

# The potential of *A. Muricata* Bioactive Compounds to Inhibit HIF1 $\alpha$ Expression Via Disruption of Tyrosine Kinase Receptor Activity: an In Silico Study

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## ABSTRACT

**Background:** Cancer is a debilitating disease that is on the increase in both developed and developing countries. The plant extract of *A. muricata* have been known to have a variety of anticancer effects, including anti-angiogenic potential. An in silico study is needed as a preliminary study to understand the mechanism underline this process. **Objective:** The aim of this study was to investigate the potential of the bioactive compounds of *A. muricata* in regulating angiogenesis process, primarily by the regulation of hypoxia inducible factor (HIF)-1 $\alpha$  expression by *in silico* study. **Methods:** This study was performed by in silico analysis including the bioactive compounds preparation, biological activity prediction, protein target and pathway analysis, 3D protein modelling, protein-ligand and protein-protein docking, and the visualization of docking results. **Results:** There are 3 bioactive compounds of *A. muricata* with the ability to inhibit HIF-1 $\alpha$  expression, including kaempferol, genistein, and glycitein. The inhibition of HIF-1 $\alpha$  expression was associated with phosphoinositide 3-kinases (PI3K)/Akt signaling pathway, which involved tyrosine kinase receptor activity on the cell membrane. Based on the silico analysis in this study, we shown that kaempferol, genistein, and glycitein inhibit HIF-1 $\alpha$  expression through the disruption of interleukin (IL)-6R and toll-like receptor (TLR)-4 and their respective ligands interaction. **Conclusion:** The findings of this study show that *A. muricata* bioactive compounds could inhibit HIF-1 $\alpha$  expression through disruption of the tyrosine kinase receptor binding with its ligand.

**Key words:** *A. muricata*, bioactive compounds, cancer, disease, *in silico*.

## 1. BACKGROUND

Cancer is a complex and devastating disease leading to millions of death every year (1-3). Due to the disorganized and lack of structural integrity of blood vessel in tumor, some tumor areas experienced inadequate perfusion and transient hypoxia. Hypoxia inducible factor-1 (HIF1) have been reported to be involved in the response to hypoxic stress (4). HIF1 consist of subunit  $\alpha$  and subunit  $\beta$ . HIF1 $\alpha$  is upregulated in hypoxic tumor cells and activates the transcription of target genes, allowing cellular adaptation to hypoxia and tumor angiogenesis (5-6). The main signaling pathways involved in the regulation of HIF1 $\alpha$  expression is phosphoinositide 3-kinases (PI3K)/Akt pathway (7). PI3K is activated by the binding of a variety

of growth factors to their receptor following by activation of its downstream signaling such as Akt and mTOR signaling pathways (8).

Currently, the main cancer treatment are surgery, radiation-based therapy, chemotherapy, gene therapy, and/or hormonal therapy (9-10). However, these treatments mostly affect both normal and tumor cells and therefore induce side effects such as suppression of bone marrow, hair loss and cardiac toxicity (11). Hence, the identification of new anti-cancer agents with higher selectivity with little or no side effects is a pressing goal.

The use of anti-inflammatory herbal products for cancer prevention and therapy is an interesting area of study in the last decades. *Graviola* (*Annona muricata*) is one of the trop-

ical plant that have been studied due to their anti-inflammatory and anti-cancer effects (9, 12). Many studies have linked *A. muricata* derived compounds as well as its crude extracts to a variety of anticancer effects including induction of apoptosis (13) and inhibition of proliferation (14) on a variety of cancer cell lines, including breast (15) and colorectal cancer (16). Latest study reported the anti-angiogenic potential of *A. muricata* crude extract on chick chorioallantoic angiogenic (CAM) assays in dose dependent manner (17). However, to date, the study exploring the potency of single bioactive compounds of *A. muricata* are very limited, hence an in silico study is needed for preliminary screening of the involvement of *A. muricata* bioactive compounds during angiogenesis.

## 2. OBJECTIVE

The aim of the study was to investigate the potential of the bioactive compounds of *A. muricata* in regulating angiogenesis process, primarily by the regulation of HIF1 $\alpha$  expression by *in silico* study.

## 3. MATERIALS AND METHODS

### Bioactive Compounds Preparation

Nineteen of *A. muricata* bioactive compounds were analyzed in this study, including annomuricin E (CID 3083520), annonacin (CID 354398), muricoreacin (CID 44559047), kaempferol (CID 5280863), glycitein (CID 5317750), murihexocin (CID 44559048), genistein (CID 5280961), catechin (CID 9064), epicatechin (CID 72276), argentinine (CID 10085878), asimilobine (CID 160875), anonaine (CID 160597), coclaurine (CID 160487), isolauriline (CID 12311076), reticuline (CID 439653), xylopinine (CID 160503), annohexocin (CID 10054746), murihexocin C (CID 10258454), squamocin (CID 441612).

### Biological Activity Prediction

Biological activity of each active compounds was predicted using the Prediction of Activity Spectra for Substances (PASS) Server (<http://www.pharmaexpert.ru/passonline/>) (18). The compounds were predicted for human intestinal absorption (HIA) for evaluating the potency for oral use by using Laboratory of Molecular Modeling and Design webserver (<http://lmm.d.ecust.edu.cn>). The lethal dose (LD50) of each compound was also evaluated to predict the lethal dose when applied *in vivo* in rat model animal (19).

### Protein Target and Pathway Analysis

The protein target of the bioactive compounds was evaluated using hit identification and target prediction using HITPICK Server (<http://mips.helmholtz-muenchen.de/hitpick/>). Analysis of the molecular pathway prediction was performed using STITCH webserver (<http://stitch.embl.de>).

### Obtaining the amino acids sequences of IFN $\gamma$ , IFN $\gamma$ R, IL-6, IL6R, LPS and TLR4

The amino acid sequences of *Homo sapiens* interferon (IFN)- $\gamma$  (GI: 56786138), IFN $\gamma$ R (GI: 124474), IL-6 (GI: 4261586), IL-6R subunit  $\alpha$  (GI: 124343), lipopolysaccharide (LPS) *Sinorhizobium meliloti* (GI: 152264), and TLR-4 (GI: 6175873) were obtained from NCBI database (<https://www.ncbi.nlm.nih.gov>).

### 3D modeling of IFN $\gamma$ , IFN $\gamma$ R, IL-6, IL6R, LPS and TLR4 protein structure

The 3D structure of IFN $\gamma$ , IFN $\gamma$ R, IL-6, IL6R, LPS and TLR4 proteins was predicted by using homology modeling method provided by SWISS-MODEL web server (<https://swissmodel.expasy.org>) (20).

### Protein-ligand and protein-protein docking,

Docking of the active compounds of *A. muricata* with IFN $\gamma$ , IFN $\gamma$ R, IL-6, IL6R, LPS and TLR4 protein was performed by using SwissDock webserver (<http://www.swissdock.ch>). The protein-protein docking simulation was then performed using ClusPro Webserver (<https://cluspro.org>) (21).

### Visualization and Analysis of the Interactions

The results of the docking were visualized using UCSF Chimera software (<https://www.cgl.ucsf.edu/chimera>), and the ligand bond interactions between bioactive compounds and protein was analyzed using LigPlot+ software (<https://www.ebi.ac.uk/thornton-srv/software/LigPlus>).

## 4. RESULTS

### The Biological Activity of *Annona muricata* active compounds

HIF1 $\alpha$  expression and activity plays a crucial role in the angiogenesis, so we first analyzed the potency of the bioactive compounds of *A. muricata* in repressing HIF1 $\alpha$  expression. The screening was based on the Pa score, which if the score of Pa is 0.3 means that the bioactive compound has minimum potency for the specific activity. And if the score of Pa is more than 0.7, the laboratory experiments result will be similar to computational prediction results. There are 5 compounds that have a Pa score above 0.7 in the activity of HIF1 $\alpha$  expression inhibitor. However, we screened the best three compounds that have high probability, including kaempferol (Pa: 0.969), genistein (Pa:

Active compounds	Pa score	HIA+	LD50 (mol/kg)
Kaempferol	0.969	0.986	3.08
Genistein	0.939	0.988	3.30
Glycitein	0.914	0.989	2.82
Catechin	0.883	0.965	1.87
Epicatechin	0.883	0.965	1.87
Argentinine	0.589	0.989	2.69
Squamocin	0.539	0.989	2.79
Annomuricin E	0.494	0.916	2.36
Annonacin	0.494	0.964	2.42
Asimilobine	0.481	0.990	2.61
Annohexocin	0.473	0.937	2.61
Coclaurine	0.472	0.984	2.52
Reticuline	0.460	0.918	2.69
Murihexocin	0.432	0.916	2.36
Murihexocin C	0.432	0.916	2.36
Muricoreacin	0.412	0.858	2.50
Isolauriline	0.347	0.994	2.68
Xylopinine	0.341	0.992	2.69
Anonaine	0.261	0.995	2.78

Table 1. *A. muricata* bioactive compounds based on Pa Score, HIA, and LD50 analysis

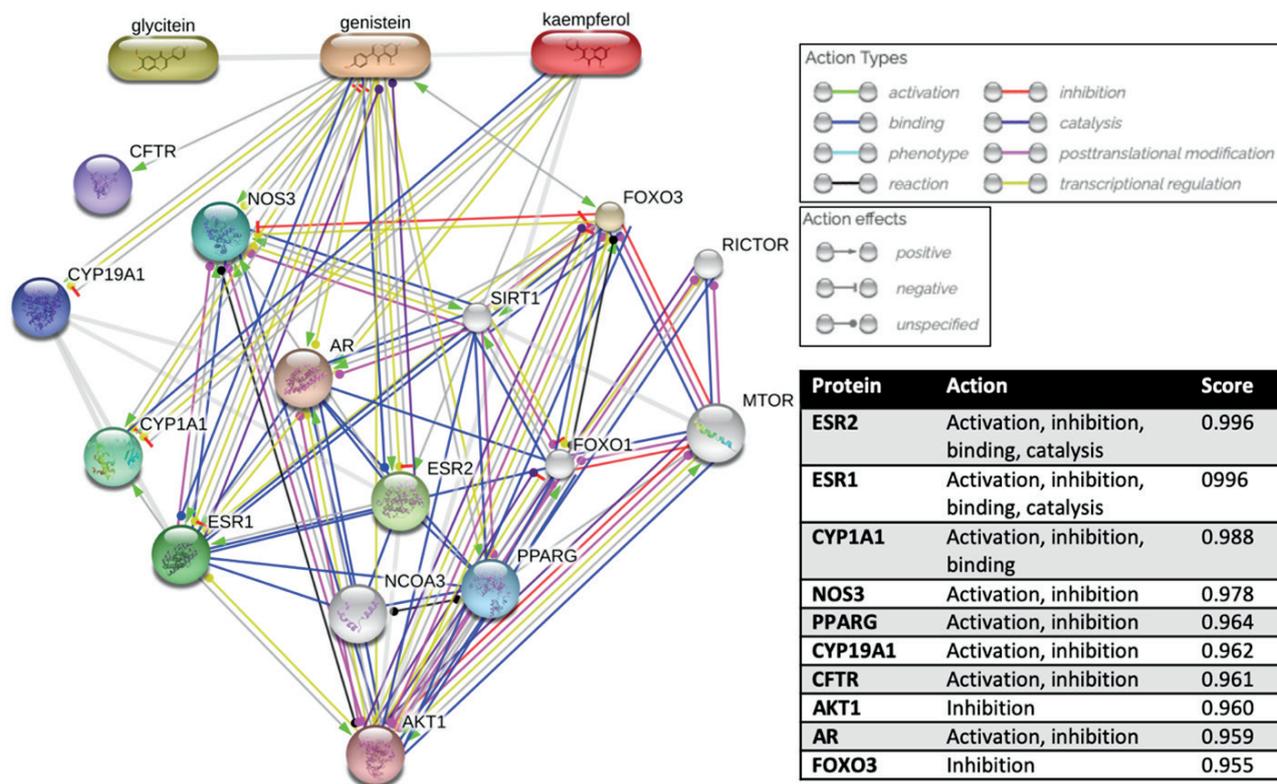


Figure 1. The potential signaling pathways affected by glycitein, genistein, and kaempferol.

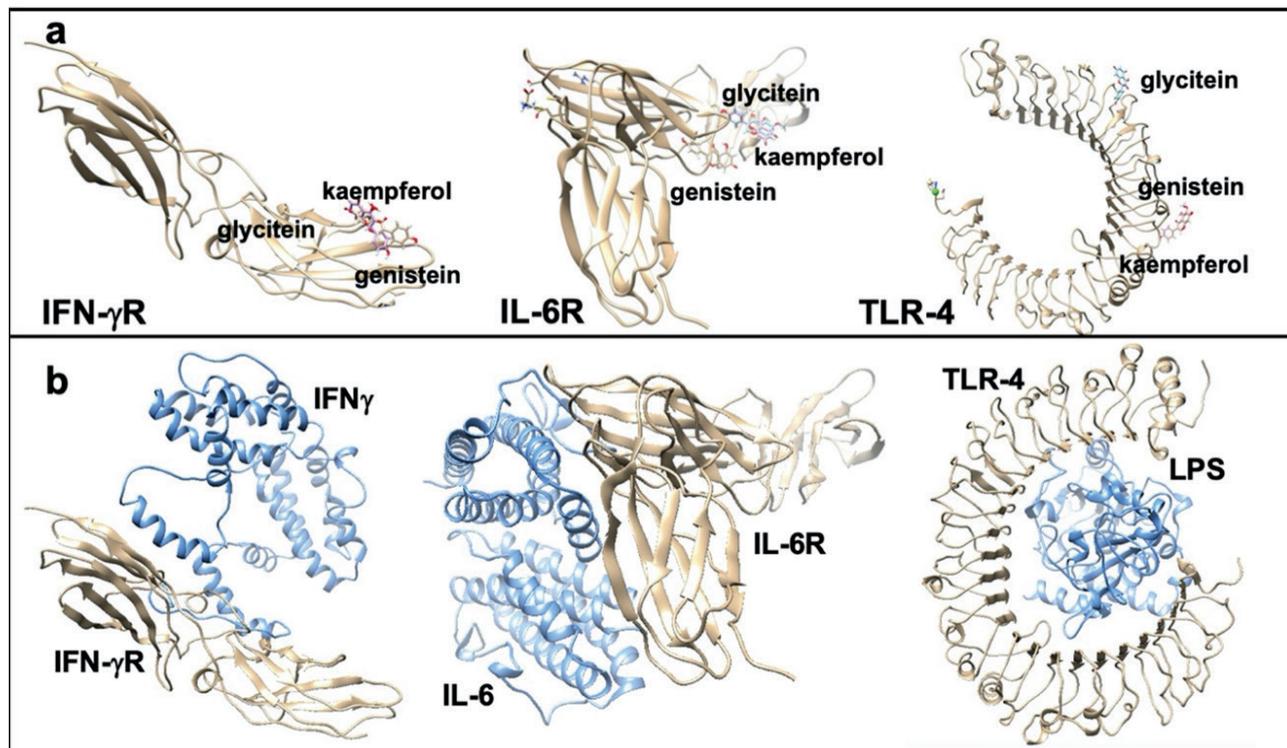


Figure 2. Binding site of kaempferol, genistein, and glycitein on IFN $\gamma$ R, IL6R and TLR4 (A). Interaction between tyrosine kinase receptors with their respective ligand.

0.939) and glycitein (Pa: 0.914) (Table 1).

HIA analysis was performed for evaluating the pharmacokinetics properties of the bioactive compounds. Among 19 bioactive compounds of *A. muricata* analyzed, 95% of them have HIA score above 0.9. This means that

the extract can be easily absorbed in the human intestine. The lethal dose parameter is important information before conducting *in vivo* experiment. Lethal dose prediction analysis showed that all of the compounds have LD50 below 3.5 mol/kg (Table 1).

***Annona muricata* bioactive compounds as anti-angiogenic factor via PI3K/Akt signaling pathway**

The biological activity prediction showed that three active compounds of *A. muricata* possesses as anti-angiogenic factor by inhibit HIF1 $\alpha$  expression. To investigate the pathway behind this process, we performed molecular pathway prediction analysis. The result showed that glycitein, genistein, and kaempferol could affect several proteins including RAC-alpha serine/threonine-protein kinase (AKT1), androgen receptor (AR), and forkhead box O3 (FOXO3). As previously mentioned that the main signaling pathways involved in the regulation of HIF1 $\alpha$  expression is PI3K/Akt pathway, here we confirmed that glycitein, genistein, and kaempferol have the ability to inhibit Akt1 protein (score: 0.960). In addition, the three compounds also can inhibit Foxo-3 transcription activator, another downstream target of Akt1 signaling responsible for triggering apoptosis in the absence of survival factors (Figure 1).

In order to further investigate the effect of kaempferol, genistein, and glycitein binding to IFN $\gamma$ R, IL-6R, and TLR4, next we performed the docking of ligand and its receptor, and also ligand with its receptor that already binds by kaempferol, genistein, or glycitein. The result in Table 2 showed that the binding free energy value (weighted score) for IFN $\gamma$ -IFN $\gamma$ R interaction is -783.2 kcal/mol, with the lowest energy in that cluster is -908.2 kcal/mol. There are 13 hydrogen bonds and one hydrophobic bond formed between IFN $\gamma$ -IFN $\gamma$ R interaction. The binding of kaempferol, genistein, or glycitein to IFN $\gamma$ R reduced the binding free energy (-839.8 kcal/mol) as well as the lowest energy (-910.8 kcal/mol) for IFN $\gamma$ -IFN $\gamma$ R interaction. It also increased the number of hydrogen and hydrophobic bonds formed between IFN $\gamma$ -IFN $\gamma$ R. This result showed that IFN-IFN $\gamma$  is not the target molecules of kaempferol, genistein, or glycitein, because the active compounds is not capable to disrupt IFN-IFN $\gamma$  interaction.

The docking result between IL6 and its receptor showed that the binding free energy for their interaction is -624.1 kcal/mol with the lowest binding energy in that cluster is -734.9 kcal/mol. There are 36 hydrogen bonds and 2 hydrophobic bonds that formed in the interaction. The

Ligand - Receptor	Weighted score (kcal/mol)	Interaction (bond)	Residue (Ligand – Receptor)
IFN $\gamma$ - IFN $\gamma$ R receptor (IFN $\gamma$ R)	Center: -783.2 Lowest: -908.2	Hydrogen  Hydrophobic	Arg130-Glu197; Asn127-Val152; His134-Gln199; Ser155-Glu197; Gly161-Glu197; Lys153-Glu164; Arg160-Asp155; Arg160-Tyr161; Lys151-Asp155; Gly150-Asp160; Lys148-Glu158, Lys148-Val159, Lys148-Gln157. Lys151-Glu156
IFN $\gamma$ - IFN $\gamma$ R, kaempferol; IFN $\gamma$ - IFN $\gamma$ R, genistein; IFN $\gamma$ - IFN $\gamma$ R, glycitein	Center: 839.8 Lowest: -910.8	Hydrogen  Hydrophobic	Arg130-Glu197; Asn127-Val152; His134-Gln199; Ser155-Glu197; Gly161-Glu197; Met157-Glu197; Phe159-Glu197; Arg160-Asp155; Arg160-Tyr161; Lys151-Asp155; Lys153-Pro163; Gly150-Tyr161; Gly150-Val159; Lys148-Gln157; Lys148-Asp89. His134-Asp144; Lys153-Glu164; Lys148-Glu156; Lys148-Glu158.
IL6 – IL6 receptor (IL6R)	Center: -624.1 Lowest: -734.9	Hydrogen  Hydrophobic	Ile164-Gln152; Gln155-Gln152; Asp168-Gln144; Asp168-Arg141; Thr170-Arg141; Asp162-Lys156; Asp162-Arg52; Lys159-Lys157; Lys159-Lys156; Ala158-Lys156; Leu161-Lys156; Leu161-Arg52; Gln144-Asp168; Arg141-Asp168; Arg141-Asp168; Asn160-Arg52; Asn160-Arg52; Asn131-Thr170; Gln130-Thr170; Gln152-Ile164; Gln152-Ile164; Gln152-Gln155; Lys157-Lys159; Lys156-Lys159; Lys156-Ala158; Lys156-Asp162; Arg52-Asn160; Arg52-Leu161; Arg52-Asp162; Arg52-Lys159; Asn88-Lys159; Asn88-Arg151; Asn88-Arg151; Asn88-Arg23; Leu85-Arg23; Leu85-Arg23; Glu87-Arg23; Glu87-Arg23. Asp168-Arg141; Glu87-Arg23.
IL6 – IL6R, kaempferol; IL6 – IL6R, genistein; IL6 – IL6R, glycitein	Center: -628.1 Lowest: -731.2	Hydrogen  Hydrophobic	Asn88-Arg151; Asn88-Arg151; Asn88-Arg23; Leu85-Arg23; Leu85-Arg23; Glu85-Arg23. Glu87-Arg23.
LPS – TLR4	Center: -890.9 Lowest: -1217.2	Hydrogen  Hydrophobic	Glu402-Arg264; Glu244-His456; Asp245-His529; Arg256-Gln505; Arg256-His529; Glu129-Lys477; Tyr257-Asp580; Asn196-Glu42; Lys289-Gly40; Val250-Gln578; Arg91-Asp181; Arg91-Asp181; Arg91-Asn156; Gln160-Ser360; Arg131-Asp550; Gln120-Asn58; Gln120-Thr37; Met251-Arg606; Ser385-Glu266; Ser385-Asn265; Asp177-Arg606. Glu402-Arg264; Lys134-Glu603.
LPS – TLR4, kaempferol; LPS – TLR4, genistein; LPS – TLR4, glycitein	Center: -841.8 Lowest: -1199.3	Hydrogen  Hydrophobic	Lys139-Gln547; Ser179-Arg606; Asp177-Arg606; Asp177-Arg606; Arg131-Ser528; Arg131-Asp550; Thr105-Gln523; Thr105-Gln523; Asn104-Thr499; Asn104-Glu474; Trp107-Glu474; Ser385-Asn265; Ser385-Arg234; Ser385-Arg234; Leu386-Arg234; Thr198-Glu42; Thr198-Glu42; Asn196-Glu42; Val81-Arg382; Lys85-Asp405; Lys85-Tyr403; Lys85-Arg382; His82-Gln430; His82-Arg382; His161-Lys362. Lys134-Asp550; Glu402-Arg264; Lys85-Asp379

**Table 2. The docking result of tyrosine kinase receptor with their respective ligand in the absence or presence of kaempferol, genistein and glycitein**

binding of kaempferol, genistein, or glycitein to IL6R slightly reduce the binding free energy (-628.1 kcal/mol) but increase the lowest energy (-731.2 kcal/mol) for IL6 $\gamma$ -IL6R interaction. It also significantly reduced the number of hydrogen and hydrophobic bonds formed between IL6-IL6R (Table 2). These results showed that IL6R

was presumed to be the target molecules of kaempferol, genistein, or glycitein, because the active compounds was be able to disrupt IL6-IL6R interaction.

The last docking analysis was performed between LPS from *Sinorhizobium meliloti* bacteria to TLR4. The result showed that the binding free energy for their interaction is -890.9 kcal/mol with the lowest binding energy in that cluster is -1217.2 kcal/mol. There are 21 hydrogen bonds and 2 hydrophobic bonds that formed in the interaction. The binding of kaempferol, genistein, or glycitein to TLR4 significantly increased the binding free energy (-841.8 kcal/mol) as well as the lowest energy (-1199.3 kcal/mol) for LPS – TLR4 interaction (Table 2). These results showed that TLR4 was presumed to be the target molecules of kaempferol, genistein, or glycitein, because the active compounds was be able to disrupt LPS-TLR4 interaction.

### DISCUSSION

HIF1 $\alpha$  is the key factor in regulating angiogenesis process through its activity as transcription factor of several angiogenic factors. HIF-1 $\alpha$  overexpression is associated with treatment failure and increased mortality. Several studies reported that HIF1 $\alpha$  was overexpressed in common cancers (22) and associated with vascular endothelial growth factor (VEGF) expression and vascularization (23). Due to its significant role in regulating angiogenesis and metastasis process, HIF1 $\alpha$  often targeted as therapeutics target for cancer (24). In this study, we found that kaempferol, genistein, and glycitein has the potential to inhibit HIF1 $\alpha$  expression (Table 1).

HIF-1 $\alpha$  protein synthesis is regulated by activation of the PI3K/Akt and mitogen-activated protein kinase (MAPK) pathways. These pathways can be activated by signaling via receptor tyrosine kinases, non-receptor tyrosine kinases or G-protein-coupled receptors. We analyzed three tyrosine kinase receptors which are the upstream of PI3K/Akt signaling pathway, including IFN $\gamma$ R, IL-6R, and TLR4R. We found that kaempferol, genistein, and glycitein might affect IL6 – IL6R binding as well as LPS – TLR4, but not IFN $\gamma$ -IFN $\gamma$ R binding (Table 2). Previous study reported that kaempferol inhibits angiogenic ability by targeting VEGF receptor-2 and downregulating the PI3K/AKT and extracellular signal-regulated kinase (ERK) pathways in VEGF-stimulated human umbilical vein endothelial cells (25). Another study reported that genistein was found to inhibit angiogenesis through regulation of multiple pathways, such as PI3K/Akt and ERK1/2 signaling pathways (26). Most of the previous studies that reported about these bioactive compounds was focused only on the pathways affected, but did not explain how the bioactive compounds act. Here we are the first to reported that kaempferol, genistein, and glycitein affect PI3K/Akt signaling pathway due to their ability to disrupt IL6 – IL6R and LPS – TLR4 interaction.

### 5. CONCLUSION

There are 3 bioactive compounds of *A. muricata* with the ability to inhibit HIF-1 $\alpha$  expression, including kaempferol, genistein, and glycitein. Based on the silico analysis in this study, we found that kaempferol, genistein, and glycitein inhibit HIF-1 $\alpha$  expression through the dis-

ruption of IL6R and TLR4 and their respective ligands interaction.

- **Author's contribution:** F.R.P.D has a major role in the designing and data collection of this work. R.F.A, N.I.A, N.S and S.P.A.W had a part in data analysis and article preparing for drafting.
- **Conflict of Interest:** There are no conflicts of interest.
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