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Molecular Mechanisms of Increased Platelets: An In Silico of the Active Compounds in *Psidium guajava*

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Abstract. Dengue virus infection causes thrombocytopenia. *Psidium guajava* is widely used by people to increase platelet counts. This research aims to analyze in silico the molecular mechanisms of compounds in guava fruit in increasing platelets. The compounds in guava fruit were taken from Dr. Duke's Phytochemical and Ethnobotanical Databases, which include secondary metabolites such as flavonoids, terpenoids, tannins, alkaloids, and fatty acids. Target proteins were predicted using PharmMapper. Protein interaction networks were created using STRING, visualized, and analyzed using Cytoscape. Potential target proteins were identified by topology, modularity, functional, and KEGG pathway analysis. Degree and betweenness centrality are parameters in topological analysis and the cluster with the highest score is selected as the functional module. The results showed that MAPK1, MAPK14, and AKT1 are involved in many inflammatory pathways, and MMP9 is a target protein directly involved in increasing vascular permeability. The compounds arjunolic acid, farnesene, beta-carotene, and alpha-linolenic acid inhibit MAPK1, citral, ellagic acid, palmitic acid, and oleanolic acid inhibit MAPK14, guaijaverin, pantothenic acid, and citric acid inhibit AKT1, guaijaverin and pantothenic acid inhibit MMP9. It was concluded that the bioactive compounds in guava fruit play a role in increasing platelets by inhibiting the MAPK, PI3K-Akt pathways, and leukocyte trans-endothelial migration, thereby inhibiting or reducing the production and expression of inflammatory mediators and vascular permeability.

Keywords: dengue virus, protein interaction network, *Psidium guajava*, thrombocytopenia, vascular permeability

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INTRODUCTION

Dengue fever is an arboviral disease caused by the dengue virus (DENV) which is transmitted to humans through the bite of female Aedes aegypti and Aedes albopictus mosquitoes (Wang et al., 2020). Infection with one of the DENV serotypes can cause various clinical manifestations with different levels of severity (Srikiatkhachorn et al., 2017), ranging from mild hemorrhagic fever or dengue fever to more fatal diseases, such as dengue hemorrhagic fever or dengue shock syndrome. Dengue fever is still a health problem in both urban and semi-urban areas (Nazri et al., 2013). Dengue hemorrhagic fever has also caused morbidity and mortality, especially in tropical and subtropical regions, so it has become one of the main concerns of the Indonesian government and WHO (Harapan et al., 2019).

Thrombocytopenia is a characteristic symptom of dengue fever, with varying degrees of severity and prevalence (Quirino-Teixeira et al., 2020). The platelet count can decrease drastically to below 30,000 platelets/μL (normal range 150,000-450,000 platelets/μL), thereby causing bleeding (Ojha et al., 2017). The inflammatory response triggered by the release of chemokines and cytokines causes increased vascular permeability, platelet dysfunction, thrombocytopenia, and plasma leakage (Sanyaolu, 2017). Damage to the vascular endothelium causes platelet adhesion and aggregation, so many platelets are consumed and become one of the causes of thrombocytopenia in dengue hemorrhagic fever (DHF) (Retnowati et al., 2018). In vitro research by Krishnamurti et al. (2002) showed that in conditions of vascular endothelial damage, there is an increase in platelet adhesion to

reduce more severe vascular leakage so that the number of platelets decreases.

DENV infection of monocytes/ macrophages, mast cells, and dendritic cells causes the secretion of cytokines, chemokines, and/or other factors that can affect endothelial integrity. This causes an increase in vascular permeability and plasma leakage (Vervaeke et al., 2015). Several pro-inflammatory mediators that play a role include IL-1β, IL-6, IL-8, IFN-α, IFN-β, MCP-1, MIF, MMP-2, MMP-9, MMP-13, RANTES, TNF-α, and VEGF (Vervaeke, 2014).

Guava (*Psidium guajava*) is a plant that is widely used by people to increase the number of platelets due to DENV infection. Compounds in guava fruit have antioxidant, anti-inflammatory, and anti-viral activities (Naseer et al., 2018). The results of the study showed an increase in the number of platelets in dengue fever patients after administering red guava juice (Widhawati & Solehah, 2018; Marisa & Suriani, 2019; Rahayuningrum & Morika, 2019; Az-Zahra & Al Jihad, 2022). Guava fruit contains many compounds, and each compound has many targets. This research aims to analyze the molecular mechanisms of active compounds in guava fruit (Psidium guajava) which have the potential to increase platelets based on a protein interaction network approach. This research provides an understanding of the molecular mechanisms of compounds in guava fruit that are involved in increasing platelet numbers. This information can help develop therapy to overcome the problem of decreased platelets occurring in dengue hemorrhagic fever patients. This research has the novelty of applying an in silico approach to analyze active compounds in guava fruit to understand the complex interactions between various compounds and target proteins regarding their potential and effects on increasing platelets.

METHODS

The compounds in guava fruit were obtained from Dr. Duke's phytochemical and ethnobotanical database accessed via https:// phytochem.nal.usda.gov. The search is carried out using the keyword "Psidium guajava", then selecting fruit in the part filter column. The chemical structure of each compound in SMILES and 3D format (downloaded in .sdf format) was obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/).

Biological Activity Prediction

Prediction of the biological activity of compounds in guava fruit as anti-inflammatory was carried out using prediction of activity spectra for substances (PASS) online (http://www.pharmaexpert.ru/passonline/predict.php) by entering SMILES of the compound in the search column and clicking “get prediction”.

Target Protein Prediction

Prediction of the target protein for each compound in guava fruit was carried out using the PharmMapper server (http://www.lilabecust.cn/pharmmapper/) by entering the 3D structure of the compound. GeneCards (https://www.genecards.org/) and DisGeNET (https://www.disgenet. org/) were used to obtain DHF-related genes. Venn diagram 2.1 (http://bioinfogp.cnb.csic.es/tools/venny/) was used to filter overlapping targets between dengue-related genes and target compounds in guava fruit.

Construction of Protein Interaction Networks

Creating a protein interaction network is carried out to determine the relationship between target proteins. Protein interaction networks were constructed using STRING (https://string-db.org/). The protein interaction network in the form of TSV was imported into Cytoscape software. Compounds input to Cytoscape are compounds with a z'score >1.Topological analysis was carried out using the Analyze Network plugin in Cytoscape to predict potential proteins based on degree and betweenness centrality parameters. Modularity analysis was carried out using the molecular complex detection (MCODE) plugin in Cytoscape to identify functional modules from previously built networks. Functional analysis was carried out using the biological networks gene ontology (BiNGO) plugin on Cyoscape to determine biological processes, molecular functions, and cellular components based on gene ontology (GO). The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was carried out using STRING to determine the pathways involved in the inflammatory mechanism.

RESULTS AND DISCUSSION

Screening compounds to determine biological activity is the first step to exploring the potential of herbs computationally (Poerwosusanta et al., 2019). Prediction of the anti-inflammatory activity of compounds in guava fruit was carried out using PASS Online based on

the relationship between the structure of the compound and its biological activity (Filimonov et al., 2014).

Based on the prediction results, as many as 40 compounds have anti-inflammatory activity with Pa values between 0.3 to 0.9 (Table 1). These results show that guava fruit contains many compounds that have anti-inflammatory potential. The Pa value indicates a possible pharmacological effect with a value ranging from 0 to 1. A total of 20 compounds had Pa values >0.7 (had similar effects experimentally and had similarities to known pharmacological drugs). A total of 13 compounds had a value of 0.5<Pa<0.7 (having similar effects experimentally and not similar to pharmacological drugs). A total of 7 compounds had Pa values <0.5 (not in accordance with experimental activity). However, this activity can be confirmed by experimental data and can become a new object for research (Stasevych et al., 2020).

Table 1. Prediction results of anti-inflammatory activity of compounds in guava fruit

No	Pa Value	Number of compounds
1.	0.3<Pa<0.5	7
2.	0.5<Pa<0.7	13
3.	0.7<Pa	20

Target Protein Prediction

The target protein prediction for each compound is selected which has a z'score value > 1. According to Kim *et al*. (2019), the greater the positive z'score value, the more significant the protein target is to the compound being tested. Figure 1 shows the results of predicting target proteins from compounds in guava fruit. There were 885 target proteins associated with DBDs obtained from the GeneCards (768) and DisGeNET (117) databases (Figure 1A). A total of 68 target proteins that overlapped with DBD-related proteins were selected for protein interaction network analysis (Figure 1B).

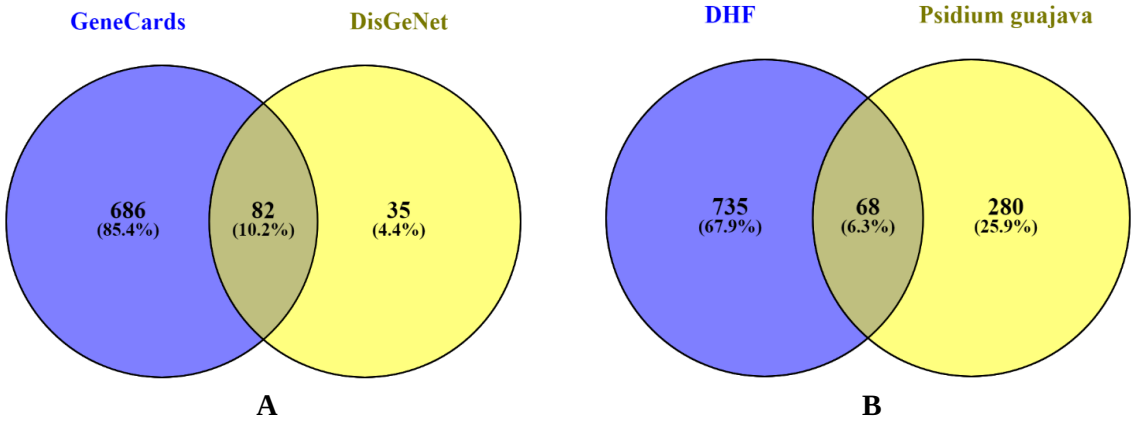


Figure 1. Target protein prediction results. (A) Target protein related to dengue hemorrhagic fever (B) Target protein from compounds in Psidium guajava fruit.

Construction of Protein Interaction Networks

A protein interaction network-based approaches are generally used to predict candidate genes that have potential as major drug targets (Han *et al*., 2021). This network consists of 68 nodes representing target proteins and 456 edges representing interactions between proteins (Figure 2). These results show that 66 of the 68 target proteins have interactions with each other**.** Compound-protein interaction network visualization is displayed in the form of nodes and edges. Nodes depict proteins and compounds, while edges are connecting lines between nodes that illustrate interactions between compounds and proteins (Figure 2).

Figure 2. Protein interactions show 66 target proteins that interact with each other.

The visualization results of the compound and protein interaction network (Figure 3) show that there is an interaction between multi-compounds and multitargets. One compound can interact with more than one target protein, and one target protein can be the target of several compounds.

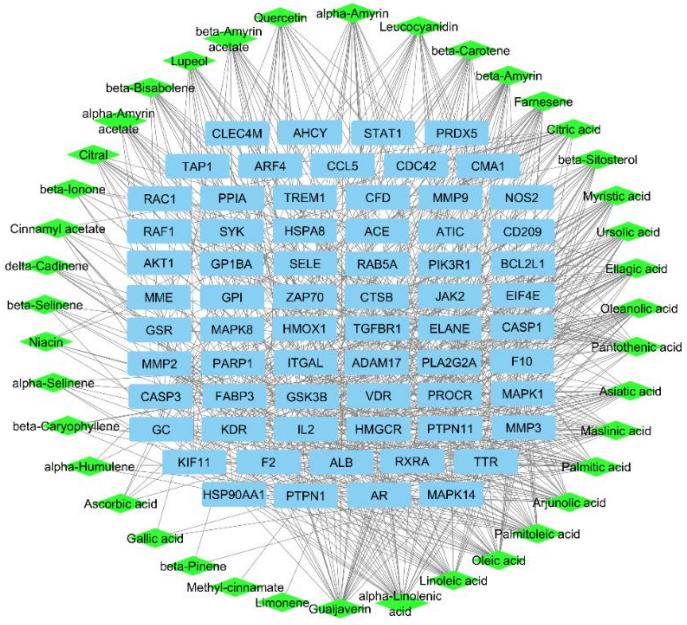


Figure 3. Visualization of the compound-protein interaction network, consisting of 106 nodes (66 proteins and 40 compounds). Blue nodes indicate target proteins, and green nodes indicate compounds in guava fruit.

There are 27 compounds with the highest levels or having the most targets (Table 2). In this table, the degree value is defined as the number of target proteins that can interact with the compound. This shows that these compounds can play an effective role in the treatment of dengue hemorrhagic fever. By having multiple targets, these compounds may be able to overcome the complexity of dengue hemorrhagic fever by involving more than one pathway or biological

mechanism. These compounds may also have synergistic interactions between them, where their combination together produces a stronger therapeutic effect than when used separately. In this study, compounds that had more than ten target proteins were selected as potential compounds.

Table 2. Compounds in guava fruit with degree values > 10.

No.	Compounds	*Degree*	No	Compounds	*Degree*
1.	alpha-Linolenic acid	27	15.	beta-Amyrin	20
2.	Guaijaverin	27	16.	beta-Sitosterol	20
3.	Linoleic acid	27	17.	Citric acid	20
4.	Oleic acid	26	18.	Farnesene	20
5.	Palmitoleic acid	26	19.	alpha-Amyrin	19
6.	Arjunolic acid	25	20.	beta-Carotene	19
7.	Palmitic acid	25	21.	Leucocyanidin	19
8.	Maslinic acid	24	22.	Quercetin	18
9.	Asiatic acid	23	23.	beta-Amyrin acetate	16
10.	Oleanolic acid	23	24.	Lupeol	16
11.	Pantothenic acid	23	25.	beta-Bisabolene	15
12.	Ellagic acid	22	26.	alpha-Amyrin acetate	14
13.	Ursolic acid	22	27.	Citral	13
14.	Myristic acid	21			

A protein interaction network approach and topological analysis were applied to identify the most significant proteins and analyze the molecular pharmacology of the compounds (Dhasmana *et al*., 2020). Topological analysis is a quantitative method for identifying key proteins involved in the mechanism of a disease. The parameters used in topological analysis are degree and betweenness centrality (Ren *et al*., 2016). The degree is the number of direct interactions a node has. The node with the largest degree is considered a hub protein that is hypothesized to play a key role in the network (Azuaje *et al*., 2010). Betweenness centrality shows the importance of nodes based on the number of shortest paths passing through each node (Nair *et al*., 2014).

The protein interaction network is visualized into different sizes and colors based on the degree and betweenness centrality parameter values (Figure 4). The larger the node size, the higher the degree value. The more intense the red color, the higher the betweenness centrality value.

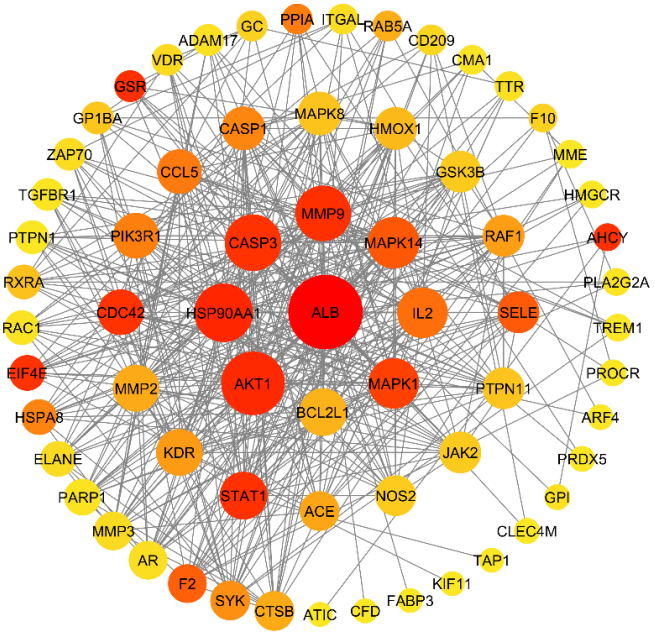


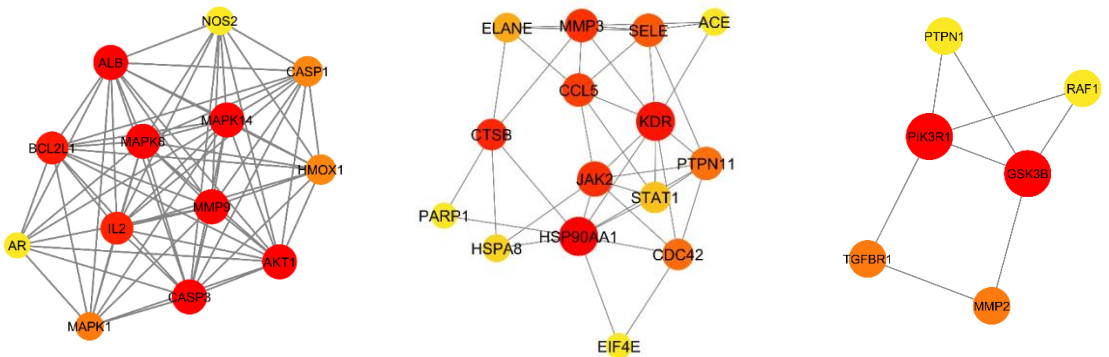
Figure 4. Topological analysis of protein interaction networks with a degree and betweenness centrality parameters

Based on the average degree value > 13 and average betweenness centrality > 0.0128, the top 16 hub targets (potential targets) were obtained with parameter values above the average (Table 3). The top 16 hub target genes ranked based on node degree and betweenness centrality were ALB, AKT1, HSP90AA1, CASP3, MMP9, MAPK14, MAPK1, IL2, STAT1, CDC42, PIK3R1, CCL5, CASP1, SELE, SYK, and F2. The average degree value > 13 implies that, on average, each node (gene or protein) in the network is connected to more than 13 other nodes. A higher degree indicates a greater number of interactions. Betweenness centrality measures how often a node acts as a bridge along the shortest path between two other nodes. An average value above 0.0128 suggests that nodes in the network often serve as important intermediaries. The identified hub target genes may play a key role in the overall network. They might be involved in regulating various biological processes or pathways. Additional analyses, such as functional and pathway analysis, could be performed on these genes to elucidate their roles in specific biological pathways or processes.

Table 3. Results of topological analysis

No.	Target proteins	*Degree*	*Betweenness Centrality*
1.	ALB	51	0.279111278
2.	AKT1	40	0.073725160
3.	HSP90AA1	35	0.092939230
4.	CASP3	33	0.033569438
5.	MMP9	32	0.043196965
6.	MAPK14	28	0.021064921
7.	MAPK1	27	0.024491363
8.	IL2	27	0.017611699
9.	STAT1	24	0.038759324
10.	CDC42	22	0.031884611
11.	PIK3R1	22	0.015202249
12.	CCL5	21	0.016063735
13.	CASP1	20	0.014198366
14.	SELE	18	0.020670302
15.	SYK	16	0.012870037
16.	F2	15	0.019971518

To filter significant modules from the network, modularity analysis was carried out using the molecular complex detection (MCODE) plugin in Cytoscape (Ye *et al*., 2019). The term modulation means finding clusters or highly interconnected regions in a network (Dhasmana *et al*., 2020). The modules obtained were three clusters. Cluster 1 (score 11.7) consists of 13 nodes and 70 edges, cluster 2 (score 5.6) consists of 15 nodes and 39 edges, and cluster 3 (score 3.2) consists of 6 nodes and 8 edges (Figure 5).



A B C

Figure 5. The results of the module analysis produce 3 clusters, namely cluster 1 (A), cluster 2 (B) and cluster 3 (C)

The nodes in cluster 1 are identified as the most important nodes because the module scores are the highest. The module score indicates how dense the module is to its surrounding nodes. A higher module score indicates the node is more important (Lin *et al*., 2021). Eight hub proteins in cluster 1 interact with 5 other proteins (MAPK8, NOS2, HMOX1, BCL2L1, and AR) (Figure 5).

The results of functional analysis show that most of the target proteins in cluster 1 are involved in biological processes, including AKT1, MAPK1, MAPK14, CASP1, CASP3, IL2, MMP9, HMOX1, BCL2L1, and NOS2 (Figure 6).

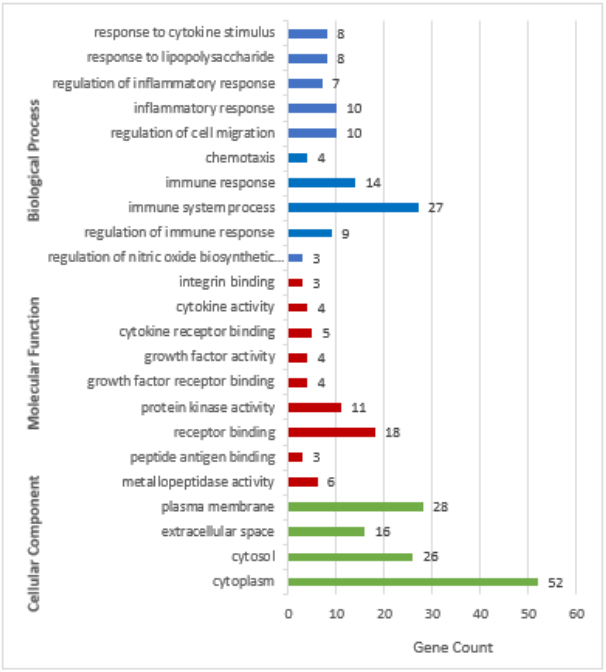


Figure 6. Results of functional analysis based on gene ontology. Blue bars indicate biological processes, red bars indicate molecular functions and green bars indicate cellular components.

The results of KEGG pathway analysis show that the anti-inflammatory target compounds in guava fruit are involved in several inflammatory pathways such as PI3K-Akt (hsa04151), MAPK (hsa04010), TNF (hsa04668), chemokine (hsa 04062), NLR (NOD-like receptor) (hsa04621), JAK-STAT (hsa04630), leukocyte trans-endhotelial migration (hsa04670), VEGF (hsa04370), TLR (Toll-like receptor) (hsa04620), and NF-kB (hsa04064) (Figure 7). Understanding the pathways involved in the production of inflammatory mediators (Ngabire *et al*., 2018) is very necessary for analyzing the anti-inflammatory role of compounds in guava fruit.

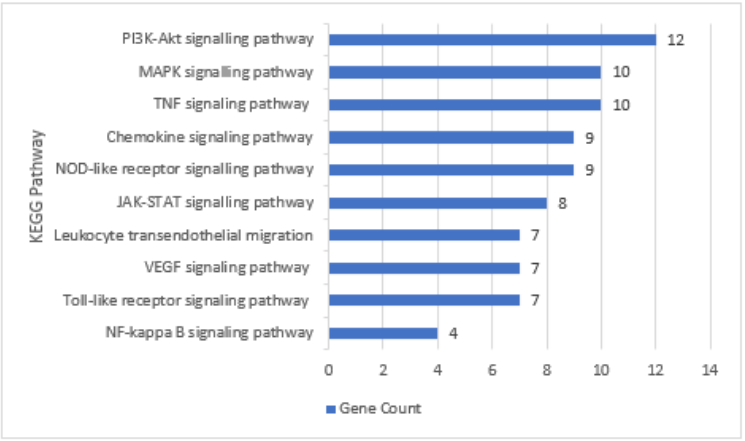


Figure 7. KEGG pathway from compounds in guava fruit

The results of KEGG analysis showed that the target proteins MAPK1, MAPK14, and AKT1 were most involved in the expression and production of inflammatory mediators, such as TNF-α, IL-6, IL-1β, RANTES, etc. While MMP9 is directly involved with leukocyte trans-endothelial migration which leads to endothelial hyperpermeability and plasma leakage. In this study, 11 compounds were selected based on their interactions with potential target proteins (Table 4).

Table 4. Compounds in guava fruit that interact with potential target proteins.

Potential target proteins			
MAPK1	MAPK14	AKT1	MMP9
Arjunolic acid	Citral	Guaijaverin	Guaijaverin
Farnesene	Ellagic acid	Pantothenic acid	Pantothenic acid
beta-Carotene	Palmitic acid	Citric acid	
alpha-Linolenic acid	Oleanolic acid		

Anti-Inflammatory Molecular Mechanisms of Compounds in Guava Fruit

Research in recent years shows that plants have various biological activities including anti-inflammatory effects through the regulation or inhibition of inflammatory mediators (such as cytokines) and the pathways involved in their production (Ngabire *et al.*, 2018). DENV infection is associated with an excessive or prolonged inflammatory response and causes endothelial cell contraction, permeability, and changes in endothelium adhesion (Aloia *et al*., 2015).

Immune cells such as monocytes, macrophages, lymphocytes, and dendritic cells play an important role in the immune response to DENV infection, thereby triggering the release of various inflammatory mediators. Endothelial cells are also involved in platelet adhesion and aggregation, as well as leukocyte adhesion and migration to infected tissues. Inhibition of signaling pathways involved in the inflammatory response is one approach in the development of anti-inflammatory therapy. MAPK and PI3K-Akt pathways are involved in the activation of immune cells and endothelial cells, making them potential targets for anti-inflammatory therapy.

MAPK Signaling Pathway

In this research, several compounds in guava fruit can inhibit the MAPK signaling pathway through interactions with MAPK1 (arjunolic acid, farnesene, beta-carotene, and alpha-linolenic acid) and MAPK14 (citral, ellagic acid, palmitic acid, and oleanolic acid) (Figure 8). Mitogen-activated protein kinase (MAPK), belonging to the large family of serine/threonine kinases, is a major inflammatory signaling pathway from the cell surface to the nucleus. The MAPK signal transduction pathway plays an important role in many immune-mediated inflammatory responses (Hommes *et al.*, 2003).

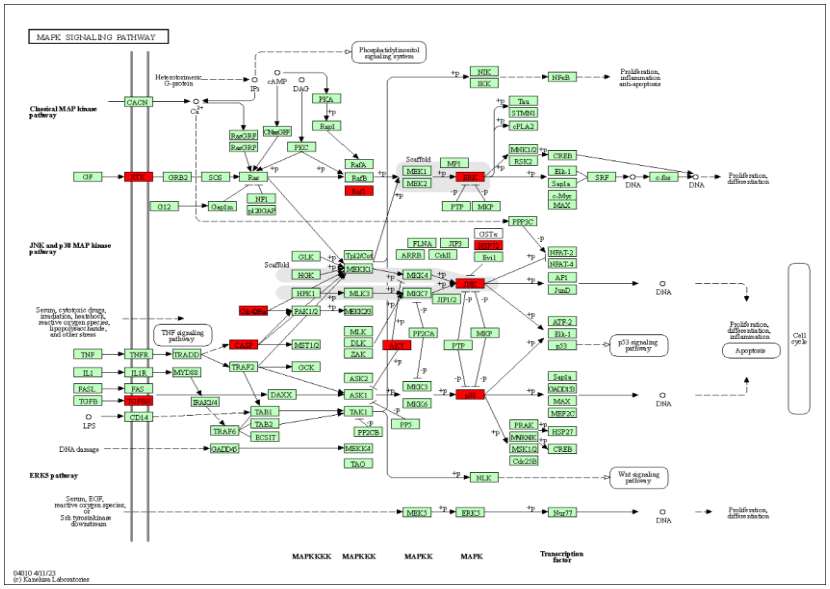


Figure 8. MAPK signaling pathway. The red color is the target compound in guava fruit.

The MAPK pathway plays an important role in the regulation of proinflammatory cytokine production and downstream signaling events that lead to inflammation. The MAPK family consists of three subfamilies, namely extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 MAPK. The MAPK signaling pathway can be triggered by stress, growth factors, pathogen-associated molecular patterns, and inflammatory cytokines. Stimulation of MAPK KKs such as A-Raf, B-Raf, and C-Raf causes phosphorylation and activation of MAPK (MEK1/2) which then stimulates MAPK activity (ERK1/2). Activation of ASK1, TAK1, and MEKK1/2 results in activation of MEK4/7 which is responsible for JNK activation. Activation of MTK1 and ASK1 will activate MKK3/6, causing phosphorylation and activation of p38. ERK1/2, JNK and p38 are responsible for the activation of transcription factors including TCF/ELK1, ATF-2, and AP-1, among others. These transcription factors cause the expression of genes encoding inflammatory cytokines, cell differentiation, growth, and apoptosis (Manzoor & Koh, 2012).

Activation of the MAPK pathway can stimulate immune cells to release inflammatory mediators such as IL-1β, TNF-α, VEGF, and IL-6. The MAPK pathway also influences the proliferation and differentiation of T lymphocyte cells, causing a cytokine storm in DHF sufferers. Cytokines produced by T cells can modulate the function of other immune cells that play a role in inflammation.

DENV infection causes an adaptive immune response by activating naïve CD4+ and CD8+ T cells to differentiate into effector T cells. Effector T cells cause cytokine production or lysis of virus-infected cells. Activated CD4+ cells can differentiate into Th1 cells and Th2 cells, which have an important role in the regulation of immune responses (Roy & Bhattacharjee, 2021). Th1 cells mostly secrete IL-2 and IFN-γ, while Th2 cells secrete IL-4, IL-5, IL-6, IL-10, and IL-13 (Chaturvedi et al., 2006). DENV infection can trigger an inflammatory response by activating the MAPK signaling pathway (Sreekant et al., 2018). Oral administration of the SB203580 compound to DENV-infected mice was shown to prevent increases in hematocrit and lymphopenia, inhibit inflammation and pathology (including intestinal leakage), and significantly increase survival (Fu et al., 2014). The compound SB203580 is a MAPK p38 (MAPK14) inhibitor. DENV induces ERK1/2 (MAPK3/1) phosphorylation and increased apoptosis. Research shows that inhibiting ERK1/2 using FR180204 can limit hepatocyte apoptosis and reduce liver cell damage. FR180204 treatment also showed improvement in clinical parameters, such as leukopenia, thrombocytopenia, transaminases, and histology (Sreekanth et al., 2014).

PI3K-AKT Signaling Pathway

In this study, the compounds guaijaverin, pantothenic acid, and citric acid in guava fruit can inhibit the PI3K-Akt signaling pathway through interaction with AKT1 (Figure 9). Activation of AKT1 causes various inflammation-related effects through activation of the NF-κB pathway. Activation of the PI3K-Akt pathway plays an important role in the immune response to viral infections including SARS-CoV-2, SARS-CoV, dengue, and Japanese encephalitis virus. Activation of this pathway is required for viral entry and replication in host cells. Therefore, Akt is a potential therapeutic target for various disease conditions (Shrimali *et al*., 2021).

PI3K is a key molecule in the signal transduction pathway that begins with the binding of extracellular signals to cell surface receptors. PI3K has serine/threonine kinase and phosphatidylinositol kinase activities. There are eight catalytic subtypes in the PI3K family divided into three categories. Among them, the most studied is PI3K class I, which consists of classes IA and IB. Class I PI3Ks are activated by several cell surface receptors (Zhao *et al*., 2021).

Akt, also known as protein kinase B (PKB), is the main effector downstream of PI3K. Activation of PI3K leads to the formation of PIP3 as a second messenger that activates downstream proteins. Phosphoinositide-dependent protein kinase-1 (PDK1) is one of the important downstream proteins activated by PIP3, which then activates PKB/Akt signal transduction. Once activated, Akt will activate or inhibit downstream target proteins such as Bad, Caspase9, NF-κB, and GSK3β through phosphorylation, thereby regulating cell proliferation, differentiation, apoptosis, and migration (Zhao *et al*., 2021). Flavivirus triggers NF-κB activation via PI3K activation. DENV initiates the host's innate immune response through molecular mechanisms involving the PI3K/NF-κB signaling pathway. The specific inhibitor of PI3K, LY294002 can reduce nuclear translocation of NF-κB (Chang *et al.,* 2006).

Inhibition of the MAPK and PI3K-Akt pathways by compounds in guava fruit via MAPK1, MAPK14, and AKT1 can inhibit or reduce the production of inflammatory mediators

and vascular permeability. During DENV infection, endothelial cells are activated by various signaling molecules, including VEGF, TNF-α, and IL-1β due to activation of the MAPK and PI3K-Akt pathways. Activation of this pathway causes a cellular response resulting in increased production of inflammatory mediators and vascular permeability. Activation of endothelial cells by growth factors and inflammatory cytokines during DENV infection causes upregulation of adhesion molecules on the surface of endothelial cells resulting in migration of leukocytes from the bloodstream to the infected tissue. Increased leukocyte migration affects endothelial cell integrity and contributes to inflammation and vascular leakage.

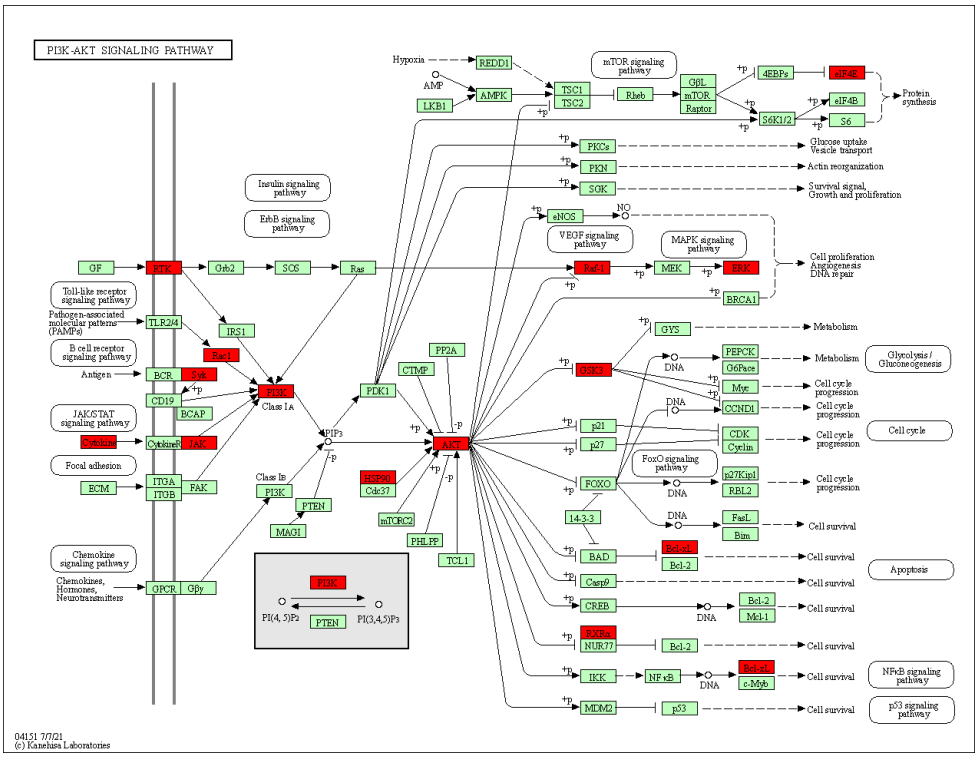


Figure 9. PI3K-AKT signaling pathway. The red color is the target compound in guava fruit.

Leucocyte Trans-Endothelial Migration

In this study, guaijaverine and pantothenic acid in guava fruit can interact with MMP-9 thereby preventing leukocyte migration and increasing vascular permeability (Figure 10). Matrix metalloproteinase-9 (MMP9) or known as gelatinase B (GelB) produced by DENV-infected dendritic cells can trigger vascular leakage (Pan *et al*., 2021), thereby facilitating the migration of leukocyte cells to inflammatory areas. MMP-9 activity affects vascular permeability by destroying extracellular matrix components and endothelial cell junctions (Cabral-Pacheco *et al*., 2020). Trans-endothelial migration of leukocytes plays an important role in DENV infection. This can increase disease severity, including causing a cytokine storm, excessive complement activation, coagulation abnormalities, and increased vascular permeability (Zheng *et al*., 2022).

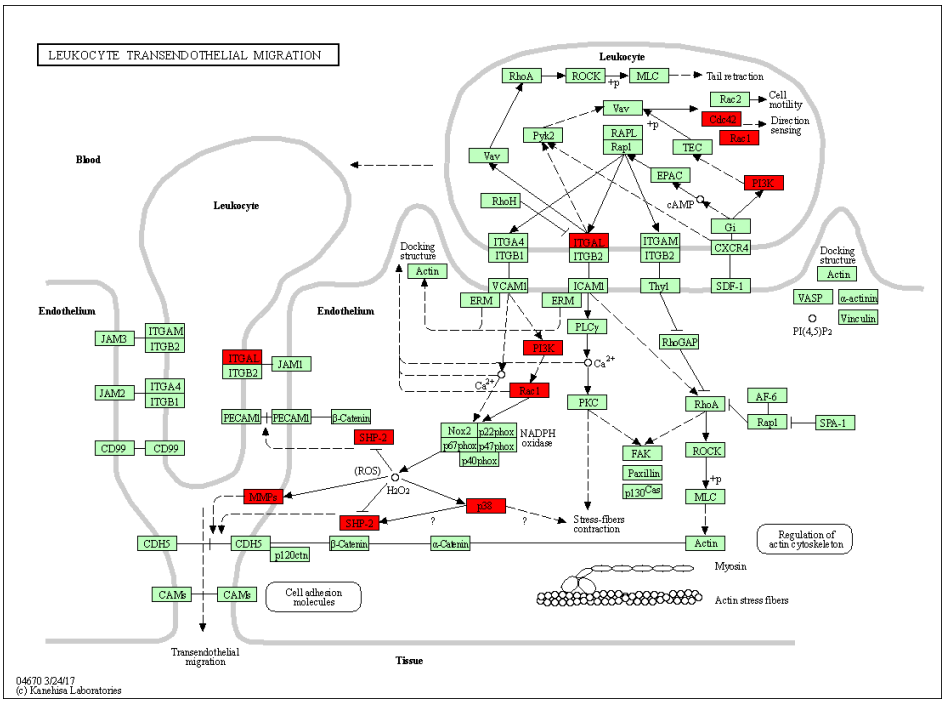


Figure 10. Leukocyte transendothelial migration. The red color is the target compound in guava fruit.

Inflammatory cytokines and growth factors released during inflammation or tissue damage lead to increased expression of MMP9 (Li *et al*., 2007). Increased proinflammatory cytokines IL-6, TNF-α, IL-8, VEGF, and sVCAM-1 in the serum of DBD/DSS patients correlate with vascular damage. Most of these cytokines stimulate the production of gelatinolytic MMPs. Thus, many pathways trigger the overproduction of gelatinolytic MMPs that cause the vascular damage observed in DHF/DSS (Luplerdlop *et al*., 2006).

Pan *et al*. (2021) revealed the molecular mechanism of DENV NS1 protein regulating MMP9 to induce endothelial hyperpermeability and vascular leakage in humans and mice. NS1 DENV increases MMP9 expression through activation of the NF-κB signaling pathway. NS1 also recruits MMP9 to interact with β-catenin and Zona occludens protein-1/2 (ZO-1 and ZO-2) which degrade adhesion and tight junction proteins, resulting in endothelial hyperpermeability and vascular leakage.

The pathogenesis of DENV infection is associated with complex interactions between the virus, host genes, and the host immune response (Bhatt *et al*., 2021). Thrombocytopenia in DHF is caused by increased platelet use due to damage to the vascular endothelium. Endothelial damage stimulates platelet adhesion and aggregation (Retnowati *et al*., 2018). On the other hand, DENV infection of monocytes, macrophages, and mast cells also triggers increased vascular permeability and vascular leakage due to the secretion of various mediators such as cytokines, chemokines, proteases, lipid mediators, etc. (Wan *et al*., 2018).

In silico is widely applied in the fields of pharmacology molecular (Kusuma *et al*., 2022; Lisdiana & MUstikaningtyas, 2023), toxicology (Hemmerich & Ecker, 2020), and germplasm diversity (Mursyidin *et al*., 2021). The novelty of this research is the in silico approach to provide a more comprehensive understanding of the interactions of proteins and active compounds in guava fruit. The active compounds in guava fruit as well as the explanation of their molecular mechanisms and specific targets in the treatment of dengue fever, especially the effect of increasing platelets, are still limited and have not been fully explored. This research contributes to providing an understanding of the molecular mechanisms underlying the increase in platelet numbers by active compounds in guava fruit. This research provides a scientific basis for the use of active compounds in guava fruit for dengue fever therapy, especially in terms of increasing platelets.

CONCLUSION

Compounds in guava fruit (Psidium guajava) that have the potential to increase platelets through anti-inflammatory activity based on protein interaction networks are arjunolic acid, farnesene,

beta-carotene, alpha-linolenic acid, citral, ellagic acid, palmitic acid, oleanolic acid, guaijaverin, pantothenic acid, and citric acid. The compounds arjunolic acid, farnesene, beta-carotene, and alpha-linolenic acid act as anti-inflammatories by inhibiting MAPK1, citral, ellagic acid, palmitic acid, and oleanolic acid through inhibiting MAPK14, guaijaverin, pantothenic acid, and citric acid through inhibiting AKT1, and guaijaverine and pantothenic acid through inhibition of MMP-9. Through this anti-inflammatory mechanism, compounds in guava fruit play a role in increasing platelets by inhibiting the MAPK, PI3K-Akt pathway, and leukocyte transendothelial migration, thus inhibiting or reducing the production and expression of inflammatory mediators and vascular permeability. The results of this in silico research need to be continued with in vitro and in vivo research, to clarify the understanding of the molecular mechanism of increasing platelets by active compounds in guava fruit.

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