# A NETWORK PHARMACOLOGY OF LEMONGRASS (*Cymbopogon citratus*) ON COVID-19 CASES

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## Abstract

Various ways and treatment efforts are carried out to avoid the severe impact of COVID-19 cases, one of which is using plants as natural immunomodulatory agents. One of the plants that is proven to act as a natural immunomodulator is lemongrass (*Cymbopogon citratus*). This study aimed to determine the protein tissue associated with the body's immune system activated by *C. citratus*. The secondary metabolites of *C. citratus* were identified using the KNApSAck and Dr. Duke databases. Target proteins associated with plant-secondary metabolite compounds from the SwissTargetPrediction database and immunomodulatory-associated target proteins were obtained from the GeneCards database. The intersected proteins were put into StringDB and analyzed using KEGG to obtain network pharmacology. 98 secondary metabolite compounds of *C. citratus* were obtained from the database. Proteins associated with *C. citratus* contain 1096 compounds, and those related to immunomodulators contain 1380 proteins. The intersection results obtained 244 proteins predicted to interact with *C. citratus* and are related to immunomodulators. From the results of KEGG analysis, five pathways related to *C. citratus* were obtained, namely PD-L1 expression and PD-1 checkpoint pathway in cancer, Fc epsilon RI signaling pathway, Th17 cell differentiation, T cell receptor signaling pathway, and IL-17 signaling pathway. MAPK 1, MAPK 3, and MAPK 14 proteins are predicted to be in all five related pathways, and Mol 13 compounds are predicted to be able to interact with these three proteins. Thus, it can be concluded that the compound Mol 13 is the compound that plays the most role in acting as an immunomodulator in *C. citratus*.

***Keywords:*** *Lemongrass; Cymbopogon citratus; COVID-19; immunomodulator; network pharmacology*

## Backgroud

Corona viruses are known to cause disease in humans and animals, usually infecting only the upper respiratory tract and causing relatively minor symptoms (Syauqi, 2020). COVID-19 (Corona Virus Disease 2019), the disease that first appeared in Wuhan at the end of 2019 (Lena *et al*., 2023), can destroy lung cells and trigger local immune responses, recruiting macrophages and monocytes that respond to infection, release cytokines, and prime adaptive T and B cell immune responses. In most cases, this process can resolve the infection. However, in some cases, a dysfunctional immune response can lead to severe and even systemic lung pathologies (Tay *et al*., 2020).
Various ways and treatment efforts are carried out to avoid the severe impact of COVID-19 cases, one of which is using plants as natural immunomodulatory agents (Morley, 2020). Immunomodulators are essential to the treatment process amid the current pandemic and disasters. Immunomodulators help the body optimize the immune system's function, the central system that plays a role in the body's defence against viruses (Erniati and Ezraneti, 2020).Immunomodulators are compounds that have a role in the human immune system, specifically and non-specifically, that can improve the immune system and increase cellular and humoral defences in the human body (Erniati & Ezraneti, 2020). In addition, immunomodulators also function in improving the immune system in the body by restoring immune system function, which is generally called immune restoration, stimulating a disturbed immune system (immunostimulant), then immunodepression, which suppresses and normalizes immune reactions that experience abnormal or abnormal conditions (Fujiati *et al*., 2022).
One plant that is empirically used by the community to improve the body's immune system is lemongrass (*Cymbopogon citratus*). The ease of lemongrass in tropical regions such as Indonesia makes lemongrass a plant that is easily found in the yards of Indonesian people's homes and is often used as a spice for cooking. The phytochemical composition of lemongrass includes tannins, saponins, anthraquinones, phenols, flavonoids and alkaloids (Ernis *et al*., 2020).
Exploration of the activity of a compound of a plant can be done through three approaches: in vitro, in vivo, and in silico tests. Research is carried out with the in silico method for developing new drugs. It is more effective, efficient and economical because it is tested by computer (Zhang *et al*., 2019). The use of in silico methods is more like a computational predictive model. The purpose of in silico prediction is as a preliminary test before proceeding to in vitro and in vivo testing, screening several test compounds before proceeding to the in vitro and in vivo stages to predict the toxicity of these compounds (Ekins *et al*., 2007). Network Pharmacology have been explicitly used to explore protein/gene-disease connectivity pathways. It can illustrate the complexity between biological systems, drugs and disease to network and provide a comprehensive approach. Recent years have seen an overall increase in traditional medicinal plant research due to their natural resources and the ability to use these plants to complement existing pharmacological approaches (Veda *et al*., 2023). Based on the background description that has been described, this study aims to determine the protein network associated with the body's immune system that is activated due to the administration of lemongrass (Sharma *and* Kaur, 2022)

## Methods

### *Tools*

Some of the online databases was used in this research include: KNApSAcK (http://www.knapsackfamily.com/KNApSAcK/), Dr. Duke's Phytochemical and Ethnobotanical Databases (https://phytochem.nal.usda.gov/), PubChem (https://pubchem.ncbi.nlm.nih.gov/), 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***

The secondary metabolite compounds of plants were identified using the KNApSAck and Dr. Duke's Phytochemical and Ethnobotanical Databases, then looking for the SMILES code of each compound using PubChem (Lena *et al*., 2023). Proteins with a probability of > 0 that were predicted using SwissTargetPrediction were selected for further analysis (Daina *et al*., 2019). The immunomodulators-linked proteins have been searched using Genecards (Stelzer *et al*., 2016). Then, look for the intersection between the predicted proteins from SwissTargetPrediction and immunomodulator-linked proteins using Venny (Daina *et al*., 2019). The list of proteins that appear is then entered into the StringDB (Szklarczyk *et al*., 2021),after which predictions of proteins related to the immune system are searched using KEGG (Kanehisa *et al*., 2023) by looking at which proteins interact with the most with pathways related to the immune system and then looking at secondary metabolite compounds of lemongrass that interact with these proteins.

## Results and Discussion

***Identification and Bioavailability Prediction of Secondary Metabolites of Lemongrass***

Secondary metabolites of lemongrass were obtained using the KNApSAcK Family and Dr. Duke's Phytochemicals and Ethnobotanical Databases. There are 76 (KNApSAcK Family) and 34 (Dr. Duke's Phytochemicals and Ethnobotanical Databases), with a total of 98 secondary metabolite compounds obtained from those databases (**Table 1**). KNApSAcK is the database with the highest number of compounds. KNApSAcK ranks first with more than 10,500 entries for therapeutic efficacy and biological activity records (Nguyen-Vo *et al*., 2020). Dr. Duke's Phytochemicals and Ethnobotanical Databases was widely used to characterize bioactive compounds in plants (Anand *and* Gokulakrishnan, 2012).

**Table 1. A list secondary metabolite of lemongrass from KNApSAcK Family (1) and Dr. Duke's Phytochemicals and Ethnobotanical Databases (2)**

| No | Compound Name | Compound Code | Source |
|---|---|---|---|
| **1** | (-)-beta-Caryophyllene epoxide | Mol 1 | [1] |
| **2** | (E)-Ferulic acid | Mol 2 | [1] |
| **3** | 1,8-cyneal | Mol 3 | [1,2] |
| **4** | 2-methyl-5-(1-propenyl)pyrazine | Mol 4 | [1] |
| **5** | 2-Methyl-benzoxazole | Mol 5 | [1] |
| **6** | 3,7-Dimethyl-2,6-octadien-1-ol | Mol 6 | [1] |
| **7** | 3-O-Caffeoylquinic acid | Mol 7 | [1] |
| **8** | 3-O-Feruloylquinic acid | Mol 8 | [1] |
| **9** | 5-Caffeoylquinic acid | Mol 9 | [1] |
| **10** | Alpha,4-Dimethylstyrene | Mol 10 | [1] |
| **11** | Alpha-beta-dihydropseudoionone | Mol 11 | [2] |
| **12** | Alpha camphorene | Mol 12 | [1,2] |
| **13** | Alpha-pinene | Mol 13 | [1,2] |
| **14** | Alpha-terpineol | Mol 14 | [2] |
| **15** | Astragalin | Mol 15 | [1] |
| **16** | Beta-Myrcene | Mol 16 | [1] |
| **17** | Beta-Nerol | Mol 17 | [1] |
| **18** | Beta-Pinene | Mol 18 | [1] |
| **19** | Beta-Thujene | Mol 19 | [1] |
| **20** | Beta-dihydropseudoionone | Mol 20 | [2] |
| **21** | Beta-sitosterol | Mol 21 | [2] |

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|---|---|---|---|
| **22** | Biochanin A | Mol 22 | [1] |
| **23** | Biochanin A 7-O-glucoside | Mol 23 | [1] |
| **24** | Capric-acid | Mol 24 | [2] |
| **25** | Caprylic-acid | Mol 25 | [2] |
| **26** | Caryophyllene | Mol 26 | [2] |
| **27** | Caffeic acid | Mol 27 | [1] |
| **28** | Carlinoside | Mol 28 | [1] |
| **29** | Carveol | Mol 29 | [1] |
| **30** | Cassiaoccidentalin B | Mol 30 | [1] |
| **31** | Cinaroside | Mol 31 | [1] |
| **32** | Cis-beta-Ocimene | Mol 32 | [1] |
| **33** | Cis-Citral | Mol 33 | [1] |
| **34** | Cis-p-Coumaric acid | Mol 34 | [1] |
| **35** | Cis-Verbenol | Mol 35 | [1] |
| **36** | Citral | Mol 36 | [1,2] |
| **37** | Citronellal | Mol 37 | [1,2] |
| **38** | Citronellol | Mol 38 | [1,2] |
| **39** | Cyclopentadiene | Mol 39 | [1] |
| **40** | Cyclopentene | Mol 40 | [1] |
| **41** | Cymbopogone | Mol 41 | [1,2] |
| **42** | Cymbopogonol | Mol 42 | [1,2] |
| **43** | Farnesal | Mol 43 | [2] |
| **44** | Farnesol | Mol 44 | [2] |
| **45** | Fucosterol | Mol 45 | [2] |
| **46** | Furfural | Mol 46 | [2] |
| **47** | Genistein | Mol 47 | [1] |
| **48** | Genistein 7-O-glucoside | Mol 48 | [1] |
| **49** | Genistein 8-C-glucoside | Mol 49 | [1] |
| **50** | Geranic acid | Mol 50 | [1,2] |
| **51** | Geranyl butyrate | Mol 51 | [1] |
| **52** | Geraniol | Mol 52 | [2] |
| **53** | Geranyl-acetate | Mol 53 | [2] |
| **54** | Gomphrenin II | Mol 54 | [1] |
| **55** | Gomphrenin III | Mol 55 | [1] |
| **56** | Hirsutrin | Mol 56 | [1] |
| **57** | Isogomphrenin II | Mol 57 | [1] |
| **58** | Isogomphrenin III | Mol 58 | [1] |
| **59** | Isoorient | Mol 59 | [1] |
| **60** | Isopulegol | Mol 60 | [2] |
| **61** | Isovaleraldehyde | Mol 61 | [2] |
| **62** | Isovaleric-acid | Mol 62 | [2] |
| **63** | Isoorientin 2''-O-rhamnoside | Mol 63 | [1] |
| **64** | Isorhamnetin 3-O-beta-D-glucopyranoside | Mol 64 | [1] |
| **65** | Isorhamnetin 3-O-rutinoside | Mol 65 | [1] |
| **66** | Isoshaftosides | Mol 66 | [1] |
| **67** | Isoscoparin | Mol 67 | [1] |
| **68** | Kurilensin A | Mol 68 | [1] |
| **69** | Linalool | Mol 69 | [1,2] |
| **70** | Lonicerin | Mol 70 | [1] |

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|---|---|---|---|
| **71** | Luteolin | Mol 71 | [1,2] |
| **72** | L-linalool | Mol 72 | [2] |
| **73** | Limonene | Mol 73 | [2] |
| **74** | Linalyl-acetate | Mol 74 | [2] |
| **75** | M-Toluamide | Mol 75 | [1] |
| **76** | Myrtanal | Mol 76 | [1] |
| **77** | Myrtenol | Mol 77 | [1] |
| **78** | Myrcene | Mol 78 | [2] |
| **79** | Nerolic acid | Mol 79 | [1] |
| **80** | Nicotiflorin | Mol 80 | [1] |
| **81** | Nerol | Mol 81 | [2] |
| **82** | Orientin | Mol 82 | [1] |
| **83** | P-Coumaric acid | Mol 83 | [1] |
| **84** | P-Cymene | Mol 84 | [1] |
| **85** | Pearls | Mol 85 | [1] |
| **86** | Photocitral A | Mol 86 | [1] |
| **87** | Phytol | Mol 87 | [1] |
| **88** | Pinacol | Mol 88 | [1] |
| **89** | P-Mentha-3,8-diene | Mol 89 | [1] |
| **90** | Prunet's | Mol 90 | [1] |
| **91** | Quercetin | Mol 91 | [2] |
| **92** | Routine | Mol 92 | [1,2] |
| **93** | Swertiajapony | Mol 93 | [1] |
| **94** | Trans-4-Hydroxycinnamic acid | Mol 94 | [1] |
| **95** | Trans-alpha-Bergamotene | Mol 95 | [1] |
| **96** | Trans-beta-Ocimene | Mol 96 | [1] |
| **97** | Trans-Carveol | Mol 97 | [1] |
| **98** | Trans-Citral | Mol 98 | [1] |

***Immunomodulator-linked Protein That Predicted Interact With Lemongrass***

A search for target protein predictions of each prediction compound was carried out using SwissTargetPrediction. The search results found that there are 909 proteins that are predicted to interact with secondary metabolites of the lemongrass. Immunomodulator-linked protein was searched using GeneCard. There were 1340 proteins obtained related to immunomodulators. Furthermore, intersections were carried out using Venny between proteins that were predicted to interact with secondary metabolites of lemongrass and immunomodulator-linked proteins, and there are 244 proteins that are predicted to interact with secondary metabolites of lemongrass related to immunomodulators (**Figure 1**).

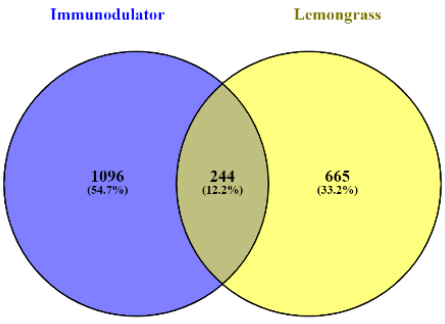

**Figure 1. The protein is predicted to interact with secondary metabolites of lemongrass with proteins related to immunomodulators**

**Network Pharmacology Analysis**

The protein obtained from the results of the Venn diagram intersection is then further analyzed using StringDB, which aims to create a network of interactions between selected target proteins and analyze immunostimulating biological pathways influenced by these proteins (**Figure 3**) (Lena *et al*., 2023; Veda *et al*., 2023). StringDB is a database of known and predicted protein-protein interactions integrating functional relationship data from multiple sources, including more than nine million proteins (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 five highest strength values of immunomodulators-linked pathways were selected for further analysis (**Table 3**). Three proteins are connected to five immunomodulator-linked pathways: MAPK1, MAPK3, and MAPK14.

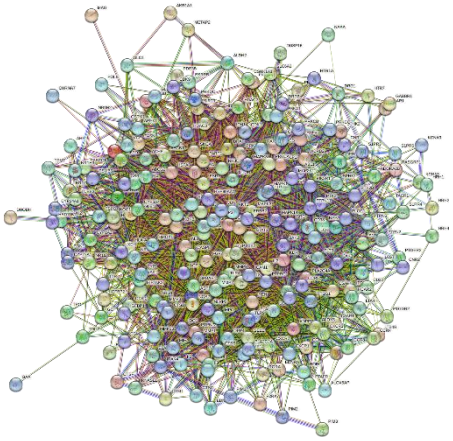

**Figure 2. Network pharmacology analysis using StringDB**

**Table 3. Five highest immunomodulator-linked pathways using KEGG enrichment**

| Code | Pathway | Strength |
|---|---|---|
| Hsa05235 | PD-L1 expression and PD-1 checkpoint pathway in cancer | 1.37 |
| Hsa04664 | Fc epsilon RI signaling pathway | 1.3 |
| Hsa04659 | Th17 cell differentiation | 1.28 |
| Hsa04660 | T cell receptor signaling pathway | 1.25 |
| Hsa04657 | IL-17 signaling pathway | 1.21 |

In further analysis, there is one compound (Mol 13) that has the most potential to be developed into immunomodulators (**Table 4**). This compound (alpha-pinen) connect to three immunomodulator-linked protein. Therefore, lemongrass has the potential to be further explored and developed as a promising immunomodulating agent.

**Table 4. A list of secondary metabolites that was predicted can interact with immunomodulator-linked pathways.**

| Compound | Molecule |
|---|---|
| MAPK 1 | Mol 2, Mol 7, Mol 8, Mol 9, Mol 13, Mol 14, Mol 27, Mol 34, Mol 41, Mol 60, Mol 69, Mol 72, Mol 81, Mol 83, Mol 89, Mol 94 |

| Compound | Molecule |
|---|---|
| MAPK 3 | Mol 6, Mol 13, Mol 21, Mol 35, Mol 45, Mol 52, Mol 73, Mol 81 |
| MAPK 14 | Mol 1, Mol 5, Mol 6, Mol 8, Mol 11, Mol 13, Mol 20, Mol 33, Mol 41, Mol 42, Mol 43, Mol 44, Mol 45, Mol 51, Mol 52, Mol 57, Mol 61, Mol 69, Mol 85, Mol 89 |

## Conclusion

Based on the network pharmacology analysis, alpha-pinene has the most potential to be developed into immunomodulators because it interacts with three essential immunomodulator-linked proteins, which interact with five immunomodulator-related pathways.

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## References

Anand, T. *and* Gokulakrishnan, K. (2012) 'GC–MS Analysis and Anti-Microbial of Bioactive Components of Hybanthus enneaspermus', *Int J Pharm Pharm Sci*, 4, p. 646-650.

Daina, A. *et al*. (2019) 'SwissTargetPrediction: Updated Data and New Features for Efficient Prediction of Protein Targets of Small Molecules', *Nucleic Acids Res*, 47, p. 357-366. doi: 10.1093/nar/gkz382.

Ekins, S. *et al*. (2007) 'In Silico Pharmacology for Drug Discovery: Methods for Virtual Ligand Screening and Profiling', *Br J Pharmacol*, 152, p. 9-20. doi: 10.1038/sj.bjp.0707305.

Erniati, E. *and* Ezraneti, R. (2020) 'Aktivitas Imunomodulator Ekstrak Rumput Laut' *Acta Aquatica: Aquatic Sciences Journal*, 7, 79. doi: 10.29103/aa.v7i2.2463.

Ernis, G. *et al*. (2020) 'Uji In Vitro Aktivitas Imunomodulator Minyak Atsiri Serai Dapur (Cymbopogon citratus) Terhadap Proliferasi Sel Limfosit Mencit', *BIOEDUSAINS: Jurnal Pendidikan Biologi dan Sains*, 4, p. 129-135.

Fujiati, F. *et al*. (2022) 'Pemanfaatan Tanaman Herbal Sebagai Imunomodulator Dalam Rangka Meningkatkan Imunitas Bagi Lansia Di Panti Sosial Tresna Werdha Banjabaru', *Jurnal Pengabdian Al-Ikhlas*, 7. doi: 10.31602/jpaiuniska.v7i3.6872.

Grabowski, P. *and* Rappsilber, J. (2019) 'A Primer on Data Analytics in Functional Genomics: How to Move from Data to Insight?', *Trends Biochem Sci*, 44, 21-32. doi: 10.1016/j.tibs.2018.10.010.

Kanehisa, M. *et al*. (2023) 'KEGG for Taxonomy-Based Analysis of Pathways and Genomes', *Nucleic Acids Res*, 51, p. 587-592. doi: 10.1093/nar/gkac963.

Lena, N. *et al*. (2023) 'Analisis Jejaring Farmakologi Tanaman Jati Belanda (Guazuma ulmifolia Lamk.) Sebagai Imunomodulator', *Journal of Islamic Pharmacy*, 8, p. 1-6. doi: 10.18860/jip.v8i1.20782.

Morley, J. E. (2020) 'COVID-19 — The Long Road to Recovery', *Journal of Nutrition, Health and Aging*, 24, p. 917-919. doi: 10.1007/s12603-020-1497-y.

Nguyen-Vo, T. H. *et al*. (2020) 'Plant Metabolite Databases: From Herbal Medicines to Modern Drug Discovery', *J Chem Inf Model*, 60, p. 1101-1110. doi: 10.1021/acs.jcim.9b00826.

Sharma, A. D. *and* Kaur, I. (2022) 'GC-FID Based Aromatic Profiling and Molecular Docking Studies of Lemon Grass (Cymbopogon citratus L.) Essential Oil as Novel Therapeutic for SARS-Cov2 Spike Protein', *Arabian Journal of Medicinal and Aromatic Plants*, 8, p. 1-20. doi: 10.48347/IMIST.PRSM/ajmap-v8i2.30261.

Stelzer, G. *et al*. (2016) 'The GeneCards suite: From Gene Data Mining to Disease Genome Sequence Analyses', *Curr Protoc Bioinformatic*, 1, p. 30-33. doi: 10.1002/cpbi.5.

Syauqi, A. (2020) 'Jalan Panjang Covid-19 (Sebuah Refleksi Dikala Wabah Merajalela Berdampak pada Perekonomian)', *JKUBS: Journal of Chemical Information and Modeling*, 1, p. 1-19.

Szklarczyk, D. *et al*. (2021) 'Erratum: The STRING Database in 2021: Customizable Protein–Protein Networks, and Functional Characterization of User-Uploaded Gene/Measurement Sets', *Nucleic Acids Research*, 49, p. 605-612. doi: 10.1093/nar/gkab835.

Tay, M. Z. *et al*. (2020) 'The Trinity of COVID-19: Immunity, Inflammation and Intervention', *Nat Rev Immunol*, 20, p. 363-374. doi: 10.1038/s41577-020-0311-8.

Veda, A. I. H. *et al*. (2023) 'In Silico Study Potential Secondary Metabolite Candidate of Citronella Grass (Cymbopogon nardus) on Immunity Cases', *Eksakta : Berkala Ilmiah Bidang MIPA*, 24, p. 465-475.

Zhang, R. *et al*. (2019) 'Network Pharmacology Databases for Traditional Chinese Medicine: Review and Assessment', *Front Pharmacol*, 10, p. 1-14. doi: 10.3389/fphar.2019.00123.