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Molecular Docking and Molecular Dynamics Simulation of *Kaempferia galanga* Bioactive Compounds as Cancer Immunotherapy against PD-L1 Through Inhibition of RAS, JAK, ERK Protein

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Abstract

Cancer remains the world's biggest health issue which kills nearly one in six people worldwide. Several modalities have been proposed, including the conventional and recently developed approach including cancer-immunotherapy. The main challenge of the therapy is to develop effective and safer treatments with minimal side effects. Therefore, the discovery of new drugs to complement and enhance current therapies is urgently needed. In the present study, we aimed to evaluate *Kaempferia galanga* bioactive compounds as potential cancer immunotherapy agents targeting PD-L1 by inhibiting RAS, JAK, ERK proteins through molecular docking and molecular dynamics simulation. *In silico* study was performed including the ligands and target proteins preparation, drug likeness and toxicity screening, molecular docking, molecular dynamic simulation, and biological activity prediction. Inhibiting the expression of PD-L1 through blocking the activation of several target proteins such as RAS, JAK, and ERK could be a promising strategy against cancer. *In silico* study demonstrated that sandaracopimaradiene has favorable binding affinity scores compared to other bioactive compounds against the target proteins, ERK. Molecular dynamics simulation also demonstrated the stable pattern of sandaracopimaradiene on some parameters which indicate that this compound effectively bound to the target proteins. The findings indicate that sandaracopimaradiene is a compound comparable to the anticancer control drug, ruxolitinib. Finally, the biological activity prediction also strengthens the role of sandaracopimaradiene against cancer development.

Keywords: Cancer-immunotherapy, *Kaempferia galanga*, natural products, PD-L1, molecular dynamics simulation.

1. 1. Introduction

Cancer is still the major public health problem worldwide. Unfortunately, cancer is causing a high rate of death, accounting almost for one in six deaths globally (Bray *et al*., 2024; Dunn 2023; Sung *et al*., 2021). The risk factors of certain cancers might vary, but some common factors that increase the occurrence of cancer include smoking, alcohol intake, obesity, chemicals, and lack of physical exercise. These factors then promote the genetics alteration and instability which further causes cancer initiation (Marino *et al*., 2024; Lazarus *et al*., 2022; Łukasiewicz *et al*., 2021). Cancer is a complex disease which covers not only the tumor, but also the environment surrounding the tumor. Treating cancer is challenging and demonstrates rapid developments. Conventional treatments including surgery, chemotherapy, and radiotherapy have been proposed. Recently, to optimize the treatment, some advanced cancer therapies including cancer immunotherapy demonstrated promising patient outcomes (Debela *et al*., 2021; Bondhopadhyay *et al*., 2020).

Modulating the immune system to fight the cancer is the main principle of cancer immunotherapy. The modulation of the immune system could be approached by strengthening the immune cells or improving the immune function to recognize and inhibit the action of the cancer. One of the most targeted molecules for cancer immunotherapy is PD1/PD-L1 axis (Tan *et al*., 2020). PD1/PD-L1 interaction plays a pivotal role for cancer to evade from the immune system destruction. Thus, by blocking this axis or reducing the expression of the checkpoint molecules could prevent the cancer progression, and at the same time enhance the function of immune cells (Esfahani *et al*., 2020). Clinical study demonstrated that PD1/PD-L1 blockade in patient with breast cancer could improve the objective response rates (Liu *et al*., 2021; Koh, 2021). Although PD-1/PD-L1 blockade has demonstrated promising outcomes, several studies have also reported side effects and the development

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of drug resistance during therapy (Reynolds *et al*., 2021; Andrews *et al*., 2019). Therefore, finding new agent for PD1/PD-L1 blockade which more effective and has minimum unwanted effects is urgently needed.

It has been widely demonstrated that plants-derived natural products showed significant effect in treating multiple types of diseases, including cancer (Putra et al., 2023; Shrihastini et al., 2021; Putra and Rifa'i, 2019). *Kaempferia galanga* is a traditional medicinal plant known for its therapeutic properties, including anti-inflammatory and immunomodulatory activities. It originates from India and is widely distributed across several Southeast Asian countries, including Indonesia, Malaysia, Myanmar, and Thailand. Traditionally, *K. galanga* has been used for ameliorating some diseases such as cold, cough, toothache rheumatism, and hypertension (Wang *et al*., 2021; Kumar, 2020; Srivastava *et al*., 2019). Importantly, K. galanga is rich in functional bioactive compounds, including hexadecanoic acid, oleic acid, 2-propenoic acid, octadecanoic acid, glycidyl stearate, sandaracopimaradiene, nonoxynol-2, and phthalic acid (Ali *et al*., 2018). Interestingly, a study confirmed that *K. galanga* extracts have anti-cancer effects on breast cancer by inhibition of a breast cancer resistance protein (Elshamy *et al*., 2019). Another study demonstrated that *K. galanga* extract could inhibit metastasis by suppressing the MMP9 protein expression which was responsible in metastasis. Interestingly, *K. galanga* extract also inhibits the migration of breast cancer cell lines (Ahlina *et al*., 2020). However, the study of *K. galanga* bioactive compounds as cancer immunotherapy is based on limited data. Therefore, in the present study, we aimed to evaluate the activity of K. galanga bioactive compounds in inhibiting several proteins, including RAS, JAK, and ERK, which are responsible for PD-L1 expression, using in silico approaches. The in silico approach facilitates the screening of compounds according to their drug-likeness and less toxic properties. This approach also enables the selection of drugs by assessing their binding affinity and stability via molecular docking and dynamics simulation.

2. Materials and Methods

2.1. Drug likeness and toxicity prediction

Several major *K. galanga* bioactive compounds including hexadecanoic acid (CID. 985), oleic acid (CID. 445639), 2-propenoic acid (CID. 6581), octadecanoic acid (CID. 5281), glycidyl stearate (CID. 62642), sandaracopimaradiene (CID. 443469), nonoxynol-2 (CID. 24773), phthalic acid (CID. 1017) were computationally evaluated (Ali *et al*., 2018). SwissADME webserver (http://www.swissadme.ch/) was used to evaluate the drug-likeness (Daina et al., 2017). Additionally, ProTox 3.0. webserver (https://tox.charite.de/protox3/) was used to predict the toxicity level and the probability of the compounds to induce toxicity (Banerjee et al., 2024).

2.2. Ligand structure preparation

All chemicals structure of *K. galanga* bioactive compounds were retrieved from PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Tipifarnib (CID. 159324), Ruxolitinib (CID. 25126798), and GDC-0994 (CID. 154702204) were used as control for RAS, JAK, and ERK inhibitor, respectively. All chemical structures were saved on the sdf format which is compatible to the software for molecular docking.

2.3. Target protein preparation

The sequence of target proteins, including RAS, JAK, and ERK, was retrieved from UniProt database (https://www.uniprot.org/). Then, each database was collected for 3D structure modeling. In this present study, SWISS MODEL (https://swissmodel.expasy.org/) webserver was used to build the 3D structure of target protein. The model with high similarity was chosen for further *in silico* analysis (Hidayatullah et al., 2020; Putra and Rifa'i, 2020).

2.4. Molecular docking and visualization

After ligands and target proteins preparation has done, the molecular docking was performed by using PyRx 0.8 software (https://pyrx.sourceforge.io/). The docking grid was set which cover all the surface of the target proteins. Finally, Discovery Studio software (https://www.3ds.com/) was used to analyze and visualize the molecular docking data (Hidayatullah et al., 2021; Putra, 2018).

2.5. Molecular dynamics simulation

To evaluate the stability of the protein-ligand complex, molecular dynamics simulation was performed. YASARA software (http://www.yasara.org/) was used to evaluate several molecular dynamics simulation, including total energy, Coulomb energy, RMSD, SASA, surface molecule, surface VdW, radius gyration, and H-Bond (Heikal et al., 2024; Putra et al., 2024; Ozvoldik et al., 2023; Hidayatullah et al., 2022).

2.6. Biological activity prediction

Finally, biological activity prediction of *K. galanga* bioactive compounds was performed. The SMILES of each bioactive compound retrieved from PubChem database were used. Way2Drug server (http://www.way2drug.com/passonline/) was occupied to get the biological activity prediction related to the cancer incidence.

3. Results and Discussion

This study assessed the drug-likeness of bioactive compounds from *K. galanga*. Several chemical characteristics such as molecular weight, number of rotatable bonds, number of H-bond acceptors, number of H-bond donors, and molar refractivity were evaluated to assess the drug-likeness of the bioactive compounds (Figure 1A). Five drug-likeness validation parameters, Lipinski, Ghose, Veber, Egan, and Muegge, were employed to assess the number of rule violations exhibited by these bioactive compounds (Figure 1B). The drug-likeness screening of the active compounds revealed varying levels of rule violations; however, all compounds exhibited only minimal deviations from the established parameters. In addition, toxicity prediction indicated that glycidyl stearate, nonoxynol-2, phthalic acid, and sandaracopimaradiene belong to higher toxicity classes, suggesting that these compounds exert relatively low toxic effects on cells (Figure 1C, 1D).

Drug likeness screening plays crucial part in assessing and filtering the bioactive compounds for further test as

drug candidate. Through the assessment from its chemical characters, then it helps to get the reliable and accurate data about the kinetic and dynamic of the compounds (Zhu *et al*., 2020; Wang *et al*., 2019). Similarly, the toxicity prediction also plays pivotal role in drug discovery field. The prediction could help to make accurate decision for dosage related experiment, promote the safety evaluation of chemical, and reduce the use of experimental animals (Füzi *et al*., 2023; Barratt *et al*., 2000). The results indicate that the bioactive compound is a druggable chemical with low toxicity, making it suitable for further analysis.

A

Compounds	Formula	Molecular weight	Num. rotatable bonds	Num. H-bond acceptors	Num. H-bond donors	Molar Refractivity
2-Propenoic acid	C3H4O2	72.06 g/mol	1	2	1	17.83
Glycidyl stearate	C21H40O3	340.54 g/mol	19	3	0	103.32
Hexadecanoic acid	C16H32O2	256.42 g/mol	14	2	1	80.8
Nonoxynol-2	C19H32O3	308.46 g/mol	14	3	1	93.02
Octadecanoic acid	C18H36O2	284.48 g/mol	16	2	1	90.41
Oleic acid	C18H34O2	282.46 g/mol	15	2	1	89.94
Phthalic Acid	C8H6O4	166.13 g/mol	2	4	2	40.36
Sandaracopimaradiene	C20H32	272.47 g/mol	1	0	0	90.18

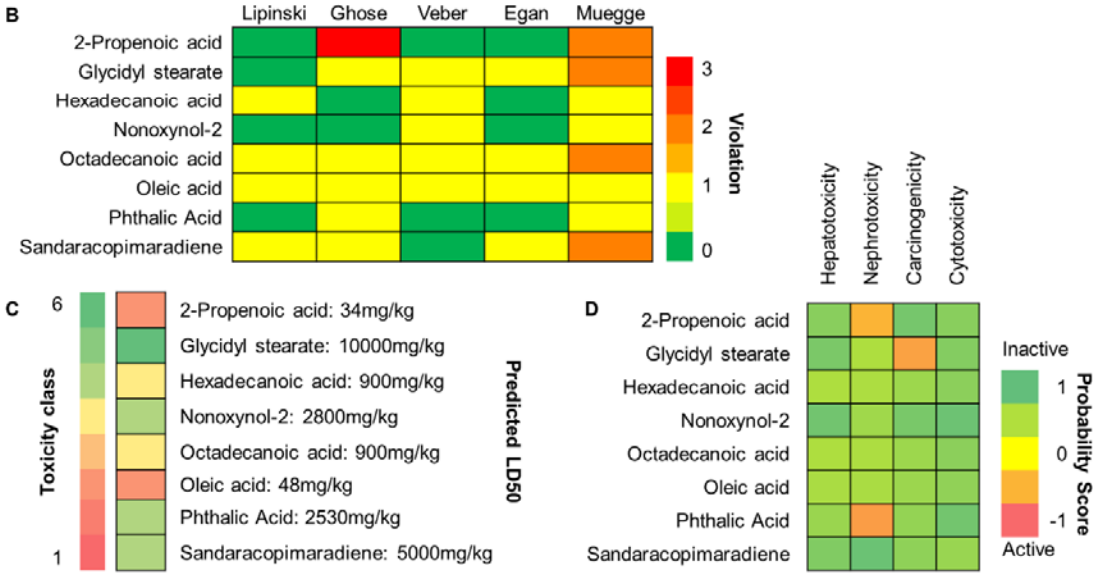

Figure 1. Chemicals characters, drug likeness and toxicity assessment. A). Chemical features of *K. galanga* bioactive compounds. B). Drug likeness evaluation of bioactive compounds. C). Toxicity assessment of bioactive compounds. D). The probability of *K. galanga* bioactive compounds to induce toxicity.

After validating the drug-likeness and evaluating the toxicity profiles of the bioactive compounds, molecular docking simulations were conducted to assess their interactions with target proteins, including RAS, JAK, and ERK (Figure 2). To the greater extent, sandaracopimaradiene have the most favorable binding affinity scores compared to the others. In the interaction with JAK protein, sandaracopimaradiene have better binding affinity compare to the control drug, ruxolitinib. These initial findings suggest that sandaracopimaradiene could be a competitive inhibitor for suppressing the PD-L1 protein by blocking some proteins, especially JAK protein.

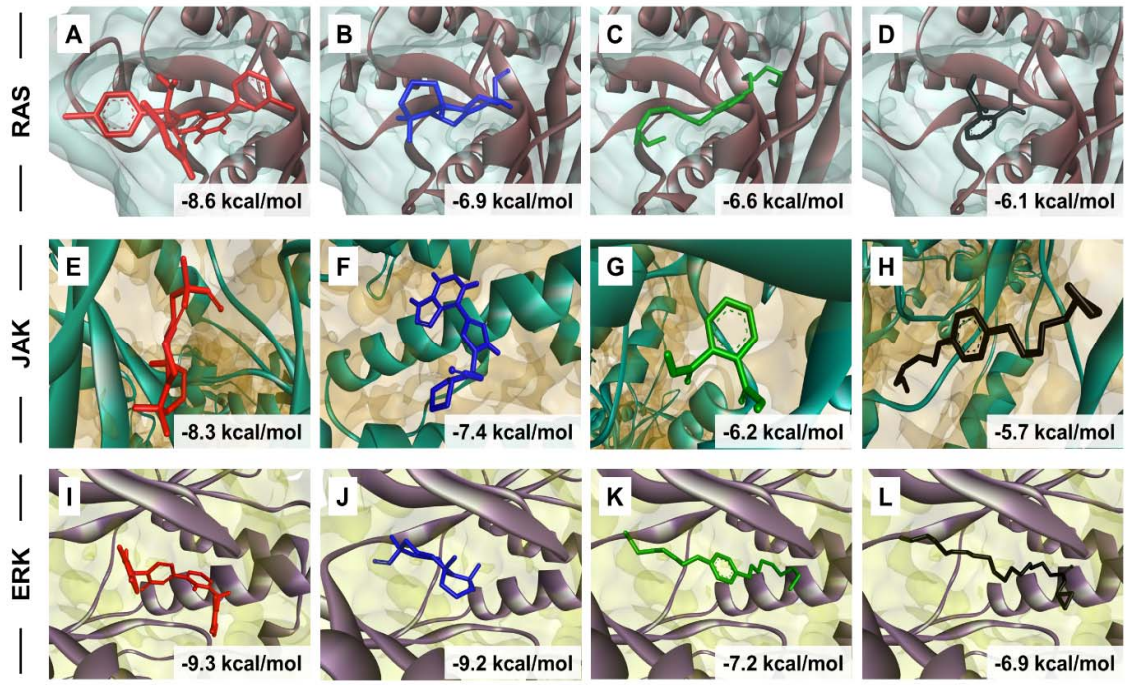

Figure 2. The 3D structure visualization of *K. galanga* bioactive compounds and control drugs toward the RAS, JAK, and ERK proteins. A. Tipifarnib, B. Sandaracopimaradiene, C. Nonoxynol-2, D. Phthalic Acid, E. Sandaracopimaradiene, F. Ruxolitinib, G. Phthalic Acid, H. Nonoxynol-2, I. GDC-0994, J. Sandaracopimaradiene, K. Nonoxynol-2, and L. Glycidyl stearate.

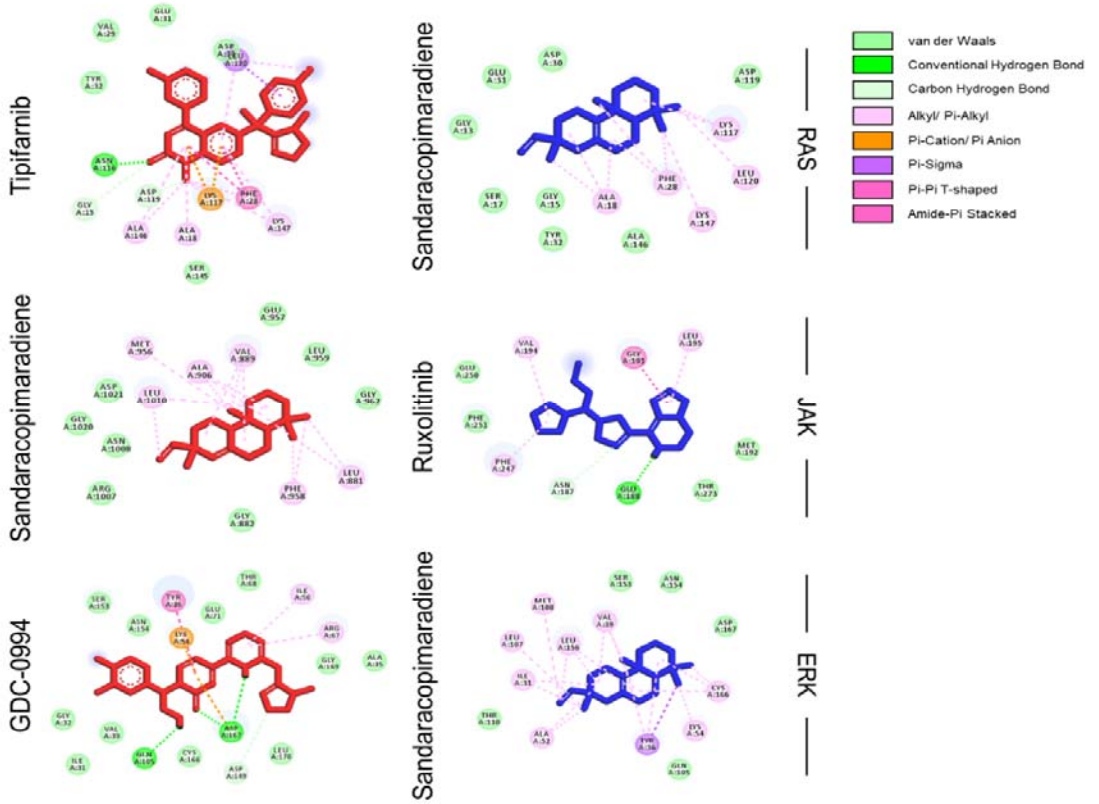

Figure 3. The interaction of *K. galanga* bioactive compound and control drug toward RAS, JAK, and ERK protein.

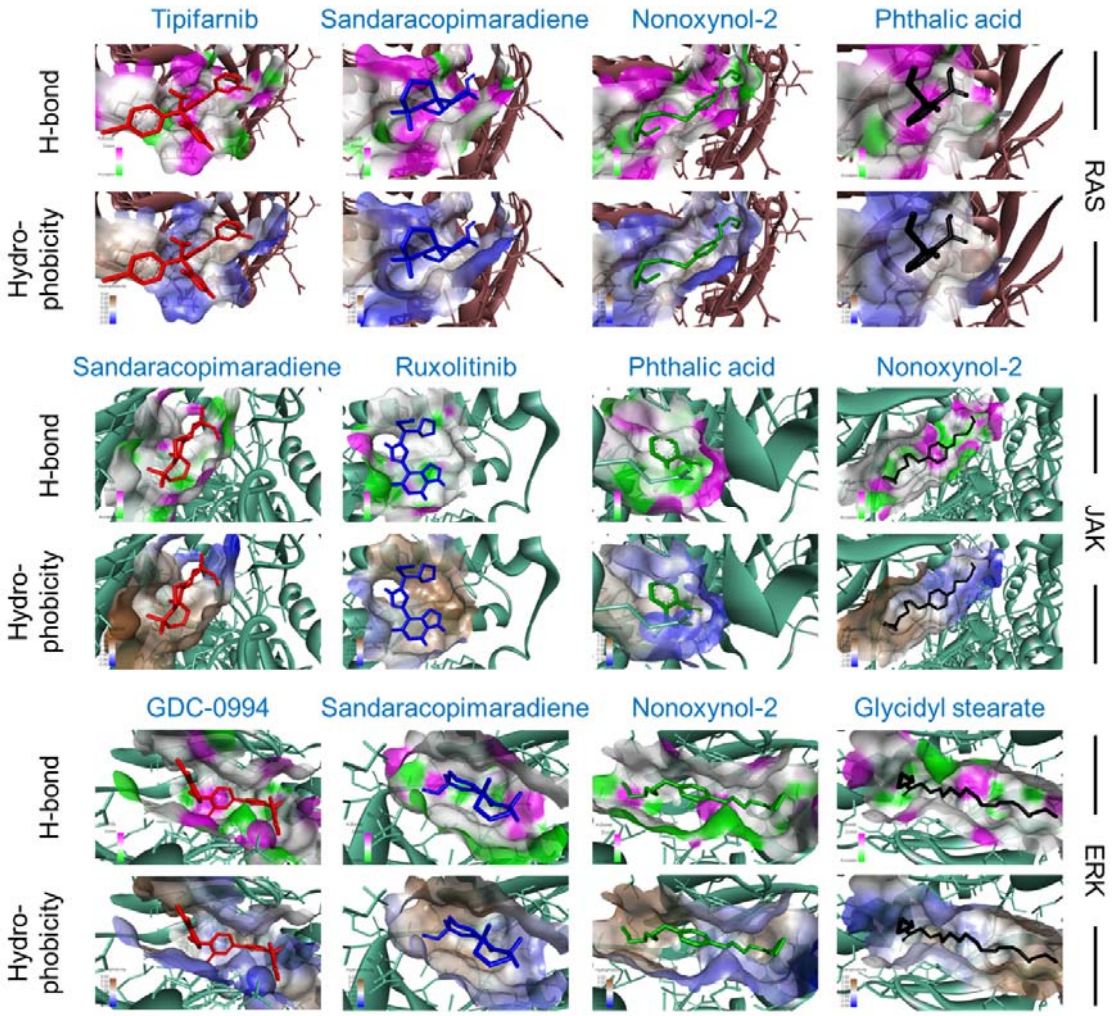

Figure 4. The physicochemical properties of *K. galanga* bioactive compounds and control drugs toward the RAS, JAK, and ERK proteins including H-bonds and hydrophobicity properties.

We examined the chemical interactions formed between sandaracopimaradiene and the RAS, JAK, and ERK proteins. Interestingly, several chemical interactions were observed, including the hydrogen bond and hydrophobic interaction (Figure 3, 4). The hydrogen bond and hydrophobic interaction plays a crucial part in the stability of the protein-ligand interaction (Ruswanto *et al*., 2024). A study has underlined the important function of these interaction in determining the effectiveness and efficacy of the drug (Patil *et al*., 2010).

To ensure the stability of the interaction between the ERK and sandaracopimaradiene, molecular dynamics simulation was performed. The ERK–control drug complex was also evaluated for comparison, to assess the stability of sandaracopimaradiene against the target protein. Several parameters, including total energy, coulomb energy, root mean square deviation (RMSD), solvent accessible surface area (SASA), surface molecule, surface Van der Waals, radius gyration, and H-bond (Figure 5). According to simulation outcomes, even though there are some fluctuations in the beginning of simulation, but almost all parameters including total energy, coulomb energy, RMSD, SASA, surface molecule, surface VdW, radius gyration, and H-bond target protein and sandaracopimaradiene or ruxolitinib were stable and reach the equilibrium in the end of simulation. These results indicate that sandaracopimaradiene exhibits stability comparable to that of the control drug.

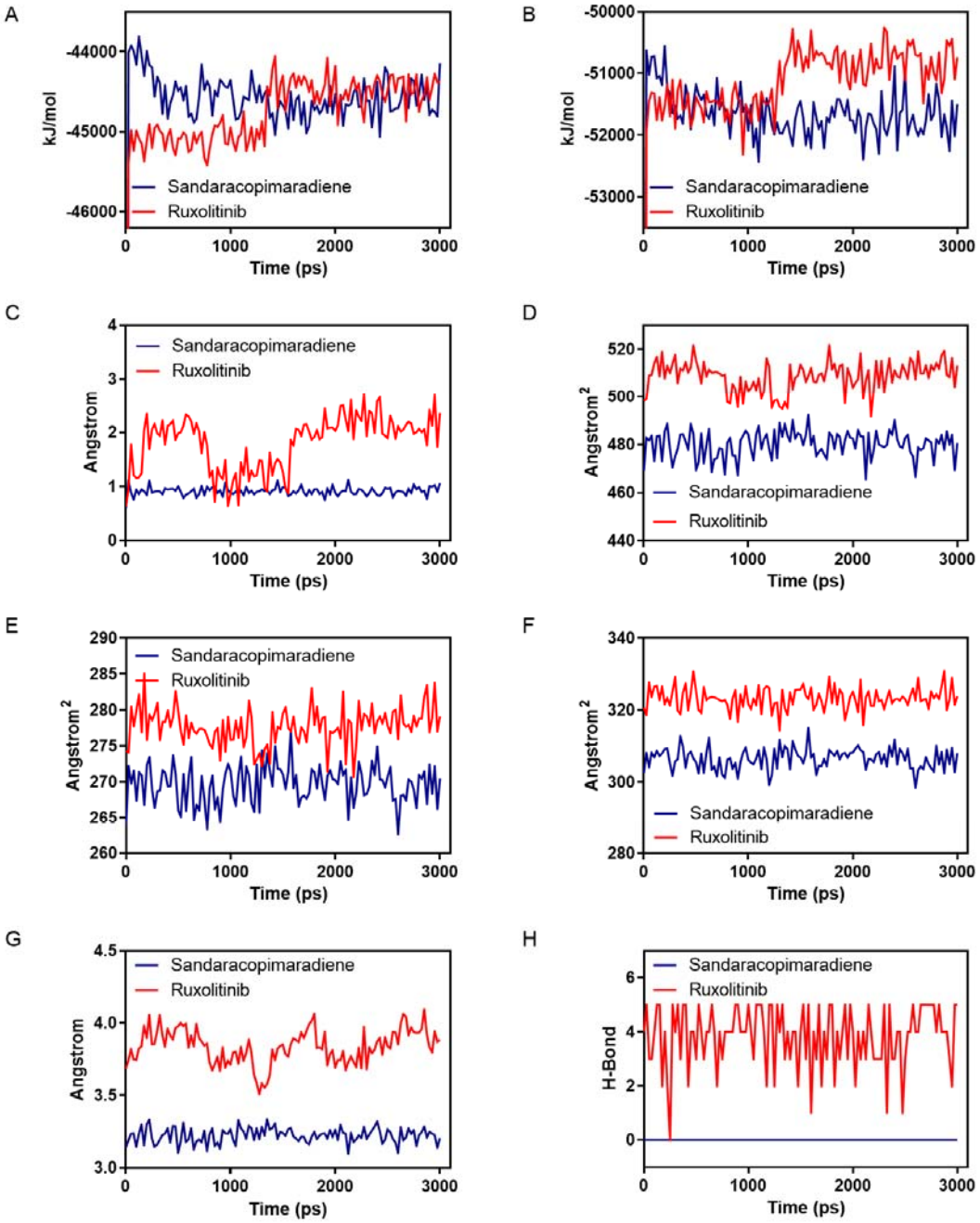

Figure 5. Molecular dynamic simulation of JAK protein - sandaracopimaradiene and JAK protein – Ruxolitinib complex. A). Total energy, B). Coulomb energy, C). RMSD, D). SASA, E). Surface molecule, F). Surface VdW, G). Radius gyration, and H). H-Bond.

Molecular dynamics simulation, a fast growing approach, has become one of the powerful tools in drug discovery study. Molecular dynamics simulation could predict the function and stability of protein, the enzymatic reaction, and also the drug-protein interaction (Son *et al*., 2024; Sinha *et al*., 2022; Hollingsworth and Dror, 2018). Specifically, molecular dynamics simulation could predict the atoms behavior and its change in biological system in time-dependent setting (Badar *et al*., 2022).

Compounds	Pa	Pi	Activity
Sandaracopimaradiene	0.525	0.012	Antineoplastic (lung cancer)
	0.472	0.022	Antineoplastic (breast cancer)
	0.449	0.003	Antineoplastic (thyroid cancer)
	0.425	0.005	Antineoplastic (endocrine cancer)
	0.290	0.020	Antineoplastic (ovarian cancer)
	0.229	0.032	Antineoplastic (glioblastoma multiforme)
	0.234	0.041	Antineoplastic (melanoma)
	0.174	0.076	Antineoplastic (colorectal cancer)
	0.169	0.071	Antineoplastic (colon cancer)
	0.162	0.074	Antineoplastic (glioma)
	0.099	0.083	Antineoplastic (carcinoma)
Nonoxynol-2	0.449	0.066	Antineoplastic (non-Hodgkin's lymphoma)
	0.244	0.146	Antineoplastic (solid tumors)
	0.224	0.208	Antineoplastic (multiple myeloma)
	0.213	0.114	Antineoplastic (bone cancer)
	0.210	0.074	Antineoplastic (liver cancer)
	0.161	0.089	Antineoplastic (lymphoma)
	0.155	0.144	Antineoplastic (sarcoma)
Phthalic Acid	0.291	0.088	Antineoplastic (multiple myeloma)
	0.165	0.115	Antineoplastic (glioblastoma multiforme)
	0.162	0.103	Antineoplastic (bladder cancer)
	0.152	0.109	Antineoplastic (renal cancer)

Figure 6. The predicted biological activities of selected *K. galanga* bioactive compounds related to the cancer.

Finally, based on the biological activity prediction, it has been demonstrated that *K. galanga* bioactive compounds including sandaracopimaradiene, nanoxynol-2, and phthalic acid demonstrate anti-cancer potency in multiple types of cancer. A report summarizes that *K. galanga* extracts exerts anti-cancer properties in several types of cancer. For instance, *K. galanga* active compounds could promote toxicity in HSC-3 and Ca922 lines. On the other hand, *K. galanga* active compounds could induce the apoptosis and influence the cell cycle of HepG2 cell lines. Finally, *K. galanga* active compounds also demonstrate anti-cancer activity in DMH-induce rat colon carcinogenesis model by modulating many pathways including proliferation, invasion, angiogenesis, apoptosis, and inflammation (Wang *et al*., 2021).

4. Conclusion

Inhibiting the expression of PD-L1 through blocking the activation of several target proteins such as RAS, JAK, and ERK could be a promising strategy against cancer. *In silico* study demonstrated that sandaracopimaradiene has favorable binding affinity scores compared to other bioactive compounds against the target proteins. Molecular dynamics simulation also demonstrated the stable pattern of sandaracopimaradiene on some parameters which indicate that this compound is effectively bound to the target proteins. Finally, the biological activity prediction also strengthens the role of sandaracopimaradiene against cancer incidence.

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Conflict of interests

We declare there is no conflict of interests in this study.

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