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In Silico Study of Pulutan (*Urena lobata*) Leaf Extract As Anti Inflammation and their ADME prediction

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Abstract

Inflammation is the basic for the pathogenesis of several diseases both of degenerative and non-degenerative disease. *Urena lobata* is a medicinal plant that can be found in Indonesia and has been used traditionally to cure influenza, inflammation and fever. However, there have been few reports about their anti-inflammatory activity and the mechanism action of this herbs are still unclear. The aim of study to evaluate the anti-inflammatory activities of active compound from *U.lobata* leaf and their pharmacokinetic property through *in silico* study. *U. lobata* leaf was extracted by digeration methods using ethanol solvent. Therefore, the active substances in the extract was analyzed by Liquid Chromatography-Mass Spectra (LC-MS). Pharmacokinetic property and physicochemical of active compounds were evaluated using pkCSM online tool. Anti-inflammatory activity of *U. lobata* active compound on phospholipase-A2 (PLA-2), cyclooxygenase-2 (COX-2) and lipoxygenase-5 (LOX-5) were evaluated by *in silico* study using. Ethanolic extract of *U. lobata* contained five active compound, there are stigmasterol, β-sitosterol mangiferin, gossypetin and chrysoeriol. Molecular docking study showed that stigmasterol and β-sitosterol of *U. lobata* have a strong activity as anti-inflammatory based on the estimation of inhibition constant (Ki) value against PLA2 and COX-2. However, mangiferin and gossypetin have a stronger anti-inflammatory effect on LOX-5 among others compound. *U. lobata* has anti-inflammatory activity through inhibition on COX-2 greater than on PLA2 and LOX-5.

Keywords: *anti-inflammation, pharmacokinetic, molecular docking, urena lobata*

Introduction

Inflammation is the immune system's response to tissue damage caused by physical trauma, chemical and microbiological substances [1]. One of the contributing factors to inflammation condition is the metabolic product of arachidonic acid. The compound is commonly found in phospholipid cell membrane and used to stabilize liquid of them. When the cell membranes damaged, the phospholipase A2 enzyme (PLA2) is activated to convert phospholipids into arachidonic acid (AA). Therefore, it is converted by cyclooxygenase-2 enzyme (COX-2) into prostaglandins, prostacycline and thromboxane. They are cytokine pro-inflammatory contributing on immune defense system and inflammation process also. On the other hand, AA is converted by 5-lipoxygenase enzyme (LOX5) to leukotriene as inflammation mediators [1,2].

The action mechanism of conventional drugs as anti-inflammatory mostly through the inhibitory activity of COX-2 and PLA2, meanwhile inhibition against LOX-5 is still limited. Synthetic anti-inflammatory drugs often cause some adverse drug reaction such as stomach pain, heartburn, nausea, vomiting, diarrhea and the others [3]. Moreover, searching another alternative medicine which less side effect is needed to overcome the problems. Herbal medicine is one of sources of drug substances which can be explored to treat the diseases.

One of the herbs that is often used to treat inflammation traditionally is Pulutan (*Urena lobata*) leaf. Pre-clinical study indicated anti-inflammatory activity, antioxidant and antimicrobial of herbs. [4,5]. Other study showed *U. lobata* inhibit the increase of free radical such as superoxide radical, hydroxyl radical and lipid peroxidation due to a flavonoid and phytosterol rich content [5,6]. However, there have been few reports about their anti-inflammatory activity especially the mechanism action.

In silico study is performed by molecular docking to predict its activity with the selected target cell. Docking is an attempt to harmonize the ligand, a protein inside the target cell such as receptor or enzyme [7]. Besides measuring docking activity, one of the principle stages in drug design is the evaluation of the pharmacokinetic properties. Pharmacokinetic analysis using an animal model is expensive, therefore we use a molecular modelling in order to evaluate both of the chemical properties and pharmacokinetic (ADME) [8,9,10]. The objective of the study was to evaluate anti-inflammatory activity of *U. lobata* by *in silico* approach and their pharmacokinetic properties.

Experimental section

Sampel preparation

Simplisia of *U. lobata* leaves were obtained from Balai Materia Medika Batu, Malang, Indonesia with certificate number 074/027/101.8/2015. Therefore, 50 g of the herbs materials was extracted in 250 ml ethanol 80 % for 4 hours using water bath shacker and it was repeated 2 times using fresh solvent. The extract is evaporated to produce a paste form and dilute with solvent according to concentration which have been fixed.

Identification of active substances

Ethanolic extract of *U. lobata* leaf was performed a qualitative analysis using Liquid Chromatography–Mass Spectra (LC-MS) Accela 1250 pump. Mobile phase contains 0.1 % formic acid in methanol and water combination. The identification included the 10 active compounds from phytosterol, flavonoid and alkaloid groups.

Prediction of physicochemical property and pharmacokinetic

Prediction of the pharmacokinetic properties (ADME: absorption, distribution, metabolism, and excretion) of the active compound was performed using the pkCSM online tool, i.e. firstly the tested compounds and the comparative compound were drawn as 2D molecular structures with ChemBio Draw Ultra and copied into ChemBio 3D Ultra to create a 3D structure, and then stored as * .sdf file or * .pdb files. Secondly, all of the tested compounds and the comparative compound were translated into SMILES format using SMILES Translator Online Help [11]. In the SMILES format, the compounds were processed using the pkCSM online tool to predict the ADME [12].

Molecular docking study

Activity of active substances in *U.lobata* leaf extracts both on PLA-2, COX-2 and LOX-5 were evaluated by *in silico* approach using a web-based software application (www.dockingserver.com) for ligand molecular docking and protein target. Inhibition constant, free energy of binding and surface interactions were observed by this method to examine their activity on PLA-2, COX-2 and LOX-5.

Result and Discussion

Identification of active substances in *U.lobata* leaf extracts

The Active compounds from ethanolic extract of *U.lobata* leaf can be seen in the figure 1 and table 1.

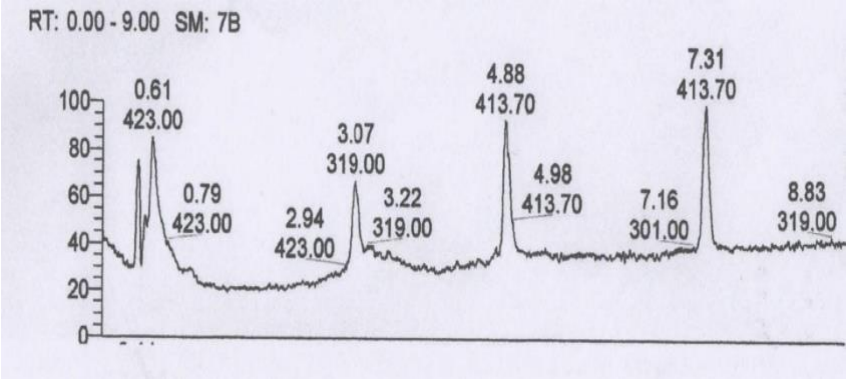

Figure 1. Chromatogram of of active compound in ethanolic extract of *U. lobata*

Table 1. Active compounds in *U.lobata* leaf extracts

No	Active compounds	Molecule weight	Ethanolic extract
1	Stigmasterol	413	(+++)
2	B-Sitosterol	415	(+)
3	Mangiferin	423	(++)
4	Quercetine	303	(-)
5	Kaempferol	286	(-)
6	Hypolaetin	302	(-)
7	Gossypetin	318	(++)
8	Luteolin	286	(-)
9	Apigenin	270	(-)
10	Chrysoeriol	300	(+)

Note : (-) : negative; (+): weak; (++): moderate; (+++); strong

The qualitative analysis using LC-MS indicated that the most abundant of active substances from *U. lobata* leaf extract were stigmasterol, mangiferin and gossypetin. Active compounds such as β-sitosterol and chrysoeriol were found also in the extracts of *U.lobata*, however the concentration was low.

All of them are classified into secondary metabolite groups and have biological activity therefore can be used to cure diseases. Stigmasterol is one of a group of phytosterols that are chemically similar to animal cholesterol. Phytosterols are insoluble in water however soluble in most organic solvents. Animal studies treated by stigmasterol showed both cholesterol and sitosterol absorption decreased 23% and 30%, respectively, over a 6-week period. It also

possesses potential antioxidant, hypoglycemic and thyroid inhibiting properties [5,6]. Gossypetin is flavonol or flavone, a type of flavonoid. Gossypetin indicates potential antioxidant, antimicrobial, anti-mutagenic and anti-atherosclerotic activities [13]. This compound is very soluble in chloroform and benzene, and also moderately soluble in ethanol and ether, but insoluble in water. β-sitosterol is one of several phytosterols or plant sterols with chemical structure similar to that of cholesterol. Sterols are isoprenoid-derived molecules that have essential functions typically in eukaryotes, and especially in higher plants. β-sitosterol are white, waxy powder with characteristic odor, soluble in ethanol and chloroform but insoluble in water [14]. It showed anti-cholesterol, anti-inflammatory and immunomodulator effects [15]. Mangiferin is a xanthonoid, and a glucoside of norathyriol. Mangiferin is soluble in hot diluted ethanol and methanol but insoluble in water. Laboratory study has identified a variety of pharmacology effect that associated with mangiferin including anti-microbial, antioxidant activity, and anti-diabetic effect in rodent [16,17]. Chrysoeriol is a flavon, one of major flavonoid classes. They exhibit many activities such as anti-inflammation and antihistamine activities. It is soluble in alkalies solution and sufficiently soluble in water [18]. The presence of active compounds in extract was influenced by polarity and extract solvent. Type of extract solvent impacts the amounts of active compounds in extract due to the difference of their solubility in solvent. Secondly, polarity of active compound also contributes to their solubility in solvent. Alkaloid, terpenoid and steroid are soluble in non- polar solvent like acetone, diethyl ether and hexane. Meanwhile, flavonoid, phenol and glycoside dissolve in polar solvent such as water and methanol [19,20]. It is appropriate with the determinate solubility theory **"**like dissolve like**"** that polar substances will dissolve in polar solvent and vice versa [19,21].

Non-nutrition compounds or secondary metabolite like alkaloid, terpenoid, flavonoid and steroid are found in a small concentration but it has pharmacology effect at certain dose [19]. Secondary metabolites are derived from metabolism of primary metabolite in plant but sometimes they have a toxic effect especially if it is used in high dose. Most of flavonoid and terpenoid in herbs have a potency as antioxidant, antiseptic and anti-inflammatory whereas steroid as anti-inflammatory and sex hormone. However, the pharmacology effect of alkaloid is difficult to be predicted in medicinal plants because they have so many biological activities [22].

Prediction of physicochemical property and pharmacokinetic of *U.lobata* leaf extract

The results of the *in silico* study of the physicochemical properties of *U. lobata* active compound can be seen in Table 2.

Table 2. Prediction of physichochemical properties of active compounds in *U.lobata* leaf extracts

Active compounds	MW	Log P	Torsion	HBA	HBD	PSA (A^2)	Water Solubility
Stigmasterol	412.702	7.801	5	1	1	186.349	-6.682
β-Sitosterol	414.718	8.025	6	1	1	187.039	-6.773
Mangiferin	422.342	-0.717	2	11	8	166,412	-2.918
Gossypetin	318.237	1.694	1	8	6	126.902	-2.900
Chrysoeriol	300.266	2.585	2	6	3	123.998	-3.237

MW=Molecular weight; LogP=logarithm of octanol/water partition coefficient; Torsion= bond between rotating atoms; HBA=H-bond acceptors; HBD=H-bond donors; PSA=polar surface activity

It can be seen that the molecular weight values of the active compound ranged from 300 to 422 (<500), the value of log of the octanol/water partition coefficient (log P) ranged from -0.717 to 8.025 (> 5), the amount of HBD ranged from 1 to 8 (>5), and the amount of HBA ranged from 1 to 11 (>10). Only chrysoeriol meet Lipinski Rules of Five completely. Meanwhile gossypetin did not meet in amount of HBD and both of stigmasterol and β-Sitosterol did not fulfill in log P value. Whereas mangiferin have 3 parameters which did not meet the rule.

Chemical databases contain many of molecules that could be suitable ligands for an enzyme. However, no matter how good the fit with the protein target, the candidate molecule is of no use if the absorption is poor or if the drug is eliminated too slowly from the body. The World Drugs Index database were analysed and it was concluded that a compound is more likely to have poor absorption or permeability if the molecular weight exceeds 500; the calculated octanol/water partition coefficient (log P) exceeds +5; there are more than 5 H-bond donors (HBD) expressed as the sum of O–H and N–H groups; and there are more than 10 H-bond acceptors (HBA) expressed as the sum of N and O atoms. The above analysis is called the Lipinski Rules of Five because all values are multiples of five [23]

Based on Table 2, this means that only chrysoeriol meet the Lipinski Rules of Five completely, meanwhile four others did not fulfill these rule [23]. Hence, it can be predicted that chrysoeriol will be easily absorbed and have high permeability.

Table 3. Prediction of pharmacokinetic properties of active compounds in *U.lobata* leaf extracts

	Absorption		Distribution		Metabolism		Excretion	
Active compounds	Intestinal absorption (%)	Skin Permeability (cm/h)	VdSS (L/kg)	Fraction Unbound	CYP2D6 inhibitor	CYP3A4 inhibitor	Total Clearance	Renal OCT2 substrate
Stigmasterol	94.970	-2.783	0.178	0	No	No	0.618	No
Sitosterol	94.464	-2.783	0.193	0	No	No	0.628	No
Mangiferin	46.135	-2.735	1.364	0.289	No	No	0.347	No
Gossypetin	68.009	-2.735	1.552	0.234	No	No	0.304	No
Chrysoeriol	82.844	-2.735	0.741	0.070	No	No	0.597	No

VdSS; (log ml/L)

Stigmasterol, β-Sitosterol and chrysoeriol have a high intestinal absorption percentage in oral administration. Meanwhile, five compound which were identified have a same value of log Kp value (> 2.5 cm/h) and indicated a good skin permeability. Mangiferin and gosypetin have VdSS value higher than other substances. Fraction unbound was showed by mangiferin and gossypetin about 20 %, meanwhile other compound in binding form absolutely. All of active compound did not indicate the inhibitory activity on both of CYP2D6 and CYP3A4. Stigmasterol and β-Sitosterol have a high total clearance and five substance not renal OCT2 substrate.

Skin permeability is an important consideration for improving drug efficacy that is particularly of interest in the development of transdermal drug delivery. A molecule will barely penetrate the skin if log Kp is more than -2.5 cm/h [24]. From Table 2 it can be seen that the skin permeability (Kp) of ferulic acid derivatives ranges from -2.678 to -2.992 cm/h (< -2.5). Therefore, it can be predicted that all derivatives have a good skin penetrability.

The volume of distribution (VD) is the calculated volume that the whole quantity of a medicine will be circulated at an equal level of blood plasma. The higher the VD is, the larger the amount of a drug is distributed to tissue rather than plasma. This model is established from the estimation of the steady-state volume of distribution (VDss), which is then revealed as log L/kg. According to Pires et al. [24], VDss higher than 2.81 L/kg (log VDss > 0.45) is categorized as high, whereas VDss lower than 0.71 L/kg (log VDss < -0.15) is categorized as low [8]. From Table 3 it can be seen that the VDss values of active compound range from -0.178 to 1.552, moreover it can be predicted that all active compounds can be distributed evenly providing an equal level of blood plasma

Cytochrome P-450 is an important detoxification enzyme in the body, mainly found in the liver. It oxidizes xenobiotics to facilitate their excretion. Many drugs are deactivated by cytochrome P450's but some can be activated by it. Inhibitors of this enzyme, such as grapefruit juice, can affect drug metabolism and are contraindicated. The cytochrome P450's is responsible for the metabolism of many drugs. However, inhibitors of the P450's can dramatically alter the pharmacokinetics of these drugs, therefore it is important to evaluate whether a given compound is likely to be a cytochrome P450 substrate. The two main isoforms responsible for drug metabolism are P2D6 cytochrome (CYP2D6) and P3A4 cytochrome (CYP3A4) [24]. From Table 3 it can be seen that almost all active substances do not affect or inhibit the CYP2D6 and CYP3A4 enzymes, so it can be predicted that the substances tend to be metabolized by the P450 enzymes in the body.

Organic cation transporter 2 (OCT2) is a protein transporter that has a vital contribution in renal uptake, disposition, and clearance of drugs and endogenous compounds. OCT2 substrates have potential for adverse interactions with codirected OCT2 inhibitors. Evaluating the transfer of a candidate compound by OCT2 offers useful information regarding not only its clearance but also potential contraindications [24]. From Table 3 it can be seen that all active compound do not affect the OCT2 substrate, so it can be predicted that the active compound are not OCT2 substrates.

Molecular docking of *U.lobata* on COX-2, LOX-5 and PLA2

Activity of active compound from *U.lobata* leaf extracts both of on COX-2, LOX-5 and PLA2 were evaluated by *in-silico* approach and the results can be seen at Table 4, 5 and 6.

Table 4. Molecular docking of active substances of *U.lobata* leaf extracts with COX-2

No	Active compounds	Est. Free Energy of Binding (Kcal/mol)	Est. Inhibition Constant Ki	Interact. Surface
1	Stigmasterol	-9.64	11.07 nM	955.50
2	β-Sitosterol	-7.01	15.48 nM	948.83
3	Mangiferin	-7.33	42.97 µM	841.18
4	Gossypetin	-6.84	8.53 µM	644.38
5	Chrysoeriol	-6.32	3.31 µM	526.63
6	Asetosal	-4.37	304.96 nM	488.49

Table 5. Molecular docking of active substances of *U.lobata* leaf extracts with LOX-5

No	Active compounds	Est. Free Energy of Binding (Kcal/mol)	Est. Inhibition Constant Ki	Interact. Surface
1	Stigmasterol	1.06	0.00	696.70
2	β -Sitosterol	0.98	0.00	679.05
3	Mangiferin	-1.06	166.79 mM	593.69
4	Gossypetin	-0.41	498.88 mM	499.92
5	Chrysoeriol	0.14	0.00	518.85
6	Asetosal	-4.78	311.52 µM	364.69

Table 6. Molecular docking of active substances of *U.lobata* leaf extracts with PLA2

No	Active compounds	Est. Free Energy of Binding (Kcal/mol)	Est. Inhibition Constant Ki (µM)	Interact. Surface
1	Stigmasterol	-7.33	4.23	703.94
2	β -Sitosterol	-7.15	5.76	674.86
3	Mangiferin	-5.83	53.15	478.32
4	Gossypetin	-4.75	331.32	536.81
5	Chrysoeriol	-5.18	160.72	500.58
6	Dexamethasone	-5.15	167.04	530.29

Molecular docking study indicated that stigmasterol and β-sitosterol of *U. lobata* have a strong activity as anti-inflammatory based on the estimation of inhibition constant (Ki) value against PLA2 and COX-2. For stigmasterol and β-sitosterol, their activity on both of PLA2 and COX-2 more potent than reference drug. Meanwhile, mangiferin and gossypetin have a stronger anti-inflammatory effect on LOX-5 among others compound. However, their potency is lower compare to reference drugs. Stigmasterol and β-sitosterol showed the low Ki value due to they have the low free energy of binding to protein target. Therefore, they have a strong binding between ligand and protein target. It is supported also by a high interaction surface between them.

In this study, stigmasterol has the highest score of surface interaction followed by β-sitosterol and mangiferin. A great result of surface interaction indicated a stronger binding between ligand and protein target, moreover the biology activity is higher. The lowest score of binding free energy produces a strong binding molecule and biology activity. Free energy binding and surface interaction between ligand and protein target influences the inhibitory

activity of *U. lobata* leaf extract on COX-2 and PLA2. On the other hand, molecular docking on LOX-5 indicated the low free energy binding score for mangiferin and gossypetin, therefore, it results a low inhibition constant.

Recently, molecular docking is used to discover new ligands for target of known structure. Potential compound can be observed by free energy binding score. It indicates binding affinity of ligand to target protein, the lower free energy binding results the higher binding affinity [25].

In addition, inhibition constant can be predicted using bioinformatics approach. The lowest score of inhibition constant shows the most potential compound. Other parameter is surface interaction, it represents the molecular recognition between ligand and target protein. The higher score of surface interaction, the higher interaction possibilities of compounds interacting with the target protein [26].

Conclusion

Based on in silico study, *U. lobata* leaf has anti-inflammatory activity through inhibition on COX-2 greater than on PLA2 and LOX-5, the prediction of ADME also indicated good properties.

Acknowledgment

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JTPC_MANUSCRIPT
DIREVIEW

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Abstract

Inflammation is the basic for the pathogenesis of several diseases both of degenerative and non-degenerative disease. *Urena lobata* is a medicinal plant that can be found in Indonesia and has been used traditionally to cure influenza, inflammation and fever. However, there have been few reports about their anti-inflammatory activity and their mechanism action are still unclear. The aim of study to evaluate the anti-inflammatory activities of active substances from *U.lobata* leaf and their pharmacokinetic property through *in silico* study. *U. lobata* leaf was extracted by digeration methods using ethanol solvent. Therefore, the active substances in the extract was analyzed by UHPLC (ACCELLA-1250 Thermo Scientific). Pharmacokinetic property and physicochemical of active compounds were evaluated using pkCSM online tool. Anti-inflammatory activity of *U. lobata* active compound on phospholipase-A2 (PLA-2), cyclooxygenase-2 (COX-2) and lipoxygenase-5 (LOX-5) were evaluated by *in silico* study. Ethanolic extract of *U. lobata* contained five active compound, there are stigmasterol, β-sitosterol mangiferin, gossypetin and chrysoeriol. Molecular docking study indicated stigmasterol and β-sitosterol of *U. lobata* have a strong activity as anti-inflammatory based on the estimation of inhibition constant (Ki) value against PLA2 and COX-2. Meanwhile, mangiferin and gossypetin have a stronger anti-inflammatory effect on LOX-5 among others compound. *U. lobata* has anti-inflammatory activity through inhibition on COX-2 greater than on PLA2 and LOX-5.

Keywords: *anti-inflammation, pharmacokinetic, molecular docking, Urena lobata*

Introduction

Inflammation is the immune system's response to tissue damage caused by physical trauma, chemical and microbiological substances [1]. the phospholipase A2 enzyme (PLA2) is activated to convert phospholipids into arachidonic acid (AA). Therefore, it is converted by cyclooxygenase-2 enzyme (COX-2) into prostaglandins, prostacycline and thromboxane. They are cytokine pro-inflammatory contributing on immune defense system and inflammation process also. On the other hand, AA is converted by 5-lipoxygenase enzyme (LOX5) to leukotriene as inflammation mediators [1,2].

The mechanism action of conventional drugs as anti-inflammatory mostly through the inhibitory activity of COX-2 and PLA2, meanwhile the inhibition against LOX-5 is still limited. Synthetic anti-inflammatory drugs often cause some adverse drug reaction such as

stomach pain, heartburn, nausea, vomiting, diarrhea and the others [3]. Moreover, searching another alternative medicine which less side effect is needed to overcome the problems. Herbal medicine is one of sources of drug substances which can be explored to treat the diseases.

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Experimental section

Sampel preparation

Simplisia of *U. lobata* leaves were obtained from Balai Materia Medika Batu, Malang, Indonesia with certificate number 074/027/101.8/2015. Therefore, 50 g of the herbs materials was extracted in 250 ml ethanol 80 % for 4 hours using water bath shacker and it was repeated 2 times using fresh solvent. The extract is evaporated to produce a paste form and dilute with solvent according to concentration which have been fixed.

Identification of active substances

Ethanolic extract of *U. lobata* leaf was performed a qualitative analysis using UHPLC (ACCELLA-1250 Thermo Scientific) and detector MS/MS Triple Q Thermo Finnigan. Mobile phase contains 0.1 % formic acid in methanol and water combination. The identification included the 10 active compounds from phytosterol, flavonoid and alkaloid groups.

Prediction of physicochemical property and pharmacokinetic

Prediction of the physicochemical properties and pharmacokinetic (ADME: absorption, distribution, metabolism, and excretion) of the active compound was performed using the pkCSM online tool, i.e. firstly the tested compounds and the comparative compound were drawn as 2D molecular structures with ChemBio Draw Ultra and copied into ChemBio 3D Ultra to create a 3D structure, and then stored as * .sdf file or * .pdb files. Secondly, all of the tested compounds and the reference drug were translated into SMILES format using SMILES Translator Online Help [11]. In the SMILES format, the compounds were processed using the pkCSM online tool to predict the ADME [12].

Molecular docking study

Activity of active substances in *U.lobata* leaf extracts both on PLA-2, COX-2 and LOX-5 were evaluated by *in silico* approach using a web-based software application (www.dockingserver.com) for ligand molecular docking and protein target. Inhibition constant, free energy of binding and surface interactions were observed by this method to examine their activity on PLA-2, COX-2 and LOX-5.

Result and Discussion

Identification of active substances in *U.lobata* leaf extracts

The active compounds from ethanolic extract of *U.lobata* leaf can be seen in the figure 1 and table 1.

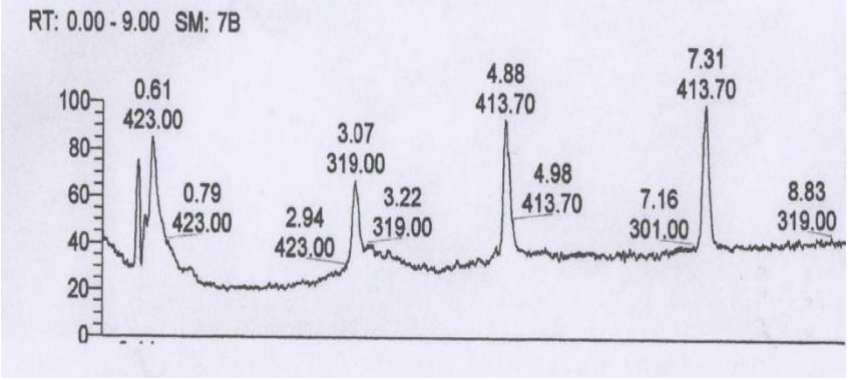

Figure 1. Chromatogram of of active compound in ethanolic extract of *U. lobata*

Table 1. Active compounds in *U.lobata* leaf extracts

No	Active compounds	Molecule weight	Ethanolic extract
1	Stigmasterol	413	(+++)
2	B-Sitosterol	415	(+)
3	Mangiferin	423	(++)
4	Quercetine	303	(-)
5	Kaempferol	286	(-)
6	Hypolaetin	302	(-)
7	Gossypetin	318	(++)
8	Luteolin	286	(-)
9	Apigenin	270	(-)
10	Chrysoeriol	300	(+)

Note : (-) : negative; (+): weak; (++): moderate; (+++); strong

The qualitative analysis using UHPLC indicated that the most abundant of active substances from *U. lobata* leaf extract were stigmasterol, mangiferin and gossypetin. Active compounds such as β-sitosterol and chrysoeriol were found also in the extracts of *U.lobata*, however the concentration was low.

All of them are classified into secondary metabolite groups and have biological activity, therefore can be used to cure diseases. Stigmasterol is one of a group of phytosterols that are chemically similar to animal cholesterol. Phytosterols are insoluble in water, however soluble in most organic solvents. Animal studies treated by stigmasterol showed both of cholesterol and sitosterol absorption decreased 23% and 30%, respectively, over a 6-week period. It also possesses potential antioxidant, hypoglycemic and thyroid inhibiting properties [5,6]. Gossypetin is flavonol or flavone, a type of flavonoid. Gossypetin indicates

potential antioxidant, antimicrobial, anti-mutagenic and anti-atherosclerotic activities [13]. This compound is very soluble in chloroform and benzene, and also moderately soluble in ethanol and ether, but insoluble in water. β-sitosterol is one of several phytosterols or plant sterols with chemical structure similar to that of cholesterol. Sterols are isoprenoid-derived molecules that have essential functions typically in eukaryotes, and especially in higher plants. β-sitosterol are white, waxy powder with characteristic odor, soluble in ethanol and chloroform but insoluble in water [14]. It showed anti-cholesterol, anti-inflammatory and immunomodulator effects [15]. Mangiferin is a xanthonoid, and a glucoside of norathyriol. Mangiferin is soluble in hot diluted ethanol and methanol but insoluble in water. Laboratory study has identified a variety of pharmacology effect that associated with mangiferin including anti-microbial, antioxidant activity, and anti-diabetic effect in rodent [16,17]. Chrysoeriol is a flavon, one of major flavonoid classes. They exhibit many activities such as anti-inflammation and antihistamine activities. It is soluble in alkalies solution and sufficiently soluble in water [18]. The presence of active compounds in the extract was influenced by polarity and extract solvent. Type of extract solvent impacts the amounts of active compounds in extract due to the difference of their solubility in solvent. Secondly, polarity of active compound also contributes to their solubility in solvent. Alkaloid, terpenoid and steroid are soluble in non- polar solvent like acetone, diethyl ether and hexane. Meanwhile, flavonoid, phenol and glycoside dissolve in polar solvent such as water and methanol [19,20]. It is appropriate with the determinate solubility theory **"**like dissolve like**"** that polar substances will dissolve in polar solvent and vice versa [19,21].

Non-nutrition compounds or secondary metabolite like alkaloid, terpenoid, flavonoid and steroid are found in a small concentration but it has pharmacology effect at certain dose [19]. Secondary metabolites are derived from metabolism of primary metabolite in plant but sometimes they have a toxic effect especially if it is used in high dose. Most of flavonoid and terpenoid in herbs have a potency as antioxidant, antiseptic and anti-inflammatory whereas steroid as anti-inflammatory and sex hormone. However, the pharmacology effect of alkaloid is difficult to be predicted in medicinal plants because they have so many biological activities [22].

Prediction of physicochemical property and pharmacokinetic of *U.lobata* leaf extract

The results of the *in silico* study of the physicochemical properties of *U. lobata* active compound can be seen in Table 2.

Table 2. Prediction of physichochemical properties of active compounds in *U.lobata* leaf extracts

Active compounds	MW	Log P	Torsion	HBA	HBD	PSA (A^2)	Water Solubility
Stigmasterol	412.702	7.801	5	1	1	186.349	-6.682
β-Sitosterol	414.718	8.025	6	1	1	187.039	-6.773
Mangiferin	422.342	-0.717	2	11	8	166,412	-2.918
Gossypetin	318.237	1.694	1	8	6	126.902	-2.900
Chrysoeriol	300.266	2.585	2	6	3	123.998	-3.237

MW=Molecular weight; LogP=logarithm of octanol/water partition coefficient; Torsion= bond between rotating atoms; HBA=H-bond acceptors; HBD=H-bond donors; PSA=polar surface activity

It can be seen that the molecular weight values of the active compound ranged from 300 to 422 (<500), the value of log of the octanol/water partition coefficient (log P) ranged from -0.717 to 8.025 (> 5), the amount of HBD ranged from 1 to 8 (>5), and the amount of HBA

ranged from 1 to 11 (>10). Only chrysoeriol meet Lipinski Rules of Five completely. Meanwhile, gossypetin did not meet in amount of HBD and both of stigmasterol and β-Sitosterol did not fulfill in log P value. Whereas mangiferin have 3 parameters which did not meet the rule.

Chemical databases contain many of molecules that could be suitable ligands for an enzyme. However, no matter how good the fit with the protein target, the candidate molecule is of no use if the absorption is poor or if the drug is eliminated too slowly from the body. The World Drugs Index database were analysed and it was concluded that a compound is more likely to have poor absorption or permeability if the molecular weight exceeds 500; the calculated octanol/water partition coefficient (log P) exceeds +5; there are more than 5 H-bond donors (HBD) expressed as the sum of O–H and N–H groups; and there are more than 10 H-bond acceptors (HBA) expressed as the sum of N and O atoms. The above analysis is called the Lipinski Rules of Five because all values are multiples of five [23]

Based on Table 2, this means that only chrysoeriol meet the Lipinski Rules of Five completely, meanwhile four others did not fulfill these rule [23]. Hence, it can be predicted that chrysoeriol will be easily absorbed and have high permeability.

Table 3. Prediction of pharmacokinetic properties of active compounds in *U.lobata* leaf extracts

	Absorption		Distribution		Metabolism		Excretion	
Active compounds	Intestinal absorption (%)	Skin Permeability (cm/h)	VdSS (L/kg)	Fraction Unbound	CYP2D6 inhibitor	CYP3A4 inhibitor	Total Clearance	Renal OCT2 substrate
Stigmasterol	94.970	-2.783	0.178	0	No	No	0.618	No
Sitosterol	94.464	-2.783	0.193	0	No	No	0.628	No
Mangiferin	46.135	-2.735	1.364	0.289	No	No	0.347	No
Gossypetin	68.009	-2.735	1.552	0.234	No	No	0.304	No
Chrysoeriol	82.844	-2.735	0.741	0.070	No	No	0.597	No

VdSS; (log ml/L)

Stigmasterol, β-Sitosterol and chrysoeriol have a high intestinal absorption percentage in oral administration. Meanwhile, five compound which were identified have a same value of log Kp value (> 2.5 cm/h) and indicated a good skin permeability. Mangiferin and gosypetin have VdSS value higher than other substances. Fraction unbound was showed by mangiferin and gossypetin about 20 %, meanwhile other compound in binding form absolutely. All of active compound did not indicate the inhibitory activity on both of CYP2D6 and CYP3A4. Stigmasterol and β-Sitosterol have a high total clearance and five substance not renal OCT2 substrate.

Skin permeability is an important consideration for improving drug efficacy that is particularly of interest in the development of transdermal drug delivery. A molecule will barely penetrate the skin if log Kp is more than -2.5 cm/h [24]. From Table 2 it can be seen that the skin permeability (Kp) of active substances range from -2.678 to -2.992 cm/h (< -2.5). Therefore, it can be predicted that all derivatives have a good skin penetrability.

The volume of distribution (VD) is the calculated volume that the whole quantity of a medicine will be circulated at an equal level of blood plasma. The higher the VD is, the larger the amount of a drug is distributed to tissue rather than plasma. This model is established from the estimation of the steady-state volume of distribution (VDss), which is then revealed as log L/kg. According to Pires et al. [24], VDss higher than 2.81 L/kg (log VDss > 0.45) is categorized as high, whereas VDss lower than 0.71 L/kg (log VDss < -0.15)

is categorized as low [8]. From Table 3 it can be seen that the VDss values of active compound range from -0.178 to 1.552, moreover it can be predicted that all active compounds can be distributed evenly providing an equal level of blood plasma

Cytochrome P-450 is an important detoxification enzyme in the body, mainly found in the liver. It oxidizes xenobiotics to facilitate their excretion. Many drugs are deactivated by cytochrome P450's but some can be activated by it. Inhibitors of this enzyme, such as grapefruit juice, can affect drug metabolism and are contraindicated. The cytochrome P450's is responsible for the metabolism of many drugs. However, inhibitors of the P450's can dramatically alter the pharmacokinetics of these drugs, therefore it is important to evaluate whether a given compound is likely to be a cytochrome P450 substrate. The two main isoforms responsible for drug metabolism are P2D6 cytochrome (CYP2D6) and P3A4 cytochrome (CYP3A4) [24]. From Table 3 it can be seen that almost all active substances do not affect or inhibit the CYP2D6 and CYP3A4 enzymes, moreover it can be predicted that the substances tend to be metabolized by the P450 enzymes in the body.

Organic cation transporter 2 (OCT2) is a protein transporter that has a vital contribution in renal uptake, disposition, and clearance of drugs and endogenous compounds. OCT2 substrates have potential for adverse interactions with codirected OCT2 inhibitors. Evaluating the transfer of a candidate compound by OCT2 offers useful information regarding not only its clearance but also potential contraindications [24]. From Table 3 it can be seen that all active compound does not affect the OCT2 substrate, so it can be predicted that the active compounds are not OCT2 substrates.

Molecular docking of *U.lobata* on COX-2, LOX-5 and PLA2

Activity of active compound from *U.lobata* leaf extracts both of on COX-2, LOX-5 and PLA2 were evaluated by *in-silico* approach and the results can be seen at Table 4, 5 and 6. The enzymes or protein target have a role in the production of cytokine pro inflammatory meanwhile several studies used them to evaluate the activity of anti-inflammation. The inhibitory activity of active substances against the enzyme indicated the potential anti-inflammatory of herbs.

Table 4. Molecular docking of active substances of *U.lobata* leaf extracts with COX-2

No	Active compounds	Est. Free Energy of Binding (Kcal/mol)	Est. Inhibition Constant Ki	Interact. Surface
1	Stigmasterol	-9.64	11.07 nM	955.50
2	β-Sitosterol	-7.01	15.48 nM	948.83
3	Mangiferin	-7.33	42.97 µM	841.18
4	Gossypetin	-6.84	8.53 µM	644.38
5	Chrysoeriol	-6.32	3.31 µM	526.63
6	Asetosal	-4.37	304.96 nM	488.49

Table 5. Molecular docking of active substances of *U.lobata* leaf extracts with LOX-5

No	Active compounds	Est. Free Energy of Binding (Kcal/mol)	Est. Inhibition Constant Ki	Interact. Surface
1	Stigmasterol	1.06	0.00	696.70
2	β -Sitosterol	0.98	0.00	679.05
3	Mangiferin	-1.06	166.79 mM	593.69
4	Gossypetin	-0.41	498.88 mM	499.92

5	Chrysoeriol	0.14	0.00	518.85
6	Asetosal	-4.78	311.52 µM	364.69

Table 6. Molecular docking of active substances of *U.lobata* leaf extracts with PLA2

No	Active compounds	Est. Free Energy of Binding (Kcal/mol)	Est. Inhibition Constant Ki (µM)	Interact. Surface
1	Stigmasterol	-7.33	4.23	703.94
2	β -Sitosterol	-7.15	5.76	674.86
3	Mangiferin	-5.83	53.15	478.32
4	Gossypetin	-4.75	331.32	536.81
5	Chrysoeriol	-5.18	160.72	500.58
6	Dexamethasone	-5.15	167.04	530.29

Molecular docking study indicated that stigmasterol and β-sitosterol of *U. lobata* have a strong activity as anti-inflammatory based on the estimation of inhibition constant (Ki) value against PLA2 and COX-2. For stigmasterol and β-sitosterol, their activity on both of PLA2 and COX-2 more potent than reference drug. Meanwhile, mangiferin and gossypetin have a stronger anti-inflammatory effect on LOX-5 among others compound. However, their potency is lower compare to reference drugs. Stigmasterol and β-sitosterol showed the low Ki value due to they have the low free energy of binding to protein target. Therefore, they have a strong binding between ligand and protein target. It is supported also by a high interaction surface between them.

In this study, stigmasterol has the highest score of surface interaction followed by β-sitosterol and mangiferin. A great result of surface interaction indicated a stronger binding between ligand and protein target, moreover the biology activity is higher. The lowest score of binding free energy produces a strong binding molecule and biology activity. Free energy binding and surface interaction between ligand and protein target influences the inhibitory activity of *U. lobata* leaf extract on COX-2 and PLA2. On the other hand, molecular docking on LOX-5 indicated the low free energy binding score for mangiferin and gossypetin, therefore, it results a low inhibition constant.

Recently, molecular docking is used to discover new ligands for target of known structure. Potential compound can be observed by free energy binding score. It indicates binding affinity of ligand to target protein, the lower free energy binding results the higher binding affinity [25]. In addition, inhibition constant can be predicted using bioinformatics approach. The lowest score of inhibition constant shows the most potential compound. Other parameter is surface interaction, it represents the molecular recognition between ligand and target protein. The higher score of surface interaction, the higher interaction possibilities of compounds interacting with the target protein [26].

Conclusion

Based on in silico study, *U. lobata* leaf has anti-inflammatory activity through inhibition on COX-2 greater than on PLA2 and LOX-5, the prediction of ADME also indicated good properties.

Acknowledgment

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In Silico Study of Pulutan (*Urena lobata*) Leaf Extract as Anti Inflammation and their ADME Prediction

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Abstract

Inflammation is the basic for the pathogenesis of several diseases both of degenerative and non-degenerative disease. *Urena lobata* is a medicinal plant that can be found in Indonesia and has been used traditionally to cure influenza, inflammation and fever. However, there have been few reports about their anti-inflammatory activity and their mechanism action are still unclear. The aim of study to evaluate the anti-inflammatory activities of active substances from *U.lobata* leaf and their pharmacokinetic property through *in silico* study. *U. lobata* leaf was extracted by digeration methods using ethanol solvent. Therefore, the active substances in the extract was analyzed by Liquid Chromatography-Mass Spectra (LC-MS). Pharmacokinetic property and physicochemical of active compounds were evaluated using pkCSM online tool. Anti-inflammatory activity of *U. lobata* active compound on phospholipase-A2 (PLA-2), cyclooxygenase-2 (COX-2) and lipoxygenase-5 (LOX-5) were evaluated by *in silico* study. Ethanolic extract of *U. lobata* contained five active compound, there are stigmasterol, β-sitosterol mangiferin, gossypetin and chrysoeriol. Molecular docking study indicated stigmasterol and β-sitosterol of *U. lobata* have a strong activity as anti-inflammatory based on the estimation of inhibition constant (Ki) value against PLA2 and COX-2. Meanwhile, mangiferin and gossypetin have a stronger anti-inflammatory effect on LOX-5 among others compound. *U. lobata* has anti-inflammatory activity through inhibition on COX-2 greater than on PLA2 and LOX-5.

Keywords: *anti-inflammation, pharmacokinetic, molecular docking, urena lobata*

Introduction

Inflammation is the immune system's response to tissue damage caused by physical trauma, chemical and microbiological substances [1]. One of the factors that contribute to inflammation condition is the metabolic product of arachidonic acid. The substance is commonly found in phospholipid cell membrane and used to stabilize liquid of them. When the cell membranes damaged, the phospholipase A2 enzyme (PLA2) is activated to convert phospholipids into arachidonic acid (AA). Therefore, it is converted by cyclooxygenase-2 enzyme (COX-2) into prostaglandins, prostacycline and thromboxane. They are cytokine pro-inflammatory contributing on immune defense system and inflammation process also. On the other hand, AA is converted by 5-lipoxygenase enzyme (LOX5) to leukotriene as inflammation mediators [1,2].

The mechanism action of conventional drugs as anti-inflammatory mostly through the inhibitory activity of COX-2 and PLA2, meanwhile the inhibition against LOX-5 is still limited. Synthetic anti-inflammatory drugs often cause some adverse drug reaction such as stomach pain, heartburn, nausea, vomiting, diarrhea and the others [3]. Moreover, searching another alternative medicine which less side effect is needed to overcome the problems. Herbal medicine is one of sources of drug substances which can be explored to treat the diseases.

One of the herbs that is often used to treat inflammation traditionally is Pulutan (*Urena lobata*) leaf. Pre-clinical study indicated anti-inflammatory activity, antioxidant and antimicrobial of this herbs. [4,5]. Other study showed *U. lobata* inhibit the increase of free radical such as superoxide radical, hydroxyl radical and lipid peroxidation due to a flavonoid and phytosterol rich content [5,6]. However, there have been few reports about their anti-inflammatory activity especially their mechanism action.

In silico study is performed by molecular docking to predict its activity with the selected target cell. Docking is an attempt to harmonize the ligand, a protein inside the target cell such as receptor or enzyme [7]. Besides measuring docking activity, one of the principle stages in drug design is the evaluation of the pharmacokinetic properties. Pharmacokinetic analysis using an animal model is expensive, therefore we use a molecular modelling in order to evaluate both of the chemical properties and pharmacokinetic (ADME) [8,9,10]. The objective of the study was to evaluate anti-inflammatory activity of *U. lobata* by *in silico* approach and their pharmacokinetic properties.

Experimental section

Sampel preparation

Simplisia of *U. lobata* leaves were obtained from Balai Materia Medika Batu, Malang, Indonesia with certificate number 074/027/101.8/2015. Therefore, 50 g of the herbs materials was extracted in 250 ml ethanol 80 % for 4 hours using water bath shacker and it was repeated 2 times using fresh solvent. The extract is evaporated to produce a paste form and dilute with solvent according to concentration which have been fixed.

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Identification of active substances in *U.lobata* leaf extracts

The active compounds from ethanolic extract of *U.lobata* leaf can be seen in the figure 1 and table 1.

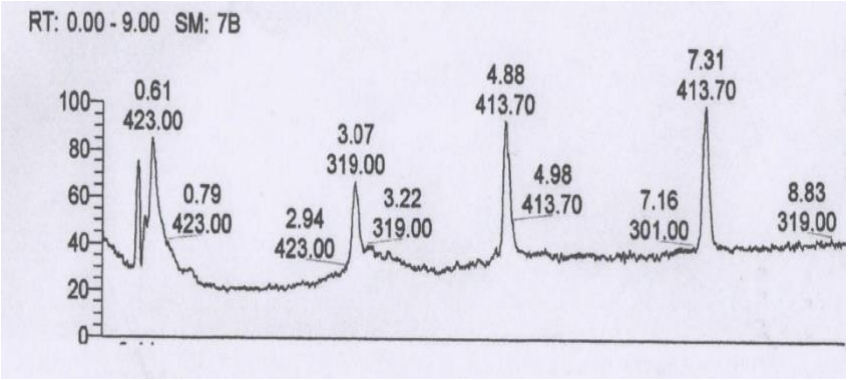

Figure 1. Chromatogram of of active compound in ethanolic extract of *U. lobata*

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The qualitative analysis using LC-MS indicated that the most abundant of active substances from *U. lobata* leaf extract were stigmasterol, mangiferin and gossypetin. Active compounds such as β-sitosterol and chrysoeriol were found also in the extracts of *U.lobata,* however the concentration was low.

All of them are classified into secondary metabolite groups and have biological activity, therefore can be used to cure diseases. Stigmasterol is one of a group of phytosterols that are

chemically similar to animal cholesterol. Phytosterols are insoluble in water, however soluble in most organic solvents. Animal studies treated by stigmasterol showed both of cholesterol and sitosterol absorption decreased 23% and 30%, respectively, over a 6-week period. It also possesses potential antioxidant, hypoglycemic and thyroid inhibiting properties [5,6]. Gossypetin is flavonol or flavone, a type of flavonoid. Gossypetin indicates potential antioxidant, antimicrobial, anti-mutagenic and anti-atherosclerotic activities [13]. This compound is very soluble in chloroform and benzene, and also moderately soluble in ethanol and ether, but insoluble in water. β-sitosterol is one of several phytosterols or plant sterols with chemical structure similar to that of cholesterol. Sterols are isoprenoid-derived molecules that have essential functions typically in eukaryotes, and especially in higher plants. β-sitosterol are white, waxy powder with characteristic odor, soluble in ethanol and chloroform but insoluble in water [14]. It showed anti-cholesterol, anti-inflammatory and immunomodulator effects [15]. Mangiferin is a xanthonoid, and a glucoside of norathyriol. Mangiferin is soluble in hot diluted ethanol and methanol but insoluble in water. Laboratory study has identified a variety of pharmacology effect that associated with mangiferin including anti-microbial, antioxidant activity, and anti-diabetic effect in rodent [16,17]. Chrysoeriol is a flavon, one of major flavonoid classes. They exhibit many activities such as anti-inflammation and antihistamine activities. It is soluble in alkalies solution and sufficiently soluble in water [18]. The presence of active compounds in the extract was influenced by polarity and extract solvent. Type of extract solvent impacts the amounts of active compounds in extract due to the difference of their solubility in solvent. Secondly, polarity of active compound also contributes to their solubility in solvent. Alkaloid, terpenoid and steroid are soluble in non- polar solvent like acetone, diethyl ether and hexane. Meanwhile, flavonoid, phenol and glycoside dissolve in polar solvent such as water and methanol [19,20]. It is appropriate with the determinate solubility theory **"**like dissolve like**"** that polar substances will dissolve in polar solvent and vice versa [19,21].

Non-nutrition compounds or secondary metabolite like alkaloid, terpenoid, flavonoid and steroid are found in a small concentration but it has pharmacology effect at certain dose [19]. Secondary metabolites are derived from metabolism of primary metabolite in plant but sometimes they have a toxic effect especially if it is used in high dose. Most of flavonoid and terpenoid in herbs have a potency as antioxidant, antiseptic and anti-inflammatory whereas steroid as anti-inflammatory and sex hormone. However, the pharmacology effect of alkaloid is difficult to be predicted in medicinal plants because they have so many biological activities [22].

Prediction of physicochemical property and pharmacokinetic of *U.lobata* leaf extract

The results of the *in silico* study of the physicochemical properties of *U. lobata* active compound can be seen in Table 2.

Table 2. Prediction of physichochemical properties of active compounds in *U.lobata* leaf extracts

Active compounds	MW	Log P	Torsion	HBA	HBD	PSA (A^2)	Water Solubility
Stigmasterol	412.702	7.801	5	1	1	186.349	-6.682
β-Sitosterol	414.718	8.025	6	1	1	187.039	-6.773
Mangiferin	422.342	-0.717	2	11	8	166,412	-2.918
Gossypetin	318.237	1.694	1	8	6	126.902	-2.900
Chrysoeriol	300.266	2.585	2	6	3	123.998	-3.237

MW=Molecular weight; LogP=logarithm of octanol/water partition coefficient; Torsion= bond between rotating atoms; HBA=H-bond acceptors; HBD=H-bond donors; PSA=polar surface activity

It can be seen that the molecular weight values of the active compound ranged from 300 to 422 (<500), the value of log of the octanol/water partition coefficient (log P) ranged from -0.717 to 8.025 (> 5), the amount of HBD ranged from 1 to 8 (>5), and the amount of HBA ranged from 1 to 11 (>10). Only chrysoeriol meet Lipinski Rules of Five completely. Meanwhile, gossypetin did not meet in amount of HBD and both of stigmasterol and β-Sitosterol did not fulfill in log P value. Whereas mangiferin have 3 parameters which did not meet the rule.

Chemical databases contain many of molecules that could be suitable ligands for an enzyme. However, no matter how good the fit with the protein target, the candidate molecule is of no use if the absorption is poor or if the drug is eliminated too slowly from the body. The World Drugs Index database were analysed and it was concluded that a compound is more likely to have poor absorption or permeability if the molecular weight exceeds 500; the calculated octanol/water partition coefficient (log P) exceeds +5; there are more than 5 H-bond donors (HBD) expressed as the sum of O–H and N–H groups; and there are more than 10 H-bond acceptors (HBA) expressed as the sum of N and O atoms. The above analysis is called the Lipinski Rules of Five because all values are multiples of five [23]

Based on Table 2, this means that only chrysoeriol meet the Lipinski Rules of Five completely, meanwhile four others did not fulfill these rule [23]. Hence, it can be predicted that chrysoeriol will be easily absorbed and have high permeability.

Table 3. Prediction of pharmacokinetic properties of active compounds in *U.lobata* leaf extracts

	Absorption		Distribution		Metabolism		Excretion	
Active compounds	Intestinal absorption (%)	Skin Permeability (cm/h)	VdSS (L/kg)	Fraction Unbound	CYP2D6 inhibitor	CYP3A4 inhibitor	Total Clearance	Renal OCT2 substrate
Stigmasterol	94.970	-2.783	0.178	0	No	No	0.618	No
Sitosterol	94.464	-2.783	0.193	0	No	No	0.628	No
Mangiferin	46.135	-2.735	1.364	0.289	No	No	0.347	No
Gossypetin	68.009	-2.735	1.552	0.234	No	No	0.304	No
Chrysoeriol	82.844	-2.735	0.741	0.070	No	No	0.597	No

VdSS; (log ml/L)

Stigmasterol, β-Sitosterol and chrysoeriol have a high intestinal absorption percentage in oral administration. Meanwhile, five compound which were identified have a same value of log Kp value (> 2.5 cm/h) and indicated a good skin permeability. Mangiferin and gosypetin have VdSS value higher than other substances. Fraction unbound was showed by mangiferin and gossypetin about 20 %, meanwhile other compound in binding form

absolutely. All of active compound did not indicate the inhibitory activity on both of CYP2D6 and CYP3A4. Stigmasterol and β-Sitosterol have a high total clearance and five substance not renal OCT2 substrate.

Skin permeability is an important consideration for improving drug efficacy that is particularly of interest in the development of transdermal drug delivery. A molecule will barely penetrate the skin if log Kp is more than -2.5 cm/h [24]. From Table 2 it can be seen that the skin permeability (Kp) of active substances range from -2.678 to -2.992 cm/h (< -2.5). Therefore, it can be predicted that all derivatives have a good skin penetrability.

The volume of distribution (VD) is the calculated volume that the whole quantity of a medicine will be circulated at an equal level of blood plasma. The higher the VD is, the larger the amount of a drug is distributed to tissue rather than plasma. This model is established from the estimation of the steady-state volume of distribution (VDss), which is then revealed as log L/kg. According to Pires et al. [24], VDss higher than 2.81 L/kg (log VDss > 0.45) is categorized as high, whereas VDss lower than 0.71 L/kg (log VDss < -0.15) is categorized as low [8]. From Table 3 it can be seen that the VDss values of active compound range from -0.178 to 1.552, moreover it can be predicted that all active compounds can be distributed evenly providing an equal level of blood plasma

Cytochrome P-450 is an important detoxification enzyme in the body, mainly found in the liver. It oxidizes xenobiotics to facilitate their excretion. Many drugs are deactivated by cytochrome P450's but some can be activated by it. Inhibitors of this enzyme, such as grapefruit juice, can affect drug metabolism and are contraindicated. The cytochrome P450's is responsible for the metabolism of many drugs. However, inhibitors of the P450's can dramatically alter the pharmacokinetics of these drugs, therefore it is important to evaluate whether a given compound is likely to be a cytochrome P450 substrate. The two main isoforms responsible for drug metabolism are P2D6 cytochrome (CYP2D6) and P3A4 cytochrome (CYP3A4) [24]. From Table 3 it can be seen that almost all active substances do not affect or inhibit the CYP2D6 and CYP3A4 enzymes, moreover it can be predicted that the substances tend to be metabolized by the P450 enzymes in the body.

Organic cation transporter 2 (OCT2) is a protein transporter that has a vital contribution in renal uptake, disposition, and clearance of drugs and endogenous compounds. OCT2 substrates have potential for adverse interactions with codirected OCT2 inhibitors. Evaluating the transfer of a candidate compound by OCT2 offers useful information regarding not only its clearance but also potential contraindications [24]. From Table 3 it can be seen that all active compound do not affect the OCT2 substrate, so it can be predicted that the active compound are not OCT2 substrates.

Molecular docking of *U.lobata* on COX-2, LOX-5 and PLA2

Activity of active compound from *U.lobata* leaf extracts both of on COX-2, LOX-5 and PLA2 were evaluated by *in-silico* approach and the results can be seen at Table 4, 5 and 6.

Table 4. Molecular docking of active substances of *U.lobata* leaf extracts with COX-2

No	Active compounds	Est. Free Energy of Binding (Kcal/mol)	Est. Inhibition Constant Ki	Interact. Surface
1	Stigmasterol	-9.64	11.07 nM	955.50
2	β-Sitosterol	-7.01	15.48 nM	948.83
3	Mangiferin	-7.33	42.97 µM	841.18
4	Gossypetin	-6.84	8.53 µM	644.38
5	Chrysoeriol	-6.32	3.31 µM	526.63
6	Asetosal	-4.37	304.96 nM	488.49

Table 5. Molecular docking of active substances of *U.lobata* leaf extracts with LOX-5

No	Active compounds	Est. Free Energy of Binding (Kcal/mol)	Est. Inhibition Constant Ki	Interact. Surface
1	Stigmasterol	1.06	0.00	696.70
2	β -Sitosterol	0.98	0.00	679.05
3	Mangiferin	-1.06	166.79 mM	593.69
4	Gossypetin	-0.41	498.88 mM	499.92
5	Chrysoeriol	0.14	0.00	518.85
6	Asetosal	-4.78	311.52 µM	364.69

Table 6. Molecular docking of active substances of *U.lobata* leaf extracts with PLA2

No	Active compounds	Est. Free Energy of Binding (Kcal/mol)	Est. Inhibition Constant Ki (µM)	Interact. Surface
1	Stigmasterol	-7.33	4.23	703.94
2	β -Sitosterol	-7.15	5.76	674.86
3	Mangiferin	-5.83	53.15	478.32
4	Gossypetin	-4.75	331.32	536.81
5	Chrysoeriol	-5.18	160.72	500.58
6	Dexamethasone	-5.15	167.04	530.29

Molecular docking study indicated that stigmasterol and β-sitosterol of *U. lobata* have a strong activity as anti-inflammatory based on the estimation of inhibition constant (Ki) value against PLA2 and COX-2. For stigmasterol and β-sitosterol, their activity on both of PLA2 and COX-2 more potent than reference drug. Meanwhile, mangiferin and gossypetin have a stronger anti-inflammatory effect on LOX-5 among others compound. However, their potency is lower compare to reference drugs. Stigmasterol and β-sitosterol showed the low Ki value due to they have the low free energy of binding to protein target. Therefore, they have a strong binding between ligand and protein target. It is supported also by a high interaction surface between them.

In this study, stigmasterol has the highest score of surface interaction followed by β-sitosterol and mangiferin. A great result of surface interaction indicated a stronger binding between ligand and protein target, moreover the biology activity is higher. The lowest score of binding free energy produces a strong binding molecule and biology activity. Free energy binding and surface interaction between ligand and protein target influences the inhibitory activity of *U. lobata* leaf extract on COX-2 and PLA2. On the other hand, molecular docking

on LOX-5 indicated the low free energy binding score for mangiferin and gossypetin, therefore, it results a low inhibition constant.

Recently, molecular docking is used to discover new ligands for target of known structure. Potential compound can be observed by free energy binding score. It indicates binding affinity of ligand to target protein, the lower free energy binding results the higher binding affinity [25]. In addition, inhibition constant can be predicted using bioinformatics approach. The lowest score of inhibition constant shows the most potential compound. Other parameter is surface interaction, it represents the molecular recognition between ligand and target protein. The higher score of surface interaction, the higher interaction possibilities of compounds interacting with the target protein [26].

Conclusion

Based on in silico study, *U. lobata* leaf has anti-inflammatory activity through inhibition on COX-2 greater than on PLA2 and LOX-5, the prediction of ADME also indicated good properties.

Acknowledgment

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In Silico Study of Pulutan (*Urena lobata*) Leaf Extract as Anti Inflammation and their ADME Prediction

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Abstract

Inflammation is the basic for the pathogenesis of several diseases both of degenerative and non-degenerative disease. *Urena lobata* is a medicinal plant that can be found in Indonesia and has been used traditionally to cure influenza, inflammation and fever. However, there have been few reports about their anti-inflammatory activity and their mechanism action are still unclear. The aim of study to evaluate the anti-inflammatory activities of active substances from *U.lobata* leaf and their pharmacokinetic property through *in silico* study. *U. lobata* leaf was extracted by digeration methods using ethanol solvent. Therefore, the active substances in the extract was analyzed by UHPLC. Pharmacokinetic property and physicochemical of active compounds were evaluated using pkCSM online tool. Anti-inflammatory activity of *U. lobata* active compound on phospholipase-A2 (PLA-2), cyclooxygenase-2 (COX-2) and lipoxygenase-5 (LOX-5) were evaluated by *in silico* study. Ethanolic extract of *U. lobata* contained five active compounds, there are stigmasterol, β-sitosterol mangiferin, gossypetin and chrysoeriol. Molecular docking study indicated stigmasterol and β-sitosterol of *U. lobata* have a strong activity as anti-inflammatory based on the estimation of inhibition constant (Ki) value against PLA2 and COX-2. Meanwhile, mangiferin and gossypetin have a stronger anti-inflammatory effect on LOX-5 among others compound. *U. lobata* has anti-inflammatory activity through inhibition on COX-2 greater than on PLA2 and LOX-5.

Keywords: anti-inflammation, pharmacokinetic, molecular docking, *Urena lobata*

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1 Introduction

Inflammation is the immune system's response to tissue damage caused by physical trauma, chemical and microbiological substances [1]. One of the factors that contribute to inflammation condition is the

metabolic product of arachidonic acid. The substance is commonly found in phospholipid cell membrane and used to stabilize liquid of them. When the cell membranes damaged, the phospholipase A2 enzyme (PLA2) is activated to convert phospholipids into arachidonic acid (AA). Therefore, it is converted by cyclooxygenase-2 enzyme (COX-2) into prostaglandins, prostacycline and thromboxane. They are cytokine pro-inflammatory contributing on immune defense system and inflammation process also. On the other hand, AA is converted by 5-lipoxygenase enzyme (LOX5) to leukotriene as inflammation mediators [1,2].

The mechanism action of conventional drugs as anti-inflammatory mostly through the inhibitory activity of COX-2 and PLA2, meanwhile the inhibition against LOX-5 is still limited. Synthetic anti-inflammatory drugs often cause some adverse drug reaction such as stomach pain, heartburn, nausea, vomiting, diarrhea and the others [3]. Moreover, searching another alternative medicine which less side effect is needed to overcome the problems. Herbal medicine is one of sources of drug substances which can be explored to treat the diseases.

One of the herbs that is often used to treat inflammation traditionally is Pulutan (*Urena lobata*) leaf. Pre-clinical study indicated anti-inflammatory activity, antioxidant and antimicrobial of this herbs. [4,5]. Other study showed *U. lobata* inhibit the increase of free radical such as superoxide radical, hydroxyl radical and lipid peroxidation due to a flavonoid and phytosterol rich content [5,6]. However, there have been few reports about their anti-inflammatory activity especially their mechanism action.

In silico study is performed by molecular docking to predict its activity with the selected target cell. Docking is an attempt to harmonize the ligand, a protein inside the target cell such as receptor or enzyme [7]. Besides measuring docking activity, one of the principle stages in drug design is the evaluation of the pharmacokinetic properties. Pharmacokinetic analysis using an animal model is expensive, therefore we use a molecular modelling in order to evaluate both of the chemical properties and pharmacokinetic (ADME) [8,9,10]. The objective of the study was to evaluate anti-inflammatory activity of *U. lobata* by *in silico* approach and their pharmacokinetic properties.

2 Materials and Methods

2.1 Sampel preparation

Simplisia of *U. lobata* leaves were obtained from Balai Materia Medika Batu, Malang, Indonesia with certificate number 074/027/101.8/2015. Therefore, 50 g of the herbs materials was extracted in 250 ml ethanol 80 % for 4 hours using water bath shacker and it was repeated 2 times using fresh solvent. The extract is evaporated to produce a paste form and dilute with solvent according to concentration which have been fixed.

2.2 Identification of active substances

Ethanolic extract of *U. lobata* leaf was performed a qualitative analysis using UHPLC (ACCELLA-1250 Thermo Scientific) and detector MS/MS Triple Q Thermo Finnigan. Mobile phase contains 0.1 % formic acid in methanol and water combination. The identification included the 10 active compounds from phytosterol, flavonoid and alkaloid groups.

2.3 Prediction of physicochemical property and pharmacokinetic

Prediction of the pharmacokinetic properties (ADME: absorption, distribution, metabolism, and excretion) of the active compound was performed using the pkCSM online tool, i.e. firstly the tested compounds and the comparative compound were drawn as 2D molecular structures with ChemBio Draw Ultra and copied into ChemBio 3D Ultra to create a 3D structure, and then stored as * .sdf file or * .pdb files. Secondly, all of the tested compounds and the reference drug were translated into SMILES format using SMILES Translator Online Help [11]. In the SMILES format, the compounds were processed using the pkCSM online tool to predict the ADME [12].

2.4 Molecular docking study

Activity of active substances in *U.lobata* leaf extracts both on PLA-2, COX-2 and LOX-5 were evaluated by *in silico* approach using a web-based software application

(www.dockingserver.com) for ligand molecular docking and protein target. Inhibition constant, free energy of binding and surface interactions were observed by this method to examine their activity on PLA-2, COX-2 and LOX-5.

3 Results and Discussion

3.1 Identification of active substances in U.lobata leaf extracts

The active compounds from ethanolic extract of *U.lobata* leaf can be seen in the figure 1 and table 1.

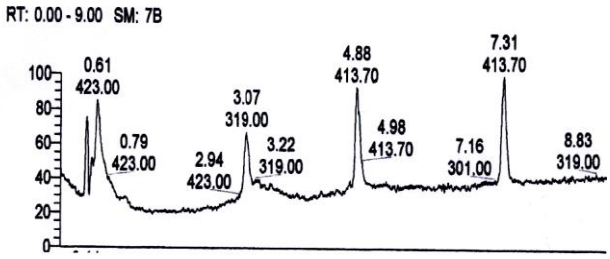

Figure 1. Chromatogram of of active compound in ethanolic extract of *U. lobata*

Table 1. Active compounds in *U.lobata* leaf extracts

No	Active compounds	Molecule weight	Ethanolic extract
1	Stigmasterol	413	(+++)
2	B-Sitosterol	415	(+)
3	Mangiferin	423	(++)
4	Quercetine	303	(-)
5	Kaempferol	286	(-)
6	Hypolaetin	302	(-)
7	Gossypetin	318	(++)
8	Luteolin	286	(-)
9	Apigenin	270	(-)
10	Chrysoeriol	300	(+)

Note : (-) : negative; (+): weak; (++): moderate; (+++); strong

The qualitative analysis using UHPLC indicated that the most abundant of active substances from *U. lobata* leaf extract were stigmasterol, mangiferin and gossypetin. Active compounds such as β-sitosterol and chrysoeriol were found also in the extracts of *U.lobata*, however the concentration was low.

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Non-nutrition compounds or secondary metabolite like alkaloid, terpenoid, flavonoid

and steroid are found in a small concentration but it has pharmacology effect at certain dose [19]. Secondary metabolites are derived from metabolism of primary metabolite in plant but sometimes they have a toxic effect especially if it is used in high dose. Most of flavonoid and terpenoid in herbs have a potency as antioxidant, antiseptic and anti-inflammatory whereas steroid as anti-inflammatory and sex hormone. However, the pharmacology effect of alkaloid is difficult to be predicted in medicinal plants because they have so many biological activities [22].

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Gossypetin	318.237	1.694	1	8	6	126.902	-2.900
Chrysoeriol	300.266	2.585	2	6	3	123.998	-3.237

MW=Molecular weight; LogP=logarithm of octanol/water partition coefficient; Torsion= bond between rotating atoms; HBA=H-bond acceptors; HBD=H-bond donors; PSA=polar surface activity

Table 3. Prediction of pharmacokinetic properties of active compounds in *U.lobata* leaf extracts

Active compounds	Absorption		Distribution		Metabolism		Excretion	
	Intestinal absorption (%)	Skin Permeability (cm/h)	VdSS (L/kg)	Fraction Unbound	CYP2D6 inhibitor	CYP3A4 inhibitor	Total Clearance	Renal OCT2 substrate
Stigmasterol	94.970	-2.783	0.178	0	No	No	0.618	No
Sitosterol	94.464	-2.783	0.193	0	No	No	0.628	No
Mangiferin	46.135	-2.735	1.364	0.289	No	No	0.347	No
Gossypetin	68.009	-2.735	1.552	0.234	No	No	0.304	No
Chrysoeriol	82.844	-2.735	0.741	0.070	No	No	0.597	No

VdSS; (log ml/L)

It can be seen that the molecular weight values of the active compound ranged from 300 to 422 (<500), the value of log of the octanol/water partition coefficient (log P) ranged from -0.717 to 8.025 (> 5), the amount of HBD ranged from 1 to 8 (>5), and the amount of HBA ranged from 1 to 11 (>10). Only chrysoeriol meet Lipinski Rules of Five completely. Meanwhile, gossypetin did not meet in amount of HBD and both of stigmasterol and β-Sitosterol did not fulfill in log P value. Whereas mangiferin have 3 parameters which did not meet the rule.

Chemical databases contain many of molecules that could be suitable ligands for an enzyme. However, no matter how good the fit with the protein target, the candidate molecule is of no use if the absorption is poor or if the drug is eliminated too slowly from the body. The World Drugs Index database were analysed and it was concluded that a compound is more likely to have poor absorption or permeability if the molecular weight exceeds 500; the calculated octanol/water partition coefficient (log P) exceeds +5; there are more than 5 H-bond donors (HBD) expressed as the sum of O–H and N–H groups; and there are more than 10 H-bond acceptors (HBA) expressed as the sum of N and O atoms. The above analysis is called the Lipinski Rules of Five because all values are multiples of five [23]

Based on Table 2, this means that only chrysoeriol meet the Lipinski Rules of Five completely, meanwhile four others did not fulfill these rule [23]. Hence, it can be predicted that chrysoeriol will be easily absorbed and have high permeability.

Stigmasterol, β-Sitosterol and chrysoeriol have a high intestinal absorption percentage in

oral administration. Meanwhile, five compound which were identified have a same value of log Kp value (> 2.5 cm/h) and indicated a good skin permeability. Mangiferin and gosypetin have VdSS value higher than other substances. Fraction unbound was showed by mangiferin and gossypetin about 20 %, meanwhile other compound in binding form absolutely. All of active compound did not indicate the inhibitory activity on both of CYP2D6 and CYP3A4. Stigmasterol and β-Sitosterol have a high total clearance and five substance not renal OCT2 substrate.

Skin permeability is an important consideration for improving drug efficacy that is particularly of interest in the development of transdermal drug delivery. A molecule will barely penetrate the skin if log Kp is more than -2.5 cm/h [24]. From Table 2 it can be seen that the skin permeability (Kp) of active substances range from -2.678 to -2.992 cm/h (< -2.5). Therefore, it can be predicted that all derivatives have a good skin penetrability.

The volume of distribution (VD) is the calculated volume that the whole quantity of a medicine will be circulated at an equal level of blood plasma. The higher the VD is, the larger the amount of a drug is distributed to tissue rather than plasma. This model is established from the estimation of the steady-state volume of distribution (VDss), which is then revealed as log L/kg. According to Pires et al. [24], VDss higher than 2.81 L/kg (log VDss > 0.45) is categorized as high, whereas VDss lower than 0.71 L/kg (log VDss < -0.15) is categorized as low [8]. From Table 3 it can be seen that the VDss values of active compound range from -0.178 to 1.552, moreover it can be predicted that all active compounds can be distributed evenly providing an equal level of blood plasma

Cytochrome P-450 is an important detoxification enzyme in the body, mainly found in the liver. It oxidizes xenobiotics to facilitate their excretion. Many drugs are deactivated by cytochrome P450's but some can be activated by it. Inhibitors of this enzyme, such as grapefruit juice, can affect drug metabolism and are contraindicated. The cytochrome P450's is responsible for the metabolism of many drugs. However, inhibitors of the P450's can dramatically alter the pharmacokinetics of these drugs, therefore it is important to evaluate whether a given compound is likely to be a cytochrome P450 substrate. The two main isoforms responsible for drug metabolism are P2D6 cytochrome (CYP2D6) and P3A4 cytochrome (CYP3A4) [24]. From Table 3 it can be seen that almost all active substances do not affect or inhibit the CYP2D6 and CYP3A4 enzymes, moreover it can be predicted that the substances tend to be metabolized by the P450 enzymes in the body.

Organic cation transporter 2 (OCT2) is a protein transporter that has a vital contribution in renal uptake, disposition, and clearance of drugs and endogenous compounds. OCT2 substrates have potential for adverse interactions with codirected OCT2 inhibitors. Evaluating the transfer of a candidate compound by OCT2 offers useful information regarding not only its clearance but also potential contraindications [24]. From Table 3 it can be seen that all active compound do not affect the OCT2 substrate, so it can be predicted that the active compound are not OCT2 substrates.

3.3 Molecular docking of U.lobata on COX-2, LOX-5 and PLA2

Activity of active compound from *U.lobata* leaf extracts both of on COX-2, LOX-5 and PLA2 were evaluated by *in-silico* approach and the results can be seen at Table 4, 5 and 6.

Table 4. Molecular docking of active substances of *U.lobata* leaf extracts with COX-2

No	Active compounds	Est. Free Energy of Binding (Kcal/mol)	Est. Inhibition Constant Ki	Interact. Surface
1	Stigmasterol	-9.64	11.07 nM	955.50
2	β-Sitosterol	-7.01	15.48 nM	948.83
3	Mangiferin	-7.33	42.97 µM	841.18
4	Gossypetin	-6.84	8.53 µM	644.38
5	Chrysoeriol	-6.32	3.31 µM	526.63
6	Asetosal	-4.37	304.96 nM	488.49

Table 5. Molecular docking of active substances of *U.lobata* leaf extracts with LOX-5

No	Active compounds	Est. Free Energy of Binding (Kcal/mol)	Est. Inhibition Constant Ki	Interact. Surface
1	Stigmasterol	1.06	0.00	696.70
2	β -Sitosterol	0.98	0.00	679.05
3	Mangiferin	-1.06	166.79 mM	593.69
4	Gossypetin	-0.41	498.88 mM	499.92
5	Chrysoeriol	0.14	0.00	518.85
6	Asetosal	-4.78	311.52 μM	364.69

Table 6. Molecular docking of active substances of *U.lobata* leaf extracts with PLA2

No	Active compounds	Est. Free Energy of Binding (Kcal/mol)	Est. Inhibition Constant Ki (μM)	Interact. Surface
1	Stigmasterol	-7.33	4.23	703.94
2	β -Sitosterol	-7.15	5.76	674.86
3	Mangiferin	-5.83	53.15	478.32
4	Gossypetin	-4.75	331.32	536.81
5	Chrysoeriol	-5.18	160.72	500.58
6	Dexamethasone	-5.15	167.04	530.29

The enzymes or protein target have a role in the production of cytokine pro inflammatory meanwhile several studies used them to evaluate the activity of anti-inflammation. The inhibitory activity of active substances against the enzyme indicated the potential anti-inflammatory of herbs.

Molecular docking study indicated that stigmasterol and β-sitosterol of *U. lobata* have a strong activity as anti-inflammatory based on the estimation of inhibition constant (Ki) value against PLA2 and COX-2. For stigmasterol and β-sitosterol, their activity on both of PLA2 and COX-2 more potent than reference drug. Meanwhile, mangiferin and gossypetin have a stronger anti-inflammatory effect on LOX-5 among others compound. However, their potency is lower compare to reference drugs. Stigmasterol and β-sitosterol showed the low Ki value due to they have the low free energy of binding to protein target. Therefore, they have a strong binding between ligand and protein target. It is supported also by a high interaction surface between them.

In this study, stigmasterol has the highest score of surface interaction followed by β-sitosterol and mangiferin. A great result of surface interaction indicated a stronger binding between ligand and protein target, moreover the biology activity is higher. The lowest score of binding free energy produces a strong binding molecule and biology activity. Free energy binding and surface interaction between ligand and protein target influences the inhibitory activity of *U. lobata* leaf extract on COX-2 and PLA2. On the other hand, molecular docking on LOX-5 indicated the low free energy binding score for mangiferin and gossypetin, therefore, it results a low inhibition constant.

Recently, molecular docking is used to discover new ligands for target of known structure. Potential compound can be observed by free energy binding score. It indicates binding affinity of ligand to target protein, the lower free energy binding results the higher binding affinity [25]. In addition, inhibition constant can be predicted using bioinformatics approach. The lowest score of inhibition constant shows the most potential compound. Other parameter is surface interaction, it represents the molecular recognition between ligand and target protein. The higher score of surface interaction, the higher interaction possibilities of compounds interacting with the target protein [26].

4 Conclusions

Based on in silico study, *U. lobata* leaf has anti-inflammatory activity through inhibition on COX-2 greater than on PLA2 and LOX-5, the prediction of ADME also indicated good properties.

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6 Conflicts of Interest

The authors declare no conflict of interest.

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