A NETWORK PHARMACOLOGY OF BROTOWALI (*Tinospora cordifolia*) ON IMMUNITY CASES

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Abstract

COVID-19 is a disease outbreak related to the human immune system. The process of spreading very quickly makes this outbreak a dangerous pandemic. COVID-19 cases in 2020 in Indonesia, positive cases were reaching 1,089,308 people with a death rate of 20,277 people as of 2 February 2021. Efforts are being made to reduce and prevent virus transmission by boosting the immune system using an immunomodulator. Based on the literature studies that have been conducted, several Indonesian local plants have potential as immunomodulator. The in silico test was carried out because the computer simulation method has scientific validity, is relatively new, and has a high level of accuracy. This study aims to determine the protein network associated with the body's immune system, which is activated due to the administration of Brotowali *(Tinospora Cordifolia)*. The research method used is exploratory descriptive with in silico analysis using a computational model with online databases, including KNApSAck, Dr. Duke, Pubchem, SwissADME, SwissTargetPrediction, Venny, StringDB, and KEGG. Based on the results of pharmacological network analysis, *T. Cordifolia* contains 33 secondary metabolites, 25 of which have high bioavailability. Proteins associated with T. cordifolia contain 640 compounds, and those related to immunomodulators contain 1380 proteins. The intersection results obtained 191 proteins predicted to interact with *T. cordifolia* and are related to immunomodulators. Based on KEGG Pathway analysis, there are five critical pathways in the immunomodulatory system, namely Th17 cell differentiation, IL-17 signaling pathway, T cell receptor signaling pathway, Fc epsilon RI signaling pathway, and TNF signaling pathway. 1-hydroxy-2-methyl-anthraquinone can be an immunomodulator because it interacts with five critical pathways in the immunomodulator system.

***Kata Kunci**: COVID-19, immunomodulator, brotowali, Tinospora cordifolia, network pharmacology*

Background

COVID-19, or Corona Virus Diseases-19, is caused by the Severe Acute Respiratory Syndrome virus (SARS-CoV-2) (Evi Kristhy *et al*., 2022). In Indonesia, the first case of COVID- 19 was announced as occurring on March 2 2020. The total number of positive COVID-19 cases in Indonesia reached 1,089,308 people, with the death rate reaching 30,277 people as of February 2 2021 (COVID-19 Handling Task Force, 2021) (Priani 2021). COVID-19 is known to be transmitted directly between humans or indirectly by droplet transfer due to physical contact with sufferers or when touching the surface of objects exposed to the virus. One way that can be done to prevent transmission of this disease, apart from implementing health protocols in daily life, canalso be done by increasing immunity (Shereen *et al*., 2020).
Immunity is the body's defence system. This system protects the body from incoming foreign objects so that body functions are not disturbed. Immunity is a reaction that will arise if foreign material enters the body either molecularly or cellularly. The cells involved in the immune system are B cells produced in the spinal cord and T cells produced in the thymus (Gede and Perdana, 2021). Immunomodulators are used to restore imbalances in the immune system. Immunomodulators are natural, biological, or synthetic substances that can increase, inhibit, or modulate the innate and adaptive functions of the immune system (Simorangkir *et al*. 2022).

Previous research showed that experts are developing several local Indonesian plants to prevent SARS-CoV-2 infection (Aziz *et al.,* 2020). Previous research results have shown significantly that certain plants increase the body's immunity by increasing the production of competent immune cells. Herbal plants that have potential as immunomodulator candidates include brotowali *(Tinospora cordifolia)* that often grows among shrubs that can be found in forests in Indonesia (Kotala and Kurnia, 2022). However, there still needs to be more information explaining this immunomodulatory activity. This research aims to determine the protein network associated with the body's immune system activated due to the administration of *T. cordifolia.*

Compound activity needs to be tested using three approaches: in silico, in vitro, and in vivo.The in silico test is carried out using a computer simulation method with scientific validity, is relatively new, and has a high level of accuracy (Istyastono, 2020). In silico testing is used to predict, provide hypotheses, and provide discoveries or advances in treatment and therapy (Johan, 2016). The bioinformatics branch of in silico screening involves adding relevant molecular structures to a target protein database. The results of this evaluation are then used to identify structures with binding and potential physiological activity, which can then be evaluated in vitro and in vivo to determine the compound's potential as a drug candidate (Shofi, 2021). Later, a network pharmacology model will be created to analyze how secondary metabolite compounds can interact molecularly with biological targets related to immunomodulation.

Methods

Tools

Some of the online databases was used in this research include: KNApSAcK (http://www.knapsackfamily.com/KNApSAcK/), Dr.Duke (https://phytochem.nal.usda.gov/), PubChem (https://pubchem.ncbi.nlm.nih.gov/), SwissADME (http://www.swissadme.ch/), SwissTargetPrediction (http://www.swisstargetprediction.ch/), GeneCards (https://www.genecards.org/), Venny (https://bioinfogp.cnb.csic.es/tools/venny/), StringDB (https://stringdb.org/), and KEGG (https://www.genome.jp/kegg/).

Research Methods

Identification of plant secondary metabolite compounds was obtained using the KNApSAck and Dr. Duke's databases (Lena *et al*., 2023), then looked for the SMILES code for each compound using PubChem and then entered into SwissADME to see bioavailability predictions using the Boiled-Egg method (Daina & Zoete, 2016; Mahanthesh *et al*., 2020). Compounds included in Boiled-Egg are selected for further analysis using SwissTargetPrediction to predict the interaction of the compound with the protein targeted in the research (Daina *et al*., 2019). Search for protein targets that interact with immunomodulators was carried out using the GeneCards (Stelzer *et al*., 2016). Then followed by looking for protein intersections predicted to have ties to compounds from the plant using the Venny database (Oliveros 2015), and the results of the intersection are entered into the StringDB for network pharmacology analysis (Szklarczyk et al. 2021). After that, we look for predictions of proteins that are interrelated with the immune system using KEGG (Kanehisa *et al*., 2023). To see which proteins interact most with pathways related to the immune system, we then looked at the secondary metabolite compounds of *T. cordifolia* that interact with those proteins.

Results and Discussion

Identification and Bioavailability Prediction of Secondary Metabolites of T. cordifolia

Secondary metabolites of *T. cordifolia* were obtained using the KNApSAck Family and Dr. Duke's Phytochemicals and Ethnobotanical Databases. There are 33 secondary metabolites obtained, of which 12 were from the KNApSAcK database and 21 from Dr. Duke's Phytochemicals Database **Table 1**. After that, the bioavailability prediction was carried out with SwissADME by using the Boiled-Egg method. Bioavailability predictions were carried out to look for secondary metabolite compounds that were predicted to have good bioavailability. This method uses image visualization to classify the absorption of a compound **Figure 1**. The egg white shows the compound's ability to be absorbed in the gastrointestinal tract, while the egg yolk shows the ability to penetrate the blood-brain barrier. This calculation refers to the lipophilic parameters (WlogP) and compound polarity (tPSA). tPSA is the topological polar surface area or representation to discriminate between well-absorbed and poorly absorbed molecules based on their lipophilicity and polarity, described by the n-octanol/water partition coefficient (log P) and the polar surface area (PSA) (Daina and Zoete, 2016). There are 25 compounds predicted to have high bioavailability **Table 2**. Bioavailability is used as a parameter to determine the amount and speed of drugs absorbed by the body (Tsamarah *et al.*, 2023).

Table 1. A list of secondary metabolites of *T. cordifolia*

No.	Compound Name	Compound code	Source
1	Betulin	Molecule 1	Dr. Duke
2	Betulinic-acid	Molecule 2	Dr. Duke
3	1,4-dihydroxy-2-carboethoxyanthraquinone	Molecule 3	Dr. Duke
4	1,4-dihydroxy-2-methyl-5-methoxyanthraquinone	Molecule 4	Dr. Duke
5	1,4-dihydroxy-2-methylanthraquinone	Molecule 5	Dr. Duke
6	1,5-dihydroxy-2-methylanthraquinone	Molecule 6	Dr. Duke
7	1-hydroxy-2-carboxy-3-methoxyanthraquinone	Molecule 7	Dr. Duke
8	1-hydroxy-2-methoxyanthraquinone	Molecule 8	Dr. Duke
9	1-hydroxy-2-methyl-anthraquinone	Molecule 9	Dr. Duke
10	Alizarin	Molecule 10	Dr. Duke

11	Anthraquinones	Molecule 11	Dr. Duke
12	Beta-sitosterol	Molecule 12	Dr. Duke
13	4-Hydroxyphenylpyruvate	Molecule 13	Knapsack
14	3'-Hydroxy-7-O-methylcoclaurine	Molecule 14	Knapsack
15	Dopamine	Molecule 15	Knapsack
16	Tyramine	Molecule 16	Knapsack
17	Magnoflorine	Molecule 17	Knapsack
18	(+)-Reticuline	Molecule 18	Knapsack
19	(+)-N-Methylcoclaurine	Molecule 19	Knapsack
20	N-trans-Feruloyltyramine	Molecule 20	Knapsack
21	Tembetarine	Molecule 21	Knapsack
22	(+)-Corytuberine	Molecule 22	Knapsack
23	Coclaurine	Molecule 23	Knapsack
24	(S)-Norcoclaurine	Molecule 24	Knapsack
25	N-trans-Feruloyl tyramine	Molecule 25	Knapsack
26	Amritoside A	Molecule 26	Knapsack
27	Amritoside B	Molecule 27	Knapsack
28	Amritoside C	Molecule 28	Knapsack
29	Amritoside D	Molecule 29	Knapsack
30	Tinocordifolin	Molecule 30	Knapsack
31	Tinocordifolioside	Molecule 31	Knapsack
32	Tinocordiside	Molecule 32	Knapsack
33	4-Hydroxyphenylacetaldehyde	Molecule 33	Knapsack

Table 2. Bioavailability prediction of the secondary metabolite of *T. cordifolia* using BOILED-Egg

No.	Bioavailability Prediction	Total Molecule	Compound Code
1.	High	25	Mol 3, Mol 4, Mol 5, Mol 6, Mol 7, Mol 8, Mol 9, Mol 10, Mol 11, Mol 13, Mol 14, Mol 15, Mol 16, Mol 17, Mol 18, Mol 19, Mol 20, Mol 21, Mol 22, Mol 23, Mol 24, Mol 25, Mol 30, Mol 31, Mol 33
2.	Low	8	Mol 1, Mol 2, Mol 12, Mol 26, Mol 27, Mol 28, Mol 29, Mol 32

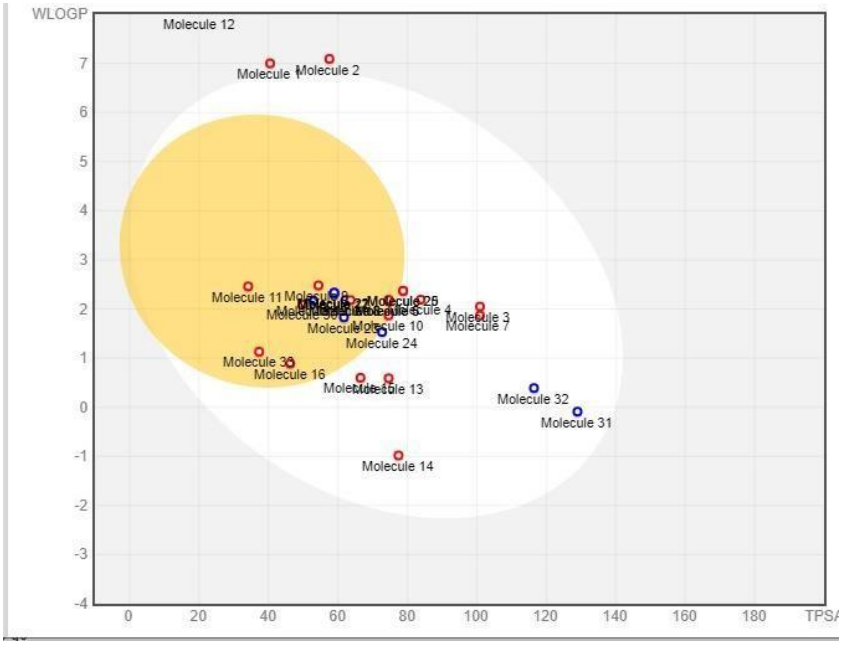

Figure 1. Bioavailability prediction of the secondary metabolite of *T. cordifolia* using BOILED-Egg

Network Pharmacology Analysis

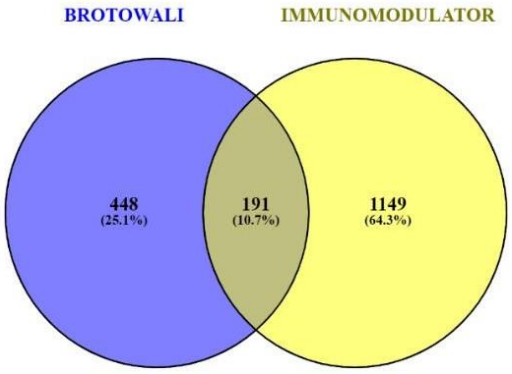

Figure 2. Venn diagram of protein that predicted linked with *T. cordifolia* and immunomodulator-linked protein

After obtaining compounds that have predicted high bioavailability, the next step was to predict target proteins that can interact with the compounds carried out by SwissTargetPrediction (Oh *et al*., 2021). The results show that 640 proteins were predicted to interact with secondary metabolites of T. cordifolia. In order to obtain related proteins with immunomodulators, it was carried out by GeneCards (Safran *et al*., 2022). The results show that 1,340 related proteins were connected with immunomodulators. GeneCards is a searchable database for human gene annotation and covers almost 90% of human protein-coding genes (Fishilevich *et al*., 2016). Next, interactions were carried out with Venn Diagram by using Venny between proteins that were predicted to interact with secondary metabolites of *T. Cordifolia* and immunomodulator-related proteins. From the interaction results, 191 immunomodulator-related proteins were predicted to interact with secondary metabolites of *T. Cordifolia* **Figure 2** and **Table 3**.

Table 3. A list of immunomodulator-linked protein

Protein Code
ABCB1, ABCG2, ABL1, ACE, ACHE, ADAM17, ADORA2B, ADRB2, AHR, AKT1, ALB, ALDH2, ALOX5, ANPEP, AOC1, APEX1, APP, AXL, BACE1, BCL2, BRAF, CASP3, CDK1, CDK9, CHRNA7, CNR1, CNR2, CSF1R, CSNK1A1, CTSB, CTSC, CTSF, CTSS, CXCL8, CXCR1, CXCR2, CXCR3, CYP1A2, CYP3A4, DHODH, DPP4, DRD1, DRD2, EDNRA, EDNRB, EGFR, EIF2AK2, EIF2AK3, ELANE, ERBB2, ESR1, ESRRB, F2, F3, FAP, FASN, FKBP1A, FLT3, G6PD, GAA, GAPDH, GUSB, HCAR2, HCK, HDAC2, HDAC3, HDAC6, HMGCR, HMOX1, HSD11B1, HSP90AA1, HSP90B1, HSPA5, HSPA8, HTR1A, HTR7, IDH1, IDO1, IGF1R, IKBKB, IL2, IMPDH2, IRAK4, ITGAL, JAK1, JAK2, JAK3, JUN, KCNA3, KCNA5, KDM6B, KDR, LCK, LDHA, LNPEP, LTA4H, MAP2K1, MAP3K7, MAPK1, MAPK10, MAPK14, MAPK3, MAPK8, MB, MERTK, MIF, MMP1, MMP13, MMP14, MMP2, MMP3, MMP7, MMP9, MPO, MSR1, MTOR, NLRP3, NOS2, NQO1, NR1H3, NR1H4, NR3C1, OPRD1, OPRM1, P2RX7, PABPC1, PARP1, PGR, PIK3CA, PIK3CG,PIM1, PIM2, PIM3, PLAA, PLEC, PNP, POLB, PPARA, PPARG, PRKCA, PRKCB, PRKCQ, PRKDC, PSMB5, PTGDR2, PTGER2, PTGER4, PTGES, PTGS1, PTGS2, PTK2, PTPN11, PTPN2, PTPN22, PTPN6, PTPRC, REN, RORA, RORC, SELE, SLC6A3, SLC9A1, SNCA, SPHK2, SRC, STAT3, SYK, TAAR1, TDO2, TGM2, TLR9, TNF, TPMT, TSPO, TTR, TYR, TYRO3, VCAM1, VCP, XDH

Network pharmacology was analyzed using StringDB. This analysis is used to create interaction networks between selected protein targets and analyze the biological pathways influenced by this protein (Veda *et al*., 2023). The StringDB systematically collects and integrates protein–protein interactions, both physical interactions as well as functional associations. All of these interactions are critically assessed, scored, and subsequently automatically transferred to less well-studied organisms using hierarchical orthology information (Szklarczyk *et al*., 2023).

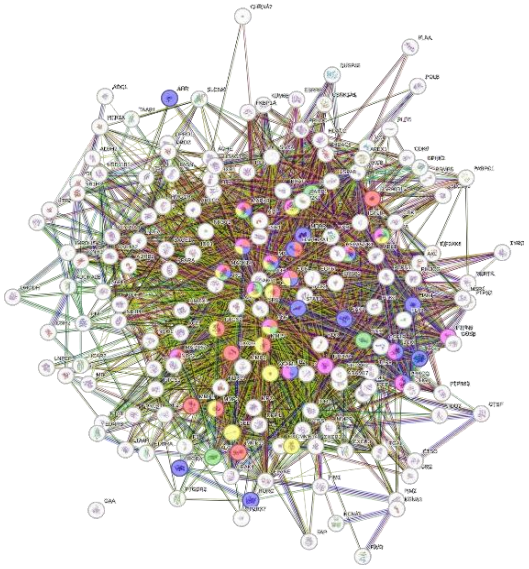

Figure 3. Network pharmacology prediction results using StringDB

After that, KEGG enrichment analysis was carried out to look at the five highest pathways associated with the immunomodulator (Veda *et al*., 2023). KEGG (Kyoto Encyclopedia of Genes and Genomes) was used for bioinformatics research and education in drug development (Hehenberger M 2020). KEGG is a collection of pathway maps drawn manually, representing ourknowledge about molecular interaction (Kanehisa *et al*,. 2023). Based on KEGG analysis,

it was found that three proteins were linked in all immunomodulator-linked selected pathways **Table 4**.

Table 4. Immunomodulator related pathway with KEGG enrichment

No.	Pathway	Strength
1.	Th17 cell differentiation	1.32
2.	IL-17 signalling pathway	1.32
3.	T Cell receptor signalling pathway	1.28
4.	Fc epsilon RI signalling pathway	1.28
5.	TNF signalling pathway	1.25

In further analysis, there is one compound (Mol 9) which connect to three immunomodulator-linked protein. These five pathways interact with the immune system because Th17 cell differentiation is a cell that is identified as pro-inflammatory and induces autoimmunity, IL-17 signaling pathway which induces and mediates pro-inflammatory responses, T Cell receptor signaling pathway is responsible for recognizing antigens as peptides, Fc epsilon RI signaling pathway In humans, it causes mast cells or basophils to release biologically active mediators that mediate hypersensitivity reactions. The TNF signaling pathway is a multifunctional cytokine that plays an important role in various cellular events such as survival, proliferation, differentiation and cell death. Therefore, the *T. Cordifolia* plant has the potential to be explored and developed further as a good immunomodulating agent. In the future, in vitro and in vivo studies can be carried out to prove the immunomodulatory activity of the plant, especially the content of the Mol 9 (1-hydroxy-2-methyl-anthraquinone), which plays an important role, as predicted in the network pharmacology.

Table 5. Compounds related to proteins based on KEGG pathway analysis is a compound related to proteins based on KEGG Pathway analysis, because it has ties to five pathways with KEGG enrichment.

Protein Code	Molecule
MAPK 1	Mol 3, Mol 4, Mol 8, Mol 9, Mol 10, Mol 11, Mol 17, Mol 21, Mol 22, Mol 30, Mol 31
MAPK 8	Mol 3, Mol 4, Mol 6, Mol 7, Mol 8, Mol 9
MAPK 10	Mol 9, Mol 11, Mol 31

Conclusion

Based on the results of network pharmacology analysis in *T. cordifolia*, the compound 1-hydroxy-2-methyl-anthraquinone is predicted to be an important compound that plays a role in the immune system because it is known to have interactions with five important pathways related to immunomodulator.

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