A NETWORK PHARMACOLOGY OF *Camellia sinensis* (GREEN TEA)

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Abstract

Network pharmacology focuses on the therapeutic concept of one-target-one-drug to network-target-components to combat complex diseases. This research uses bioinformatics and high-throughput screening methods to facilitate the prediction of various drug target networks based on the establishment of biological models and becomes more important in uncovering the underlying mechanisms of drug action. Tea (Camelia sinensis) is one of the most ancient and popular therapeutic drinks consumed throughout the world and prepared as a drink that can have many health effects. This research aims to determine the pharmacological network analysis of *C. sinensis*. The list of *C. sinensis* secondary metabolite compounds was obtained from the Dr. database Duke. Protein predictions associated with *C. sinensis* were obtained from SwissTargetPrediction. Pharmacological network analysis was performed with StringDB and KEGG enrichment. From the search results, 57 compounds were obtained. From network pharmacology analysis, 15 biomolecular pathways were obtained that were closely related to secondary metabolite compounds in *C. sinensis*. From the results of further analysis, it was found that *C. sinensis* has a role in the treatment of hypertension, cancer, and anti-inflamation.

***Keyword**: Network farmakologi, Green Tea*

Introduction

Network pharmacology is an attempt to understand the actions of drugs and their interactions with multiple targets (Hopkins, 2008). Network pharmacology aims to understand the network interactions between living organisms and drugs that affect normal and abnormal biochemical functions. Pharmacological networking exploits the pharmacological mechanisms of drug action in networks and helps discover drug targets and improve drug efficacy (Wu & Wu, 2015). With the emergence of the big data era in biomedicine and biopharmaceutical research and development, network pharmacology is a systematic approach to change the current "one drug" paradigm of discovery and development (Hopkins, 2008). Drugs can interact with many molecules in the human body, and the actions of such drugs, known as polypharmacology, may be efficacious or detrimental for treating disease. However, its meaning is limited to studying drug action in actual use. Pharmacology has been defined as an experimental science whose aim is to study the changes that occur in organisms by chemical substances (Kibble *et al*., 2015).

Recent studies state that tea (*Camelia sinensis*) has several benefits, including anticancer, antibacterial, lowering cholesterol, and increasing immunity (Hayat *et al*., 2015). The most important medical component of tea is polyphenols. The most abundant polyphenols found in green tea are flavanols, namely catechins. Tea not only acts as a drink, but various studies state that tea can also improve a person's health (Khan & Mukhtar, 2013). Epigalocatechin3-gallate is the type of catechin most commonly found in green tea and can cause apoptosis and stop the cell cycle that has experienced DNA damage (Tabaga *et al*., 2015). Green tea has the highest antioxidant activity characterized by a low IC50 value, namely 21.44 µg/mL. The IC50 (inhibition concentration) value is a number that shows the concentration of the extract that can inhibit free radical activity by 50% (Kusmiyati *et al*., 2015).

The *in silico* method is a computational approach applied to create innovation in the search, design and optimization of new drugs proposed to treat human diseases. In addition, *in silico* methods help reduce drug failure rates through pharmacovigilance computational data mining methods, medical bioinformatics approaches, and predictive computer models to identify potential drug toxicity (Hosea & Jones, 2013). The main advantage of *in silico* methodology is that it helps accelerate the rate of drug production and screening based on calculated property analysis and prediction models for drug therapy targets and safety identification while minimizing the need for costs and time as well as the work that must be done *in vitro* testing (Valerio & Choudhuri, 2012). *In silico* methods have the potential to predict undesirable side effects at early stages in the drug development pipeline, for example, based on predicted drug-target interactions. In addition, it also provides the additional benefit of generating hypotheses about the biological mechanisms of a drug or disease in predicting new drugs (Hodos et al., 2016). Therefore, this research will examine the pharmacological network in tea plants using online database materials.

Research Method

Tool

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/), SwissTargetPrediction (http://www.swisstargetprediction.ch/), StringDB (https://stringdb.org/), and KEGG (https://www.genome.jp/kegg/).

Method

Identification of plant secondary metabolite compounds was obtained using the KNApSAck and Dr. Duke's databases (Lena et al., 2023)(Lena et al., 2023), then looked for the SMILES code for each compound using PubChem and then entered into SwissTargetPrediction to predict the interaction of the compound with the protein targeted in the research (Daina *et al.*, 2019). StringDB was used 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).

Results and Discussion

This research was conducted to determine the network pharmacology of the secondary metabolite of *C. sinensis*. The first step was to obtain a list of secondary metabolites of the *C. sinensis* plant using KNApSAcK and Dr. Duke's (Kusuma *et al*., 2022; Nguyen *et al*., 2020). There are several compounds in both databases. The results of the compounds obtained from the online database were selected by excluding organic compounds and long-chain fatty acids. Compounds from the inorganic group and long-chain fatty acid compounds are removed for further processing. Fifty-six compounds found in Dr. Duke's and two in the KNaPSaCK family are found in Dr. Duke's database **(Table 1).**

Table 1. List of secondary metabolites of *C. sinensis*

No	Compound Name
1	Linalool-Oxide-(Cis-Pyranoid)
2	Linalool-Oxide-(Trans-Furanoid)
3	Linalool-Oxide-(Trans-Pyranoid)
4	Linalool-Oxide-B
5	Loliolide
6	Lutein
7	Lycopene
8	Lysine
9	M-Cresol
10	M-Digallic-Acid
11	Malic-Acid
12	Menthol
13	Methyl-Ethyl-Ketone
14	Methyl-Pyrazine
15	Methyl-Salicylate
16	Methyl-Trans-Dihydrojasmonate
17	Methyl-Xanthines
18	Myrcene
19	Myricetin
20	N,N-Dimethyl-Benzylamine
21	N-1-Dotriacontanol
22	N-1-Triacontanol
23	N-Ethyl-Acetamide
24	N-Ethyl-Aniline
25	N-Ethyl-Propionamide
26	N-Methyl-Aniline
27	N-P-Coumaroyl-Glutamic-Acid
28	N-P-Coumaryl-Glutamic-Acid
29	Neral
30	Nerol
31	Nerolidol
32	Niacin
33	Nicotiflorin
34	Nona-Trans-2,Cis-6-Dien-1-Al

35	O-Cresol
36	O-Quinone
37	O-Toluidene
38	O-Toluidine
39	O-Xylenol
40	Octa-Trans-3-Cis-5-Dien-2-One
41	Oleic-Acid
42	Oolonghomobisflavan-A
43	Oolonghomobisflavan-B
44	Oxalic-Acid
45	P-Cresol
46	P-Ethyl-Acetophenone
47	P-Ethyl-Propiophenone
48	Pectins
49	Phenylacetic-Acid
50	Phytoene
51	Phytofluene
52	Procyanidin-B-2
53	Procyanidin-B-3
54	Procyanidin-C-1
55	Prodelphinidin-B-4
56	Quercetin

The next step was to predict target proteins that can interact with the compounds carried out by SwissTargetPrediction (Oh and Cho, 2021). The selected proteins are those that have a probability value > 0. The results show that 526 proteins were predicted to interact with secondary metabolites of *C. sinensis* (**Table 2**).

Table 2. List of protein names ehich predicted to interact with secondary metabolite of *C. sinensis*

Protein Code
CA12, CA13, CA14, CA2, CA3, CA4, CA5B, CA6, CA7, CA9, CYP2A6, ACE2, ENPEP, KLK1, KLK2, MME, PRPCD, PREP, ADH1A, ADH1B, ADH1C, ADH4, ADH7, ALDH3A, AOCE, MAOA, MAOB, MIF, TPO, TYR, CDK4, DAPK1, EGFR, ERBB2, MAP2K1, MAPK1, MAPK3, MDM2, MMP1, MMP2, MMP9, RPS6KA5, SRC, AKR1C1, AKR1C2, AKR1C3, AKR1C4, CYP11B2, CYP17A1, CYP19A1, CYP1B1, CYP3A4, HSD11B1, HSD11B2, HSD17B1, HSD17B2, HSD17B3, SRD5A2, UGT2B7, AKT1, ALK, CDK4, CDK6, EGFR, ERBB2, JAK3, MAP2K1, MAPK3, MET, PIK3CD, PIK3R1, PRKCA, PRKCG, RARB, RXRA, RXRB, RXRB, RXRG, STAT3, ALDH3A1, AOC3, MAOA, MAOB, MIF, ALOX15, CYP2C9, CYP3A4, PLA2G1B, PLA2G2A, PLA2G5, ADORA1, ADORA2A, ADORA2E, ADORA3, ADRA1D, ADRA2A, ADRA2B, ADRA2C, ADRB1, AVPRA1, AVPR2, BDKRB2, BRS3, C5AR1, CHRM1, CHRM2, CHRM3, CHRM4, CHRM5, CRN1, CRN2, DRD1, DRD2, DRD3, DRD4, EDNRA, F2, F2R, F2RL1, GABBR1, GABBR2, GABRA1, GABRA5, GCG, ECGR, GLRA1, GLRA2, GPR35, GRIA1, GRIA2, GRIA4, GRIK1, GRIK2, GRIK3, GRIK5, GRM1, GRM3, GRM4, GRM5, GRM6, GRM7, GRM8, HCRTR1, HCRTR2, HRH1, HRH3, HTR1A, HTR1B, HTR1D, HTR2A, HTR2B, HTR2C, HTR5A, HTR6, HTR7, LTB4R, MC4R, MTNR1A, MTNR1B, NPY2R, NPY5R, NR3C1, OPRD1, OPRM1, OXTR, P2RX7, PLG, PTAFR, PTGER1, PTGER2, PTGER4, PTGIR, S1PR1, TAAR1, TBXA2R, THRA, TRPV1, AKT1, AXL, BCL2, AGFR, ERBB2, GSK3B, IGF1R, JAK1, JAK2, KDR, MAP2K1, MAPK1, MAP3, MET, PDGFRB, PIK3CD, PIK3R1, PRKCA, PRKCG, SRC, STAT3, ATP1A1, HSD11B2, INSR, MAPK1, MAPK3, NR3C3, PIK3CD, PIK3R1, PRKCA, PRKCG, ALOX15, ALOX5 APP, CACNA1B, CASP3, CYP2C19, CYP2C9, CYP2D6, HTR1A, HTR1B, HTR1D, HTR2A, HTR2B, HTR2C, HTR5A, HTR6, HTR7, MAOA, MAOB, MAP2K1, MAPK1, MAPK3, PRKCA, PRKCH, PTGS1MPTGS2, SLC18A2, SLC6A4, AKT1, AGFR, ERBB2, EGFR1, FLT3, G6PD, GCK, HIF1A, KIT, LDHA, MAP2K1, MAPKQ, MAPK3, MET, PDGFRB, PIK3CD, PIK3R1, SIRT3, AKR1C3, ALOX15, ALOC5, CYP2C19, CYP2C9, EPHX2, HPGDS, LTH4H, PLA2G10, PLA2G1B, PLA2G5, PTGES, PTGS1, PTGS2

The proteins obtained from SwissTargetPrediction results were then analyzed further using StringDB. which aims to create a network of interactions between selected target proteins and analyze biological pathways influenced by these proteins **(Figure 1)** (Lena *et al.*, 2023). A database of known and predicted protein–protein interactions. Integrates functional relationship data from various sources (Grabowski and Rappsilber, 2019).

After that, KEGG enrichment analysis was carried out. KEGG (Kyoto Encyclopedia of Genes and Genomes) is a collection of manually drawn pathway maps representing our knowledge of molecular interactions and reaction networks (Kanehisa *et al.*, 2023). The pathways with strength values > 1 were selected **(Table 3).** From the analysis results, it is known that there are 15 pathways with strength values > 1. Many pathways related to cancer, including tyrosine metabolism, bladder cancer, non-small cell lung cancer, phenylalanine metabolism, EGFR tyrosine kinase inhibitor resistance, and central carbon metabolism in cancer. Besides that, there are pathways related to hypertension, such as the renin-angiotensin system and the aldosterone-regulated sodium reabsorption. Other pathways related to *C. sinensis* include nitrogen metabolism, caffeine metabolism, steroid hormone biosynthesis, linoleic acid metabolism, neuroactive ligand-receptor interaction, serotonergic synapse, and arachidonic acid metabolism.

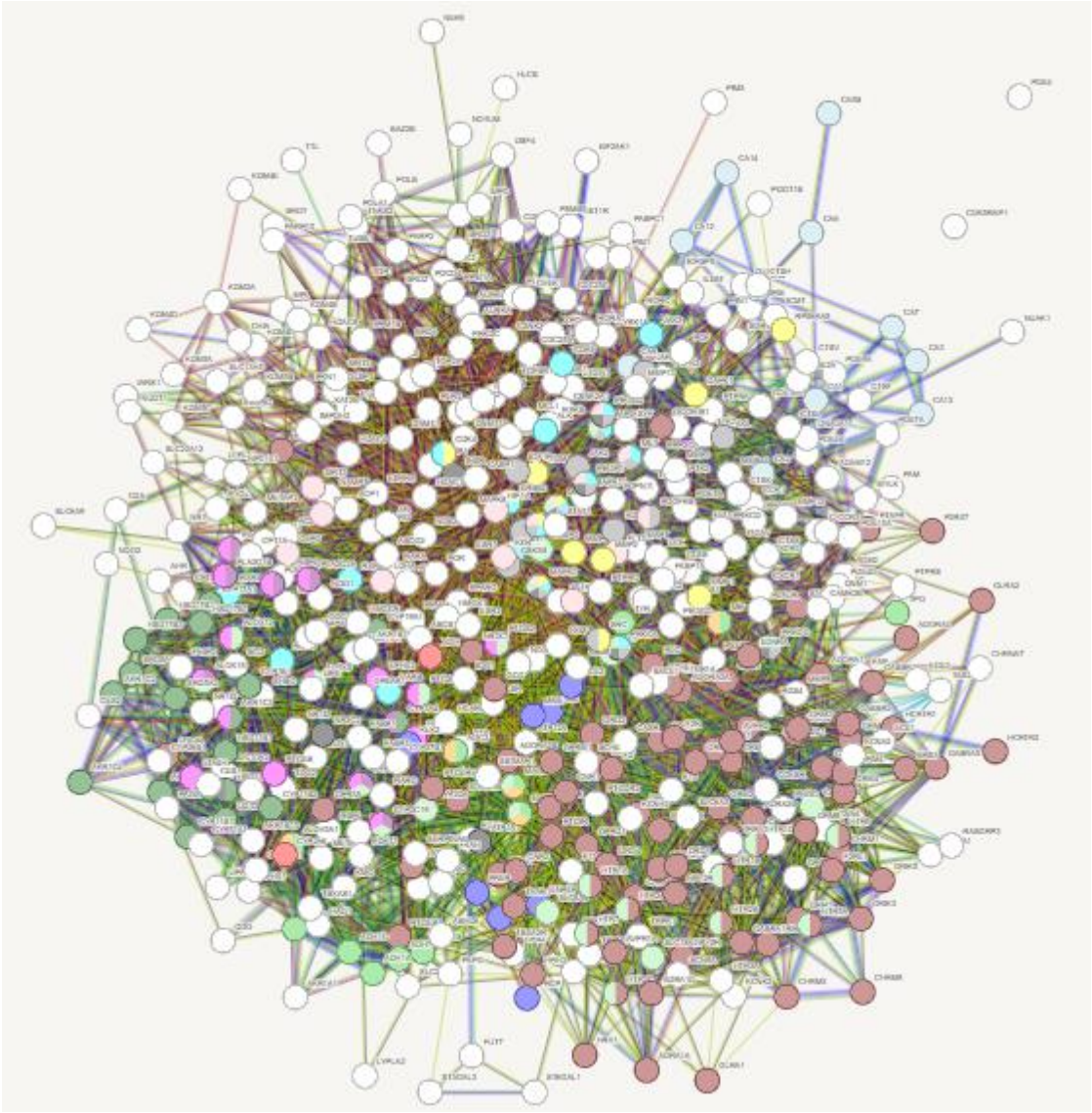

Figure 1. Network Pharmacology prediction results using StringDB

Table 3. Fifteen pathways with strength values >1 using KEGG Enrichment analysis

No	Pathway Code	Pathway Name	Strength
1	hsa00910	Nitrogen metabolism	1.41
2	hsa00232	Caffeine metabolism	1.20
3	hsa04614	Renin-angiotensis system	1.14
4	hsa00350	Tyrosine metabolism	1.13
5	hsa05219	Bladder Cancer	1.11
6	hsa00140	Steroid hormone biosynthesis	1.07
7	hsa05223	Non-small cell lung cancer	1.06
8	hsa00360	Phenylalanine metabolism	1.06
9	hsa00591	Linoleic acid metabolism	1.04
10	hsa04080	Neuroactive ligand-receptor interaction	1.03
11	hsa01521	EGFR tyrosine kinase inhibitor resistance	1.03
12	hsa04960	Aldosterone-regulated sodium reabsorption	1.03
13	hsa04726	Serotonergic synapse	1.02

14	hsa05230	Central carbon metabolism in cancer	1.02
15	hsa00590	Arachidonic acid metabolism	1.02

C. sinensis was known has anticancer activity. Flavonoids are a class of compounds responsible for tea's anticancer activity (Davalli *et al.*, 2012; Jiang *et al.*, 2021; Wang *et al.*, 2022). *C. sinensis* also was known has antihypertension (Basati *et al.*, 2021; Lai *et al.*, 2020; Verma *et al.*, 2021) and anti-inflammatory activity (Hodges *et al.*, 2020; Truong and Jeong, 2022).

Conclusion

Based on the results of network pharmacology analysis, *C. sinensis* has role in treatment hypertension, cancer, and anti-inflamation.

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