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Curcuma Longa Herbs: A Mechanism to Increase the Innate Immune Response Through a Pharmacological System Approach

Firasti Agung Nugrahening Sumadi, Nur Asyifa Azzahra, Lely Mauliana, Septia Lupita Sari, Ahmad Shobrun Jamil*

Pharmacy Department Program, University of Muhammadiyah Malang, East Java Indonesia

ORCID
Ahmad Shobrun Jamil: https://orcid.org/0000-0002-0839-7941

Abstract.
Innate immunity is the body's first line of defense against infection. One form of the innate immune response is the production of the antimicrobial peptide cathelicidin, which has many important roles in the host immunity against tuberculosis infection, as well as being an antimicrobial therapy. This study's objective was to determine the potential effect of *Curcuma longa*, an herb commonly found in Indonesia, to increase the production of the antimicrobial peptide cathelicidin, and its interaction patterns in cells using an in silico approach. The data on the chemical content of the plant were gathered from the Dr. Duke Phytochemical Database, with the prediction of absorption, distribution, metabolism, and excretion (ADME) of compounds in the plant's rhizomes conducted using the Swiss ADME Software. The Swisstargetprediction.ch website was used to predict the relationship of the plant compounds with cell proteins, and STRINGDB was used to look for pharmacological networks. The results showed that *C. longa* enhances innate immunity through the activation of TLR7/9 and other related proteins. The findings also showed that the activation of the innate immune response is followed by activation of the adaptive immune response along with its related proteins.

Keywords: antimicrobial peptide, cathelicidin, *C. longa*, pharmacological network

Corresponding Author: Ahmad Shobrun Jamil; email: shobrun@umm.ac.id

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1. Introduction

One of the important efforts in the treatment of infectious diseases is to strengthen the body's immune function by vaccination and adding nutrients that strengthen immunity. The body has both innate and adaptive immunity. Innate immunity is the body's first line of defense against infection. Innate immunity is not specific but is known to have high effectiveness in warding off infection if it is supported by a rapid and adequate response from the body's epithelial cells that are in direct contact with the infectious agent/pathogen for the first time.

One form of the innate immune response is the production of anti-microbial peptide (PAM), namely cathelicidin[1]. The cathelicidins is a group of PAM consisting of several types of peptides that have many functions in the body's immune system. Some examples of peptides belonging to the Cathelicidins family are Cathelicidins-LL-37, Cathelicidins-Bactenecin, Cathelicidins-Bac5, and Cathelicidins-PR-39. All types of peptides have broad activity against microbes (broad-spectrum)[2]. One important thing about Cathelicidin is that it has many important roles in host immunity against tuberculosis infection apart from being an antimicrobial [1, 3].

As a peptide expressed in cell biology systems. Cathelicidin production is triggered various externally. Among the external factors that influence the increased expression of cathelicidin is the availability of triggering active compounds such as retinoids, vitamin D, and several bioactive compounds from plants[4].

Currently, fast and accurate research is needed to anticipate the rapid development of infectious diseases. In silico analysis is a computational method with a fairly accurate meta-database. An in-silico approach that helps select plant materials or natural products with possible strong biological activity. This approach can also provide insight into the rationalization of the biological activity of natural compounds on body cells [5].

Based on the facts above, the problems that will be discussed in this study are what plants have the potential to increase the production of PAM cathelicidin and how their interaction patterns in cells through the in silico approach. The purpose of this study was to determine the potential natural ingredients of plant origin as candidates for immunomodulators to increase the expression of cathelicidin through an in silico bioinformatics analysis approach. This research was a preliminary study by utilizing metadata and in silico analysis which can be continued in research with in vitro and in vivo preclinical and clinical approaches.

2. Materials and Methods

2.1. Phytochemical Data Warehouse and Phytochemical Data Unification

The data source used to determine the chemical content of candidate plants is the Dr Duke Phytochemical Database warehouse page with the page https://phytochem.nal.usda.gov/ [6]. The data was obtained by entering the scientific name of the plant in the search menu and selecting the menu (p) which means phytochemical in the Latin name search list. Furthermore, the data is unified by completing the identity of

the compound including canonical smiles by entering the names of the compounds contained in the data warehouse one by one https://pubchem.ncbi.nlm.nih.gov/. The result of this unification is a list of compound names, compound codes, synonyms, canonical smiles, and other supporting data.

2.2. Prediction of Absorption, Distribution, Metabolism, and Excretion (ADME) of compounds in candidate plant rhizomes with Swiss ADME Software

ADME prediction of compounds contained in candidate plants was carried out using http://www.swissadme.ch/ [7]. Canonical smiles data for Curcuma longa compound obtained from the page https://pubchem.ncbi.nlm.nih.gov/ and unified in the previous stage, in the entry in the menu provided http://www.swissadme.ch/. Canonical smiles entries are carried out directly for all compounds with a sequence of canonical smiles entries followed by eleven compound code characters that we created ourselves in the previous unification table. Then click the ”run” menu and the results of the ADME analysis will appear.

2.3. Prediction of the relationship of candidate plant compounds with cell proteins using Swisstargetprediction software

Prediction of the relationship of plant compounds with cell proteins is used http://www.swisstargetprediction.ch/ [8]. Canonical smiles data in entries one by one. Each compound will give rise to several candidate protein relations with strong, moderate to weak probabilities.

2.4. Prediction of Pharmacological Networks to increase the body's immune response by plant induction

The STRING database in the https://string-db.org page aims to collect, assess and integrate all sources of protein-protein interaction information present in the database and to complement it with computational predictions. The aim is to show a comprehensive and objective picture of the global network of cell protein-protein relationships, including direct (physical) and indirect (functional) interactions between these proteins [9]. Proteins that have been recorded through the database http://www.swisstargetprediction.ch/in the entry in the multiple protein menu in the

STRING data. Furthermore, the program will process and generate network configurations between proteins related to the induction of candidate plant compounds in cells. The configuration of the pharmacological network is visualized with Cytoscape software.

3. Results and Discussion

3.1. Result

3.1.1. The results of screening the content of C. longa compounds from the phytochemical data warehouse

Based on data search by mining on the webserver providing data for validated phytochemical compounds on the pagehttps://phytochem.nal.usda.gov/obtained 267 active compounds C. longa. From the 267 compounds, the data was unified by completing the identity of the compounds including canonical smile, PubChem ID, synonyms, and other supporting data. The data is then screened to get the best drug-likeness (DL) data.

3.1.2. Screening results of C. longa compounds with Drug Likeness and Correlation with Innate immunity target protein

Screening for absorption, distribution of metabolism, and excretion (ADME) using softwarehttp://www.swissadme.ch/. found that there were four compounds with high drug-likeness and strong interactions with the innate immune receptor proteins TLR7 and TLR 9, namely Monodemethoxycurcumin, Bis-Demethoxycurcumin, Curcumin, and Curdione. These four compounds according to Lipinski's criteria include compounds with high DL. Based on the results of the correlation analysis of compounds and proteins using softwarehttp://www.swisstargetprediction.ch/ these compounds have a high correlation with Toll-Like Receptor (TLR) 7 and 9.

3.1.3. Results of pharmacological network analysis of C. longa with protein components of innate immunity

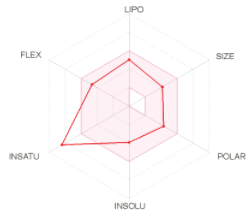
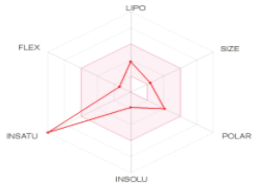
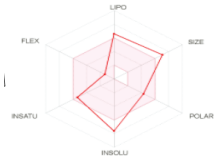
No	Compound Name	Drug Likeness	Innate immune correlated protein target
1	Monodemethoxycurcumin		TLR 7/9
2	Bis-Demethoxycurcumin		TLR 7/9
3	Curcumin		TLR 7/9
4	Curdion		TLR 7/9

Figure 1: ADME screening results, drug likeness and correlation with innate immune component proteins.

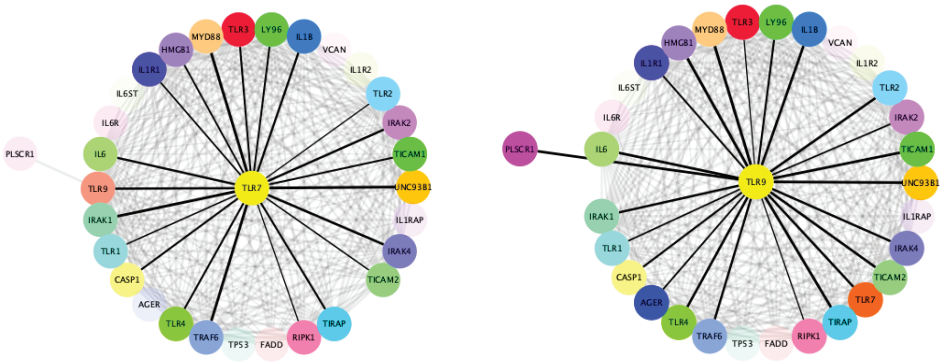


Figure 2: Pharmacological network of TLR 7 and 9 proteins with associated proteins in the cell. The thick line shows the relationship between TLR-7 and protein with the closest relationship in terms of possible co-expression, protein neighborhood, and also a correlation in various journal texts (text mining). TLR 7/9 was closely correlated with the activity of immune-related proteins including IL-6, TICAM2, TICAM1, TLR1, UNC93B1, RIPK1, PLSCR1, IRAK4, TLR4, TRAF6, CAMP, DEF10B1.

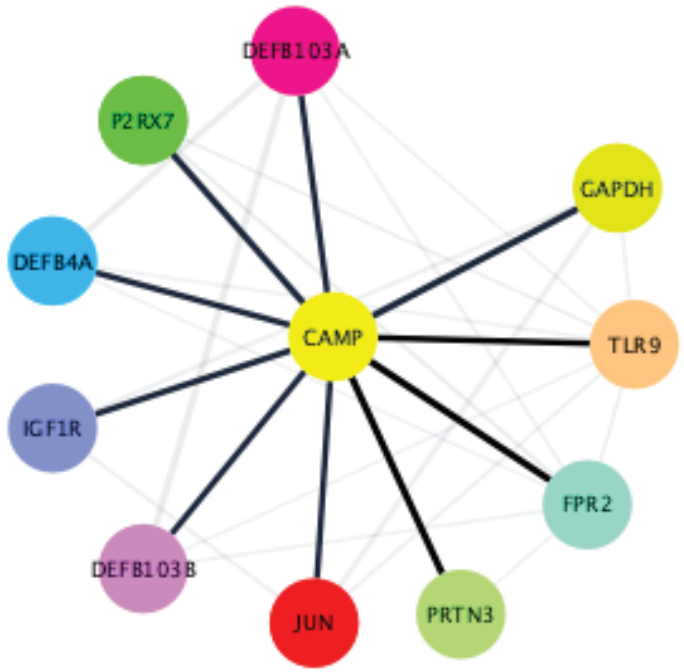


Figure 3: Relationship of CAMP with TLR 9 and several other component proteins of innate immunity.

4. Discussion

Bioinformatics analysis methods, including microarray data analysis and integrated systems pharmacology approaches, are starting to be used to predict the potential of certain compounds to interact with cell proteins for a pharmacological therapy approach [10]. Most of the pharmacologically active molecules fail to reach the market or have an inconvenient route of administration because of their chemical structure. This is especially important given the recent trend to develop drug candidates out of space similar to drugs to deal with difficult target proteins due to the need for linkage between proteins. The aim of the in silico research is to conduct an accurate study with chemical and pharmaceutical approaches so that drug candidates are known to have good ADME-Tox properties and high bioavailability [11].

In exploratory research, to determine the activity of compounds in plants and test their pharmacological efficacy, three approaches can be used. The first is an in vitro test, the second is an in vivo test and the third is an in silico test. The in silico test is both scientifically sound and fairly new and has good accuracy [12].

The linkage and association of cathelicidin as an antimicrobial peptide with TLR7 and TLR 9 was shown in the analysis usinghttp://string-db.org which can be seen in Figure 3. It appears that CAMP is co-expressed with TLR 9. Furthermore, based on ADME analysis and the relationship between molecules and compounds TLR 9 and 7 have similar relationships with immune-related proteins including IL-6, TICAM2, TICAM1, TLR1, UNC93B1, RIPK1, PLSCR1, IRAK4, TLR4, TRAF6, CAMP, DEF10B1.

Interleukin-6; Cytokines with various biological functions. IL-6 is a strong inducer of the acute phase response. Plays an important role in the final differentiation of B-cells into Ig-secreting cells.-Involved in the differentiation of lymphocytes and monocytes. Act on B cells, T cells, hepatocytes, hematopoietic progenitor cells, and CNS cells [13]. TICAM2; Serves as a sorting adapter in LPS-TLR4 signaling to regulate the MYD88 independent pathway during the innate immune response to LPS. Physically bridges TLR4 and TICAM1 and functionally transmits LPS-TRL4 signals to TICAM1; signaling is proposed to occur in early endosomes after TLR4 endocytosis. It may also be involved in IL1-triggered NF-kappa-B activation, functioning upstream of IRAK1, IRAK2, TRAF6, and IKBKB. TICAM1; Involved in innate immunity against invading pathogens. Adapters used by TLR3 and TLR4 (via TICAM2) to mediate NF-kappa-B and activation of interferon regulatory factor (IRF), and to induce apoptosis.

TLR 1; Participates in the innate immune response to microbial agents. Specifically, recognize acylated and triacylated lipopeptides. Cooperates with TLR2 to mediate the innate immune response to bacterial lipoprotein or lipopeptide [14]. UNC93B1; Plays an important role in innate and adaptive immunity by regulating nucleotide-sensing Toll-like receptor (TLR) signaling. Required for the transport of a subset of TLRs (including TLR3, TLR7, and TLR9) from the endoplasmic reticulum to the endolysosomes where they can engage pathogenic nucleotides and activate signaling cascades[15]. PLSCR1; plays a central role in the initiation of fibrin clot formation, in mast cell activation, and the recognition of apoptosis and injured cells by the reticuloendothelial system[14]. TLR4; Cooperates with LY96 and CD14 to mediate the innate immune response to bacterial lipopolysaccharide (LPS). Acting via MYD88, TIRAP, and TRAF6, leading to NF-kappa-B activation, cytokine secretion, and inflammatory response [15]. RIPK1; transduces inflammatory and cell death signals (programmed necrosis) following death receptor ligation, pathogen recognition receptor (PRR) activation, and DNA damage [16]. IRAK4 plays an important role in initiating the innate immune response against foreign pathogens. Involved in Toll-like receptor (TLR) and IL-1R. signaling pathways [17]. TRAF6; role in maturation and/or activation of dendritic cells (DCs) [18].

5. Conclusion

The pharmacological network of C. longa in enhancing innate immunity, among others, is the activation of TLR7/9 and other related proteins which show the relationship that the activation of the innate immune response will be followed by activation of the adaptive immune response along with its related proteins.

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