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Bioinformatics Analysis of Inhibition Activation SHP-2 by Galangal as Activating Agent of Cancer Immunotherapy

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Abstract

Interleukin 12 (IL-12) is a pro-inflammatory cytokine type 1 that has acted as a potential immunotherapy for cancer. The mechanism of IL-12 increases the activity of cytotoxic T cells and Natural Killer (NK) cells, reverse tumor-induced immunosuppression, prevent angiogenesis, and increases lymphocyte and antigen transport. Galangal is one of the natural ingredients that have biological activity as an anticancer and immunomodulator. In this research, researchers wanted to know the potential of the active compound of galangal to activate IL-12 by inhibiting the IL-12 analog, namely SHP-2. This research uses bioinformatics studies using several databases such as RCSB PDB, ChEMBL, Dr. Duke's Phytochemical and Ethnobotanical, UALCAN, OncoLnc and computational analysis using KNIME and MOE software. The SHP-2 structure used is taken from the RCSB PDB with the code 5EHR. The 10 compounds with the highest predictions of inhibiting SHP2 using KNIME were obtained, then molecular docking was performed using MOE and three compounds that had the potential to inhibit SHP-2 were Kaempferide, Galangin, and Riboflavin

Keywords: *cancer, computing, galangal,* Interleukin 12*, SHP-2.*

INTRODUCTION

Based on WHO data in 2018, cancer is the second leading cause of death in the world with an estimated 9.8 million deaths in 2018. One of the methods of cancer treatment is through immunotherapy. Immune system-based cancer therapy has the potential to be the best approach in the treatment of various types of cancer. Rejection from the immune system is rare against cancer and is a characteristic of cancer due to a mechanism of decreased immune function mediated by tumor cells. Immunotherapy can be developed by understanding these barriers (Curiel, 2013).

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The immune system plays a role in resistance to various kinds of pathogens such as bacteria, viruses, toxic materials, and fungi that can cause disease. The innate immune system tha involves dendritic cells and macrophage cells are one of the first to fight against pathogens. Activation of dendritic immune cells against pathogens will release various cytokine mediators (Kumar, *et al*., 2011). Since the discovery of cytokines as cancer therapeutic agents, various studies have evaluated more than 40 types of cytokines related to disease and therapy. The macrophage cells release some cytokine production due to viruses through negatively regulator of MTMR3,4 (Putri, *et al.*, 2018).

Activation of dendritic macrophage by pathogens (such as bacteria, viruses) activate the transcription factor *Janus Kinase/Signal ransducers and Activators of transcription* (JAK/STAT) family. The activation of JAK/STAT signaling pathway release the cytokine mediator Interleukin-12 (IL-12)-inducing an anti-tumor immune response. The release of IL-12 will activate *Natural Killer* (NK) cells and will affect the differentiation of T cells, especially in increasing the activation of anti-tumor CD4 Th1 (Xu., 2010). IL-12 as one of the cytokines has potential as immunotherapy because it can increase the cytotoxic activity of T cells and NK cells while reversing tumor-induced immunosuppression, preventing angiogenesis, and increasing lymphocyte and antigen transport (Nguyen, *et al*., 2020). IL-12 plays as therapeutic target in inflammatory diseases such as cancer. IL-12 expressed by activated M1 macrophage which SHP-2 plays in the differentiation of macrophage. SHP-2, Scr homology-2-containing protein tyrosine phosphate. SHP-2 activate JAK/STAT signaling pathway. SHP-2 plays role important in cancer cells invasion-metastasis, cell proliferation, cell cyle, apoptosis and drug resistant (Kanumuri, *et al*., 2022). Inhibition of SHP2 is promising targeted as cancer chemotherapeutic.

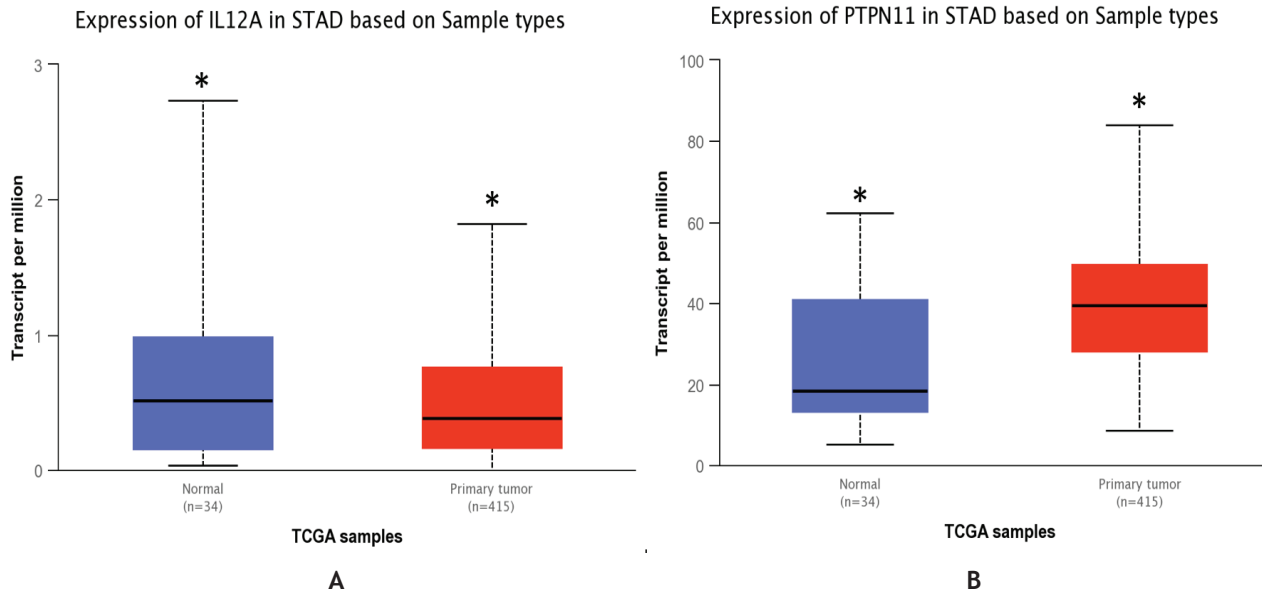

Figure 1. The protein expression of IL12A (A) and SHP-2 (encoded by PTPN11) (B) presented in normal cells (n=34) vs (b) primary tumor (n=415) sample types from UALCAN database. *$p<0.05$.

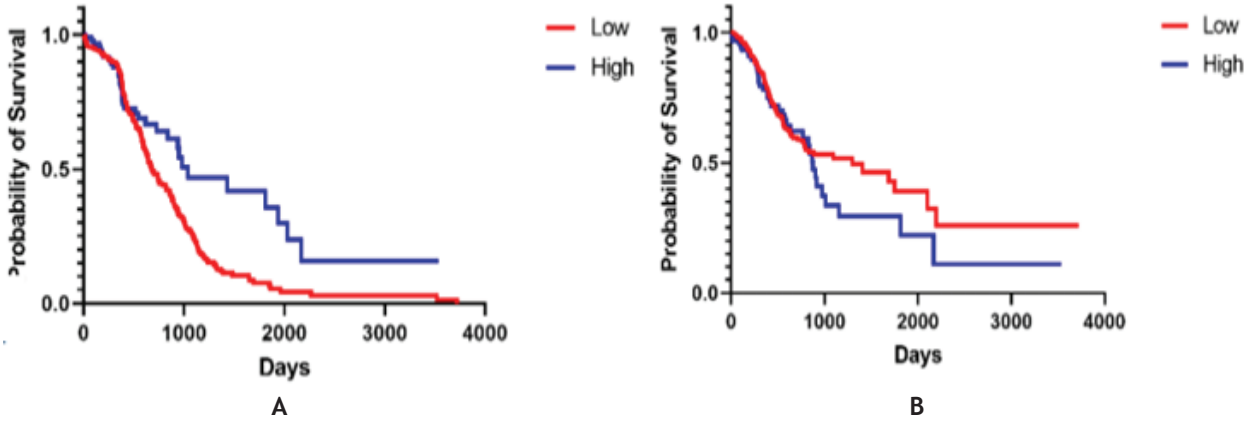

Figure 2. Survival analysis of (A) IL12A expression (B) SHP-2 expression in stomach cancer patients. The red plots present the high expression (n=189) of each individual while the blue plots present the medium/low expression (n=189) of individual (*p-value<0.05). Data was obtained from the Oncoln website (http://www.oncoln.org).

Galangal is one of the plants that is widely used by the people of Indonesia as a kitchen spice. Galangal contains flavonoids and essential oils. *Alpinia galanga* has several pharmacological activities such as antifungal, antibacterial, antiviral, immunomodulatory, and antioxidant (Chouni and Paul, 2018; Ahlina, *et al*., 2020; Alif, *et al*., 2021, Hasbiani, *et al*., 2021). Therefore, we want to develop the potential of galangal as a cancer immunotherapy drug based on local biodiversity.

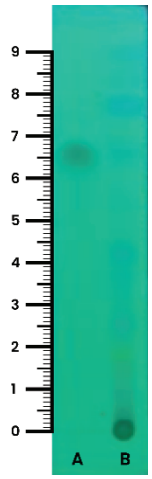

Figure 3. Qualitative analysis of galangin compound using TLC in galangal extract (B) compared to the standard reference of galangin (A). Galangal extract showed a spot in Rf 0.73 similar to a spot of the standard reference of galangin.

In this study, a study was conducted, *hybrid* namely analysis using KNIME software (*Konstanz Information Miner*) to look for compounds from the galangal rhizome that have the potential to inhibit SHP-2 and continued with a computational analysis of the interaction between SHP-2 and the active compound in the galangal rhizome using *molecular docking software*. MOE. The results of the analysis obtained are in the form of bond energies which indicate the possibility of binding between potential compounds and targets and data collection through literature study. The results obtained are expected to be a reference for developing the potential of galangal in the treatment of cancer immunotherapy through the activation of IL-12 cytokines.

MATERIALS AND METHODS

Analysis Structure of SHP-2 Protein

Structure of the SHP2 protein is traced through the Protein Data Bank (PDB) and can be accessed at (https://www.rscb.org/pdb). Structural data were used for *molecular docking* with the active compound of galangal rhizome.

Table 1. The active compound galangal rhizome.

1'-acetoxy-eugenol-acetate	Beta-sesquiphellandrene	Gamma-terpinene
1'-acetoxychavicol -acetate	borneol	Kaempferide
1.8 cineole	Cadinene	Limonene
4-Hydroxybenzaldehyde	Camphene	Linalool
Alpha-humulene	Camphor	methyl-cinnamate,
alpha-pinene	caryophyllene-oxide	Myricene
Ar-curcumene	chavicol	Niacin
Ascorbic acid	Eugenol	P-cymene
beta bisabolene	Eugenol acetate	Riboflavin
beta carotene	eugenol-methyl-ether	sabinene
beta pinene	Galangin	terpinen-4 ol
Terpinolene	Thiamin	Trans-beta -farnesene

Identification of IL12A and SHP-2 Expression in Normal and Cancer Cells

The database UALCAN was used to analyze the differences in the expression of IL12A and SHP-2 proteins in cancer cells and normal cells. The UALCAN database is publicly available and can be accessed via http://ualcan.path.uab.edu/.

Correlation Analysis Between Gene Expression Against the Probability of Survivability Stomach Cancer Patients

Database UALCAN was used to analyze the survival rate in breast cancer patients induced by IL12A protein. Then from the survival rate graph obtained, it can be obtained that the gene expression correlates to the probability of survival of gastric cancer patients. The UALCAN database is publicly available and can be accessed via http://ualcan.path.uab.edu/.

Data Collection Metabolic Profile of *Alpinia galanga*

The active compound component of the galangal rhizome was obtained through Dr. Duke's Phytochemical and Ethnobotanical (https://phytochem.nal.usda.gov). The SMILES code of each compound was traced through database Pubchem (https://pubchem.ncbi.nlm.nih.gov/). Then the active compound of the galangal rhizome along with the obtained SMILES code was entered into the table and used for process molecular docking through software MOE and compound prediction models through *software* KNIME.

Identification of Galangin Compounds in *Alpinia Galanga* Using Extraction and Thin Layer Chromatography (TLC)

Galangal simplicia was obtained from the Center for Research and Development of Traditional Medicinal and Medicinal Plants, Tawangmangu, then determined as *Alpinia galanga* (L.) Wild and then grinded to obtain 150 grams of dry powder. Extraction was carried out by maceration using 96% ethanol. The mixture is then

Table 2. Five compounds with the highest prediction score for SHP-2 inhibitor activity.

No	Compound	Predicted
1	Riboflavin	0.96
2	1.8 Cineole	0.94
3	Kaempferide	0.94
4	Galangin	0.94
5	Beta-Pinene	0.93

Figure 4. KNIME analysis workflow used to predict the most potent SHP-2 inhibitor compounds.

stirred and covered with tissue and aluminum foil and then wrapped in black plastic. The liquid is then placed on a shaker for 10 h and left for 24 h, then filtered using a vacuum to produce the filtrate. Furthermore, remaceration is carried out with the same ratio once so that the compound content in galangal can be extracted properly (Shintia, *et al*., 2019). For the results of the maserate, all were collected and concentrated using a rotary evaporator.

Ethanol extract of Galangal (*Alpinia galanga* (L.) Wild) was then analyzed qualitatively using thin layer chromatography (TLC) and compared with standard galangin (1 mg/mL in Ethanol). The sample of galangal ethanol extract was then dissolved in ethanol solvent and spotted on the bottom of the Silica Gel F254 TLC plate. Hexane and ethyl acetate as mobile phases were used with a ratio of 1:3 v/v. TLC results were detected using UV light at wave-lengths of 254 and 366 nm.

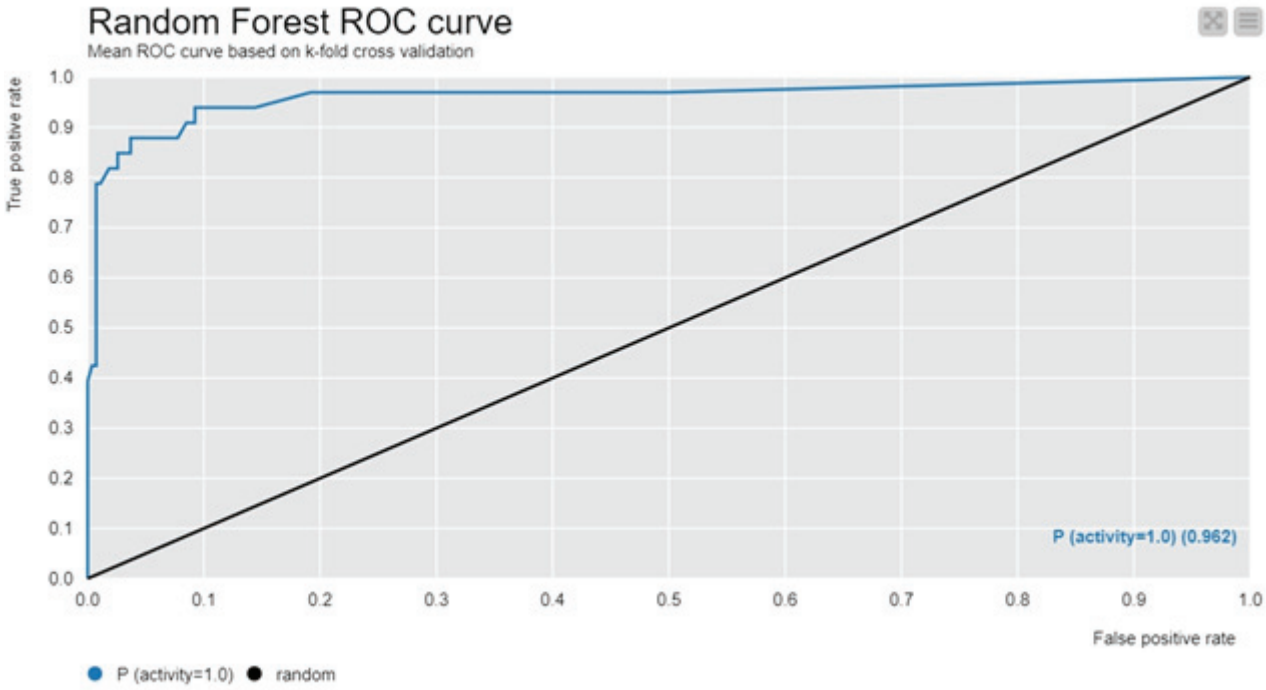

Figure 5. Results of ROC curve random forest machine learning KNIME SHP-2 inhibitor

Prediction Model of Galangal Active Compounds using KNIME Software

Analysis was carried out using KNIME Software version 4.4.0 obtained from https://www.knime.com and using machine learning with the workflow TeachOpenCADD (accessible via: https://hub.knime.com/volkamerlab/spaces/Public/latest/TeachOpenCADD%2FTeachOpenCADD~x-YhrR1mfFcGNxz7I). The compound model to be analyzed is sourced from the ChEMBL database with the target code CHEMBL 3864. The compounds obtained were analyzed by random forest classifiers to produce a model. The model was used to predict the potential of the active compound in the galangal rhizome.

Interleukin-12 (IL-12) Analog Docking Process with Galangal Active Compounds

After carrying out the molecular docking process using the MOE application, then proceed with an analysis of the docking results in the form of G values, root mean square deviation (RMSD),

Table 3. SHP-2 interaction analysis results based on the value of delta G and RMSD.

Ligand	Delta G (kcal/mol)	RMSD
5OD (*native ligand*)	-13.52	1.70
Riboflavin	-15.78	-
Kaempferide	-14.70	-
Galangin	-11.64	-
Beta-Pinene	-7.54	-
1.8 Cineole	-6.27	-

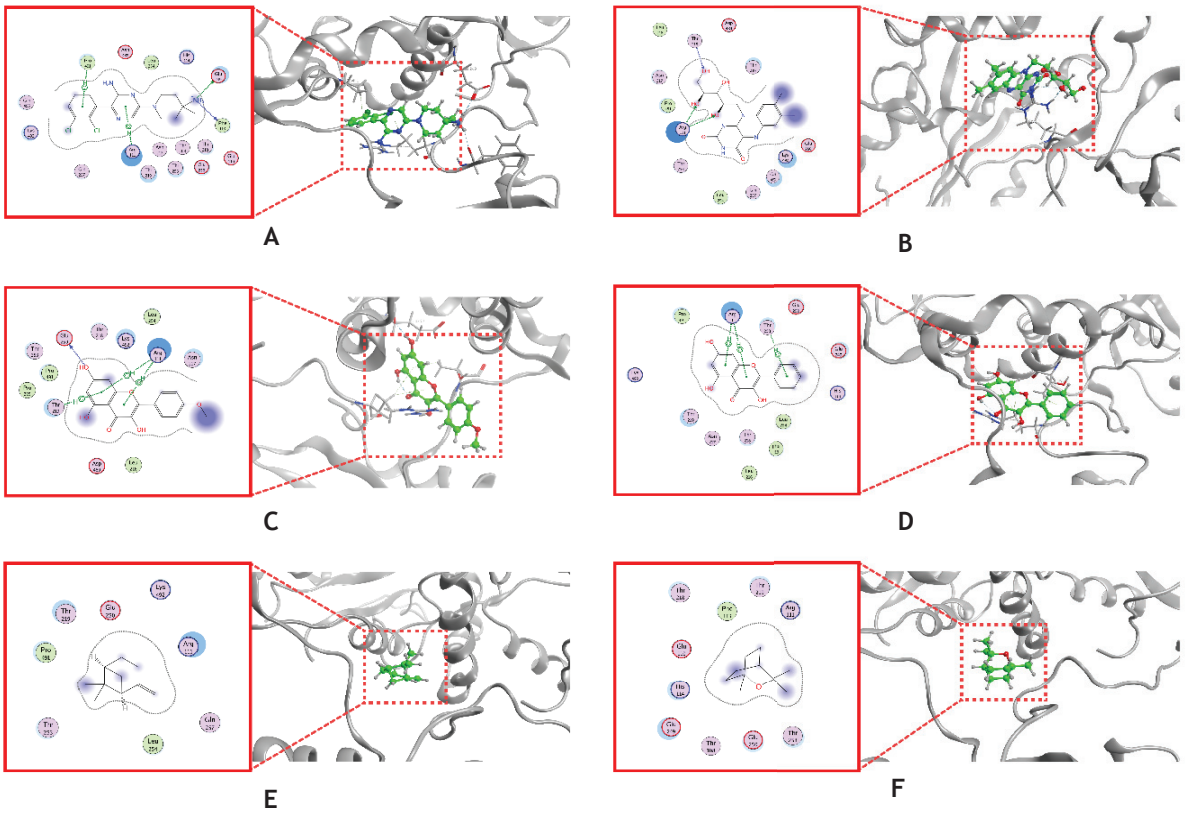

Figure 6. Molecular docking visualization of (A) Native Ligand, (B) Riboflavin, (C)Kaempferide, (D) Galangin, (E) Beta-pinene, and (F) 1.8-Cineole.

and 2D and 3D visualizations. The G value indicates the strength of the bond between the ligand and the receptor. A strong and stable bond is characterized by a low G price. The stronger the bond between the active compound of galangal and SHP-2, the higher the potential of the active compound of galangal to inhibit the protein SHP-2 in inducing gastric cancer. In addition, in the analysis of the results, the RMSD value greatly affects the validity of the data from the docking results. The process of docking is valid if RMSD < 2.

RESULTS

Differences in IL12A and SHP-2 Protein Expression in Normal Cells and Stomach Cancer Cells.

IL-12 has the potential as an immunotherapy for cancer. The expression protein of IL-12 correlated with the cytotoxic activity of T cells and NK cells that inhibit the progression of cancer cells. IL-12 as a cytokine has potential as an immunotherapy

through increasing the cytotoxic activity of T cells and NK cells as well as reverse tumor-induced immunosuppression. Reducing SHP-2 expression increases NK cell activity which will also increase the production of IL-12 cytokines which act as antitumor. We first analyzed the IL12A and SHP-2 protein expression through The Cancer Genome Atlas data portal using UALCAN database. UALCAN stomach cancer or Stomach adenocarcinoma, IL12A protein expression was lower than in normal cells with a statistical significance of 7.304×10^{-2}. While the expressions of SHP-2 protein (encoded by the PTPN11 gene) in primary tumor were higher significantly than in normal cells with a statistical significance of 8.903×10^{-7} (Figure 1A, B). The figure showed that the expression of SHP-2 significantly induces the occurrence of stomach cancer. Targeting therapy by inhibiting SHP-2 is a potent to increase the death of cancer cells.

Correlation of Protein Expression on the Probability of Survival in Stomach Cancer Patients

Survival probability data obtained from the OncoLn database. Based on Figure 2. low expression of IL12A in stomach cancer has a lower survival value when compared to high expression of IL12A in stomach cancer. Meanwhile, in SHP-2, low expression SHP-2 has a higher survival value than high expression SHP-2 in stomach cancer. A low survival value indicates that the probability of survival is smaller.

Metabolomic Profile of galangal (*Alpinia galanga*)

Metabolomic data collection of galangal rhizome was obtained from Dr. Duke's Phytochemical and Ethnobotanical. The data obtained for the sake of performing further analyzed using KNIME in order to predict its potential as immunotherapy. By collecting data through databases Dr. Duke's Phytochemical and Ethnobotanical, obtained 36 metabolomic profiles of galangal showed in Table 1.

Identification of Phytochemical Profile of Galangal Ethanol Extract

The result of determination by Faculty of Pharmacy showed that the simplicia collected was galangal or *Alpinia galanga* (L) Wild. The maceration process using 750 mL of 96% ethanol gradually has a yield of 2.89%. The extract obtained was then continued with the identification of the profile qualitative phytochemical using TLC with standard reference of galangal. The extract spots and the obtained standard reference were detected at UV 254 nm (Figure 3).

The Activity Prediction of the Most Potent Active Compound of Galangal in Inhibiting SHP-2 as an IL-12 Analog Using KNIME Software

Prediction of activity of the potential compounds of galangal in inhibiting SHP-2 as an IL-12 analog using KNIME software and algorithm from TeachOpenCADD. The analysis performed by inputing metabolomic of galangal rhizome (Table 1) in the algoritm (Figure 4) and the compound model to be analyzed was obtained from the ChEMBL. The compounds obtained were analyzed by random forest classifiers to produce a model. The analysis process was conducted by inputting 36 galangal metabolomic profiles obtained from Duke's Phytochemical and Ethnobotanical into the algorithm.

Workflow 1 (W1) is a workflow for filtering data sourced from ChEMBL by inputting ChEMBL IDs targeting the SHP-2 protein with inhibitory activity. Compounds are filtered based on their IC_{50} value and bioactivity units are converted

into nM and pIC_{50}. Compounds resulting from W1 are further analyzed for their molecular properties in W2 using Lipinski's Rule of Five to observe the ADME of the compounds. Output compounds from W2 are trained using a random forest model and are analyzed their ROC Curve accuracy values in W3. Compounds obtained in Table 1 are analyzed for their inhibitory activity with the model compounds resulting from W3 using the W4 algorithm.

The ROC Curve Random Forest data obtained was 0.962 and the overall accuracy was 97.04% that proved that machine learning was used has been valid and in accordance with the standards because the Overall Accuracy is more than 70%. The model was continued to predict the potential as SHP-2 Inhibitor.

Prediction scores mean the compounds potentially having SHP-2 inhibitors activity compared to existed compounds. Based on analysis between model and compounds from Table 1, five compounds with highest score prediction was collected. Five compounds with the highest predictions as SHP-2 inhibitors then analyzed by molecular docking to determine the interaction score of SHP-2 with galangal compounds.

Interaction of SHP-2 and Active Compounds of galangal using MOE Software

Based on the results using the KNIME software in Table 2 that showed in ten highest predictive values, we analyzed interaction of compound of galangal with SHP-2 protein. Delta G value represents the minimum energy required to generated bond in stable condition.

Based on Table 3, the docking process was valid since the RMSD value of native ligand 1.70 (<2.0). From five highest compound in galangal, three compounds with delta G values showed lower than native ligands: Riboflavin, Kaempferide, and Galangin (Table 3). Its mean that Riboflavin, Kaempferide, and Galangin has potency as inhibitor SHP2.

DISCUSSION

IL-12 therapy is a form of immunological therapy that uses interleukin-12, a cytokine produced by cells in the immune system, to stimulate and strengthen the body's immune response against cancer and infection (Portielie, *et al.*, 2003). IL-12 is a cytokine that has an important role in regulating the body's immune response to various diseases. The main function of IL-12 is to stimulate the activity of T cells and NK cells (natural killer) to produce a cytotoxic response against cancer cells and produce other cytokines that enhance the function of other immune cells (Fallon, *et al.,* 2014). SHP may be involved in modulating NK cell proliferation through regulation of signaling pathways related to cell proliferation, such as the PI3K (phosphatidylinositol 3-kinase) signaling pathway or the mTOR (mammalian target of rapamycin) signaling pathway. SHP-2 is involved in signaling pathways that regulate proliferation, differentiation, and survival of cancer cells. Inhibition of SHP-2 inhibit tumor growth, induce apoptosis (cell death), or inhibit metastasis (Dempke, *et al*., 2018). Based on our data, SHP-2 is a target for potential target in cancer therapy. Analysis by KNIME predictio shows that the active compound in galangin such as riboflavin, 1.8 Cineole, Kaempferide, Galangin, Beta-Pinene, inhibit SHP-2-prtein responsible in tumor growth. Based on Molecular docking results show that Riboflavin, Kaempferide has score docking lower than native ligand of SHP-2 and galangin shows has activity to inhibit SHP-2. The results show that Alpinia galanga has potent activity to inhibit tumor growth through SHP-2 inhibition.

CONCLUSION

From our results kaempferol, galangin, and riboflavin compounds contained in galangal rhizome have the potential as SHP-2 inhibitors and activated IL-12- inhibiting cancer progression based on bioinformatics studies.

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