar chart. The X-axis indicates the number of genes involved, while the Y-axis illustrates the distribution of gene action types. In the GO Biological Process graph (Figure 5A), the predominant genes were associated with the negative regulation of cell death. Conversely, the GO Cell Composition chart (Figure 5B) highlighted the mitochondrion as the primary location for these genes. For GO Molecular Function (Figure 5C), the majority of genes were related to transcription factor binding. Meanwhile, the KEGG pathways enrichment chart (Figure 5D) showcased cancer mechanisms as the most represented pathway. This observation aligns with findings by Mohammed et al., suggesting a connection between hypertension and cancer mechanism. Given that the hypertension is a recognized risk factor for cardiovascular disease, its prominence in cancer patient morbidity and mortality is increasingly evident (Mohammed *et al.*, 2022).

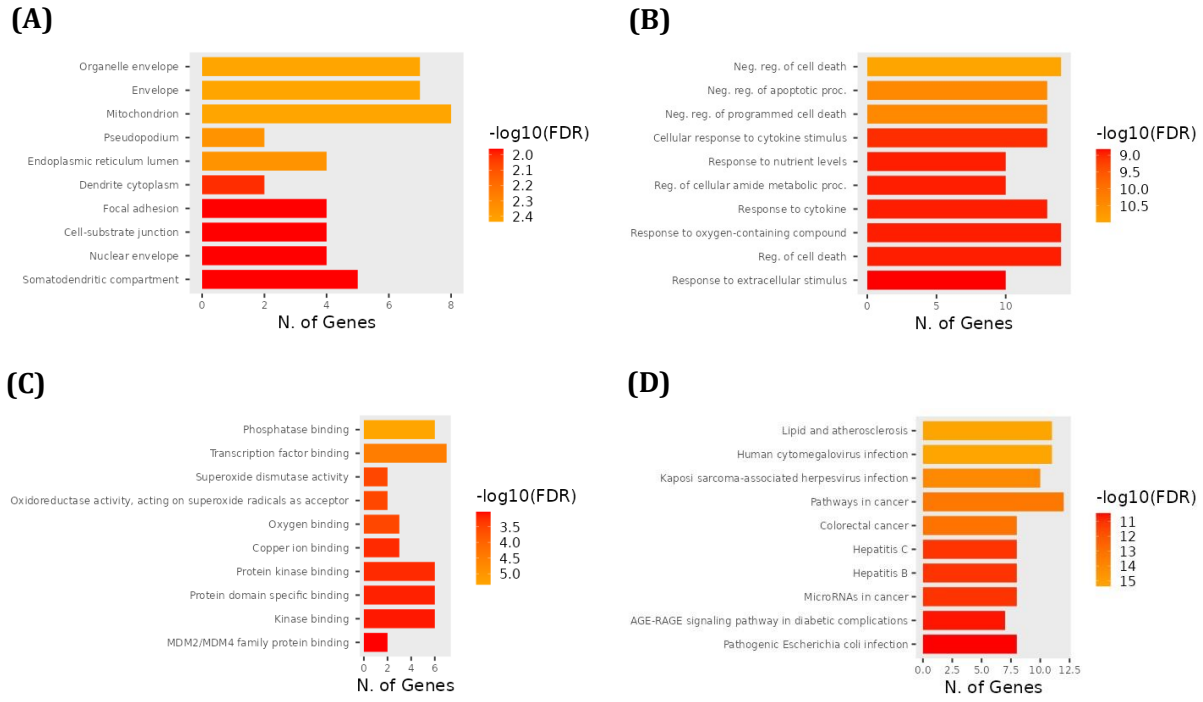


**Figure 5.** Enrichment analysis results gene ontology. A: GO biological process; B: GO Cell composition; C: GO molecular function; D: KEGG enrichment (Ge *et al*., 2020; Luo & Brouwer, 2013)

From the result of KEGG pathway, mechanism related to the hypertension disease was through cellular senescence (Figure 6). Cellular senescence refers to the cessation of regular cell division and is frequently connected with the process of getting older. It plays a crucial role in maintaining balance within the body. Nevertheless, when senescence becomes excessive and unregulated, it has the potential to promote the development and/or persistence of hypertension by accelerating the relative aging of blood vessels. For example, the role of cellular senescence in lung can stimulate pulmonary hypertension (McCarthy *et al*., 2019; Roger *et al*., 2021).

The genes in red box (Figure 6) were the genes that relate with bioactive compounds contained in SPL. Those genes were mTOR, ERK (also known as MAPK), p53, CycD and IL6. mTOR, p53 and NF-κB can develop an interaction with Akt. Akt can be a specific target for proliferation, differentiation, apoptosis and promotion related to tissue regeneration and include in PI3K-signaling pathway as an important signal transduction (Ramseyer & Garvin, 2013; Stannus *et al*., 2010). A critical component of renin-angiotensin system (RAS), named renin, can regulate blood pressure through PI3K/Akt-eNOS signaling (Cheng *et al*., 2012).

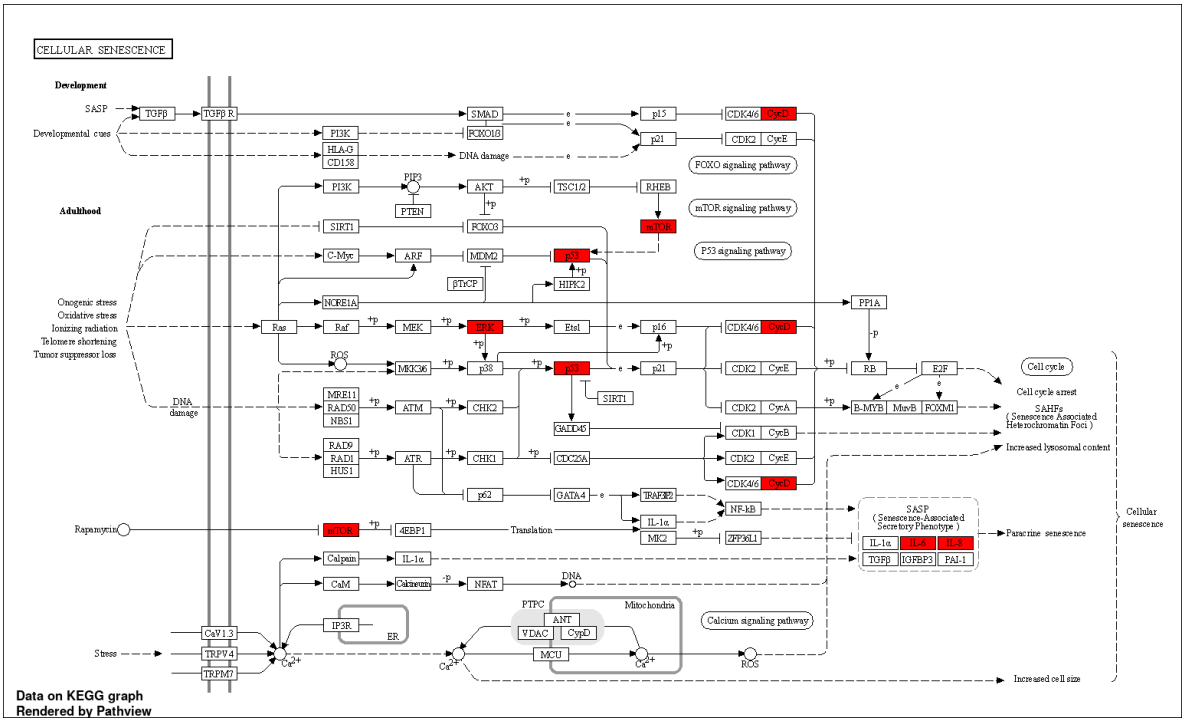


**Figure 6.** KEGG cellular senescence pathway (Kanehisa *et al*., 2021)

The bioactive compounds that have interactions with those genes mentioned before were summarized in Table 4. Those compounds were hydroxychavicol, farnesol, naphthalene, phloretin, pyrogallol, and decanal. From the previous research that had been done using betel leaf, hydroxychavicol that contained in betel leaf, showed a good inhibition of both COX-1 and COX-2. Hydroxychavicol has the potential to prevent and to treat atherosclerosis and various cardiovascular diseases due to its anti-inflammatory properties (Chang *et al*., 2007). In another study, a combination between hydroxychavicol and curcumin led to triggering the MAPK pathway and resulted in the mTOR production, ultimately leading to apoptosis in K562 cells (Chaudhuri *et al*., 2014).

**Table 4.** List of target protein and bioactive compounds from result of KEGG

| Target Protein | Bioactive Compounds in SPL |
|---|---|
| mTOR | Hydroxychavicol |
| ERK or MAPK | Farnesol, hydroxychavicol, naphthalene |
| p53 | Pyrogallol, phloretin |
| CycD | Pyrogallol |
| IL6 | Decanal, farnesol |

The second bioactive compound of SPL that interact with MAPK and IL-6, farnesol, exhibits antihypertensive properties and reduce heart rate by interacting with muscarinic receptors by conduct hydrogen bond (Silva *et al*., 2021). Muscarinic cholinergic receptors can

activate the MAP kinase (MAPK) pathway and induces cell proliferation and protein synthesis in human breast cancer cells (Jiménez & Montiel, 2005). It shows that farnesol literally have interaction with target proteins linked to hypertension. Meanwhile, naphthalene was considered as a potential factor linked to hypertension (Buckpitt *et al*., 2010), but there is still limited study on this matter.

In *in vivo* study, phloretin can protect the heart muscle from injury and prevent alterations hemodynamic parameters linked with hypertension, induced by doxorubicin in rats. Additionally, the use of phloretin can also reduce the expression of pro-inflammatory cytokines, including cytokines that play a role in the hypertension pathway (Nakhate *et al*., 2022).

Other study revealed that pyrogallol can caused cell cycle phase arrest, especially at G1 phase, and also increase p53 then triggered cell death (Park, 2016). Decanal, an aldehyde compound, exhibited the strongest positive correlation with IL-6 compared to benzyl alcohol and tetralin (Ahmed *et al*., 2021). Meanwhile, animal studies have suggested that IL-6 plays a significant role in hypertension induced by angiotensin II (Chamarthi *et al.*, 2011). This suggest that decanal has potential role in hypertension pathway.

However, this research still needs to be continued into the molecular docking stage to further explore specific mechanisms of signaling pathways in hypertension, especially bioactive compounds SPL against potential target proteins in hypertension before continue in *in vitro* and/or *in vivo* experimental.

## 4. Conclusion

In this study, 31 bioactive compounds were obtained from SPL, where 12 of these bioactive compounds belong to the terpene group. From the PPI and KEGG analysis, 6 bioactive compounds of SPL and 5 target proteins in hypertension disease were identified and predicted to have strong potential in their role in the treatment of hypertension. The six compounds were hydroxychavicol, farnesol, naphthalene, phloretin, pyrogallol, and decanal. The 5 target proteins were mTOR, ERK (also known as MAPK), p53, CycD and IL6.

## Reference

Ahmed, W., White, I.R., Wilkinson, M., Johnson, C.F., Rattray, N., Kishore, A.K., Goodacre, R., Smith, C.J., & Fowler, S.J. (2021). Breath and Plasma Metabolomics To Assess Inflammation In Acute Stroke. *Scientific Reports,* 11(1), 1–14. https://doi.org/10.1038/s41598-021-01268-5

Blowey, D.L. (2016). Diuretics In The Treatment of Hypertension. *Pediatric Nephrology,* 31(12), 2223–2233. https://doi.org/10.1007/s00467-016-3334-4

Buckpitt, A., Kephalopoulos, S., Koistinen, K., Kotzias, D., Morawska, L., & Sagunski, H. (2010). *Naphthalene. In: WHO Guidelines for Indoor Air Quality: Selected Pollutants*, in: Geneva: World Health Organization. 4.

Chamarthi, B., Williams, G.H., Ricchiuti, V., Srikumar, N., Hopkins, P.N., Luther, J.M., Jeunemaitre, X., & Thomas, A. (2011). Inflammation and Hypertension: The Interplay of Interleukin-6, Dietary Sodium, and the Renin–Angiotensin System in Humans. *American Journal of Hypertension*, 24(10), 1143–1148. https://doi.org/10.1038/ajh.2011.113

Chang, M.C., Uang, B.J., Tsai, C.Y., Wu, H.L., Lin, B.R., Lee, C.S., Chen, Y.J., Chang, C.H., Tsai, Y.L., Kao, C.J., & Jeng, J.H. (2007). Hydroxychavicol, A Novel Betel Leaf Component, Inhibits Platelet Aggregation By Suppression of Cyclooxygenase, Thromboxane Production and Calcium Mobilization. *British Journal of Pharmacology*, 152, 73–82. https://doi.org/10.1038/sj.bjp.0707367

Chaudhuri, J., Chowdhury, A.A., Biswas, N., Manna, A., Chatterjee, S., Mukherjee, T., Chaudhuri, U., Jaisankar, P., & Bandyopadhyay, S. (2014). Superoxide Activates mTOR-eIF4E-Bax Route To Induce Enhanced Apoptosis In Leukemic Cells. *Apoptosis* 19(1), 135–148. https://doi.org/10.1007/s10495-013-0904-9

Cheng, W.-H., Lu, P.-J., Hsiao, M., Hsiao, C.-H., Ho, W.-Y., Cheng, P.-W., Lin, C.-T., Hong, L.-Z., & Tseng, C.-J. (2012). Renin Activates PI3K-Akt-eNOS Signalling Through The Angiotensin AT 1 and Mas Receptors To Modulate Central Blood Pressure Control In The Nucleus Tractus Solitarii. *British Journal of Pharmacology,* 166, 2024–2035. https://doi.org/10.1111/j.1476-5381.2012.01832.x

Di Palo, K.E., Barone, N.J. (2020). Hypertension and Heart Failure: Prevention, Targets, and Treatment. *Heart Failure Clinics*, 16(1), 99–106. https://doi.org/10.1016/j.hfc.2019.09.001

Fan, H., Lu, F., Yang, A., Dong, Y., Liu, P., & Wang, Y. (2019). A Review on the Nonpharmacological Therapy of Traditional Chinese Medicine with Antihypertensive Effects. Evidence-based Complement. *Evidence-based Complementary and Alternative Medicine,* 2019, 1–7. https://doi.org/10.1155/2019/1317842

Ge, S.X., Jung, D., Jung, D., & Yao, R. (2020). ShinyGO: A Graphical Gene-Set Enrichment Tool For Animals and Plants. *Bioinformatics,* 36(8), 2628–2629. https://doi.org/10.1093/bioinformatics/btz931

Harun, N.H., Rasdi, N.A., Salleh, R.M., Safuan, S., Wan Ahmad, W.A.N., & Fuad, W.E.M. (2021). Subacute Toxicity Evaluation of Methanolic Extract of *Syzygium polyanthum* in Rats. *Tropical Life Sciences Research,* 32(2), 65–81. https://doi.org/10.21315/tlsr2021.32.2.5

Ismail, A., & Wan Ahmad, W.A.N. (2019). *Syzygium polyanthum* (Wight) Walp: A Potential Phytomedicine. *Pharmacognosy Journal,* 11(2), 429–438. https://doi.org/10.5530/pj.2019.11.67

Ismail, A., & Wan Ahmad, W.A.N. (2017). Autonomic Receptors and Nitric-Oxide Involvements in Mediating Vasorelaxation Effect Induced by *Syzygium polyanthum* Leaves Extract. *Pharmacognosy Research*, 9, 9–14. https://doi.org/10.4103/pr.pr

Ismail, A., & Wan Ahmad, W.A.N. (2016). Vasorelaxation Effect of *Syzygium polyanthum* (wight) walp. Leaves Extract On Isolated Thoracic Aorta Rings of Normal and Hypertensive Rats. *IIUM Medical Journal Malaysia,* 15, 102. https://doi.org/10.31436/imjm.v15i1.1264

Jiménez, E., & Montiel, M. (2005). Activation of MAP Kinase by Muscarinic Cholinergic Receptors Induces Cell Proliferation and Protein Synthesis in Human Breast Cancer Cells. *Journal of Cellular Physiology*, 204(2), 678–686. https://doi.org/10.1002/jcp.20326

Kanehisa, M., Furumichi, M., Sato, Y., Ishiguro-Watanabe, M., & Tanabe, M. (2021). KEGG: Integrating Viruses and Cellular Organisms. *Nucleic Acids Research,* 49, 545–551. https://doi.org/10.1093/nar/gkaa970

Kearney, P.M., Whelton, M., Reynolds, K., Muntner, P., Whelton, P.K., & He, J. (2005). Global Burden of Hypertension: Analysis of Worldwide Data. *Lancet,* 365(9455), 217–223. https://doi.org/10.1016/s0140-6736(05)17741-1

Luo, T. ting, Lu, Y., Yan, S. kai, Xiao, X., Rong, X. lu, & Guo, J. (2020). Network Pharmacology in Research of Chinese Medicine Formula: Methodology, Application and Prospective. *Chinese Journal of Integrative Medicine*, 26(1), 72–80. https://doi.org/10.1007/s11655-019-3064-0

Luo, W., & Brouwer, C. (2013). Pathview: An R/Bioconductor Package For Pathway-Based Data Integration and Visualization. *Bioinformatics,* 29(14), 1830–1831. https://doi.org/10.1093/bioinformatics/btt285

McCarthy, C.G., Wenceslau, C.F., Webb, R.C., & Joe, B. (2019). Novel Contributors and Mechanisms of Cellular Senescence in Hypertension-Associated Premature Vascular Aging. *American Journal of Hypertension,* 32(8), 709–719. https://doi.org/10.1093/ajh/hpz052

Menezes, I.A.C., Barreto, C.M.N., Antoniolli, Â.R., Santos, M.R.V, & Sousa, D.P.D. (2010). Hypotensive Activity of Terpenes Found in Essential Oils. *Z Naturforsch C J Biosci,* 65(9-10), 562–566.

Mohammed, T., Singh, M., Tiu, J.G., & Kim, A.S. (2022). Etiology and Management of Dyslipidemia in Patients With Cancer. *Frontiers in Cardiovascular Medicine,* 9, 1–13. https://doi.org/10.3389/fcvm.2022.892335

Nakhate, K.T., Badwaik, H., Choudhary, R., Sakure, K., Agrawal, Y.O., Sharma, C., Ojha, S., & Goyal, S.N. (2022). Therapeutic Potential and Pharmaceutical Development of a Multitargeted Flavonoid Phloretin. *Nutrients,* 14, 1–24.

Park, W.H. (2016). Pyrogallol Induces The Death of Human Pulmonary Fibroblast Cells Through ROS Increase and GSH Depletion. *International Journal of Oncology,* 49(2), 785–792. https://doi.org/10.3892/ijo.2016.3543

Pistoia, F., Sacco, S., Degan, D., Tiseo, C., Ornello, R., & Carolei, A. (2016). Hypertension and Stroke: Epidemiological Aspects and Clinical Evaluation. High Blood Press. *High Blood Pressure and Cardiovascular Prevention,* 23(1), 1–10. https://doi.org/10.1007/s40292-015-0115-2

Ramseyer, V.D., & Garvin, J.L. (2013). Tumor Necrosis Factor-α: Regulation of Renal Function and Blood Pressure. *American Journal of Physiology - Renal Physiology,* 304(10), 1231–1242. https://doi.org/10.1152/ajprenal.00557.2012

Roger, I., Milara, J., Belhadj, N., & Cortijo, J. (2021). Senescence Alterations in Pulmonary Hypertension. *Cells,* 10(12), 1–24. https://doi.org/10.3390/cells10123456

Rouzaud-Laborde, C., Lafitte, P., Balardy, L., Czosnyka, Z., & Schmidt, E.A. (2018). ICP and Antihypertensive Drugs. *Acta Neurochirurgica, Supplementum*, 126, 163–165. https://doi.org/10.1007/978-3-319-65798-1_34

Silva, E.A.P., Carvalho, J.S., dos Santos, D.M., Oliveira, A.M.S., de Souza Araújo, A.A., Serafini, M.R., Oliveira Santos, L.A.B., Batista, M.V. d. A., Viana Santos, M.R., Siqueira Quintans, J. de S., Quintans-Júnior, L.J., & Barreto, A.S. (2021). Cardiovascular Effects of Farnesol and Its β-cyclodextrin Complex In Normotensive and Hypertensive Rats. *European Journal of Pharmacology,* 901, 1–11. https://doi.org/10.1016/j.ejphar.2021.174060

Stannus, O., Jones, G., Cicuttini, F., Parameswaran, V., Quinn, S., Burgess, J., & Ding, C. (2010). Circulating Levels of IL-6 and TNF-α Are Associated With Knee Radiographic

Osteoarthritis and Knee Cartilage Loss In Older Adults. *Osteoarthritis and Cartilage,* 18(11), 1441–1447. https://doi.org/10.1016/j.joca.2010.08.016

Takase, H., Sugiura, T., Ohte, N., & Dohi, Y. (2015). Urinary Albumin as a Marker of Future Blood Pressure and Hypertension in the General Population. *Medicine (United States),* 94(6), 1–7. https://doi.org/10.1097/MD.0000000000000511

Tan, R., Li, J., Peng, X., Zhu, L., Cai, L., Wang, T., Su, Y., Irani, K., & Hu, Q. (2013). GAPDH Is Critical For Superior Efficacy of Female Bone Marrow-Derived Mesenchymal Stem Cells On Pulmonary Hypertension. *Cardiovascular Research*, 100(1), 19–27. https://doi.org/10.1093/cvr/cvt165

Ventura, H.O., & Lavie, C.J. (2019). Editorial: Epidemiology and Managing Aspects of Hypertension. *Current Opinion in Cardiology,* 34(4), 329–330. https://doi.org/10.1097/HCO.0000000000000631

Wenzel, U.O., Bode, M., Köhl, J., & Ehmke, H. (2017). A Pathogenic Role of Complement In Arterial Hypertension and Hypertensive End Organ Damage. *American Journal of Physiology - Heart and Circulatory Physiology*, 312(3), 349–354. https://doi.org/10.1152/ajpheart.00759.2016

Widyawati, T., Yusoff, N.A., Bello, I., Asmawi, M.Z., & Ahmad, M. (2022). Bioactivity-Guided Fractionation and Identification of Antidiabetic Compound of *Syzygium polyanthum* (Wight.)'s Leaf Extract in Streptozotocin-Induced Diabetic Rat Model. *Molecules*, 27(20), 1–15. https://doi.org/10.3390/molecules27206814