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Virtual Prediction of Phytosterol Compounds from Pegagan (*Centella asiatica* L.) As Inhibitor for Interleukin-6 (IL-6) to Prevent Rheumatoid Arthritis Disease

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ABSTRACT. *Rheumatoid arthritis (RA) is an autoimmune disease in which the joint area is inflamed which results in swelling, pain and often causes damage to the inside of the joint. Rheumatoid Arthritis is characterized by decreased Interleukin-6 (IL-6), so that Interleukin-6 (IL-6) inhibitors are needed. This strategy is convincing for controlling RA. Pegagan have to play role in overcoming the problem of joint inflammation. Phytosterols are compounds that are commonly found in pegagan and include antioxidants that are beneficial for health. This study aims to determine the mechanism pathway of pegagan's active compounds in the treatment of RA. This study used an in silico approach by analyzing target proteins and active compounds of pegagan through Lipinski rule of five and molecular docking analysis. This study used 3 phytosterol compounds including campesterol. β-sitosterol. and stigmasterol. The control used in this study was the tartaric acid compound whose receptor molecule was interleukin-6 (IL-6) with PDB ID: 1-ALU. The results of the Lipinski rule analysis found that all the test compounds used met the criteria and could be applied as drugs. Based on the results of molecular docking, stigmasterol has a good binding affinity value and is able to exceed the control compound with the highest binding affinity with score of -6.4 kcal/mol. Visualization results show that there is an interaction formed between the active compound and the target protein so that phytosterl compounds have a role that can be an alternative medicine in the prevention and treatment of RA.*

Keywords: *lipinski rule of five, molecular docking, pegagan, phytosterol, rheumatoid arthritis*

INTRODUCTION

Rheumatoid arthritis (RA) is autoimmune disease progressive with inflammation chronic attack system musculoskeletal. However, it can affect organs and systems body overall in a manner characterised by swelling, pain joints as well as destruction network accompanying synovial disorder movement followed by premature death. It is known that RA is chronic and fluctuating disease, so if not done proper and fast handling will cause damage progressive joints, deformity, disability and death [1]. When various method treatments have been done and do not succeed as well as there is reason enough strong, get done action surgery. Type treatment of RA patients generally characteristic orthopaedics, for example, synovectomy, arthrodesis, repair ulnar deviation [2].

Raising awareness about the use of medicines based on more herbal plants. As a culture and heritage nation, Indonesian traditional medicine is necessarily preserved and developed. Development of herbal medicine continues through the use of metabolites potential secondary for development to become medicine. People who like to use ingredients nature also supports such matters because the effect beside him is lower compared to drug synthetic [3].

One material known nature role in overcoming problem inflammation joints is pegagan. Pegagan (*Centella asiatica* L.) is wild plants that have prospect good enough as plant medicine [4]. Pegagan is tropical by area deployment enough wide from plains low to plains high up to 2,500 m above surface sea. Pegagan not cause effect side because can be digested by the body and its toxicity low. Pegagan contain a number of compound bioactive like asiaticoside, a form of glycosides, which are many used in portion drug traditional or herbs, either in form portion nor as material single. The plant pegagan has its own function and contributes to the improvement of the immune system and health [5].

Pegagan contains some bioactive compounds such as phytosterols. Phytosterols are triterpenoids containing a cyclopentane nucleus, perhydrophenanthrene, which consists of three cyclohexane rings and one cyclopentane ring [5]. Phytoterols play an important role for the body in guarding salt balance, controlling metabolism and increasing sexual organ function as well as

differentiating function biological other between type sex. Phytosterols in plants show effect lower cholesterol and anti-carcinogenic [6].

In silico study is research using simulation with a computer equipped with a number device. In general, it was used in activity study about studies of prediction or interaction with something drug in body and or pathogen. Such research expected can reduce risk failure in clinical trials in activity research as usual [7]. This study aims to determine the mechanism pathway of Pegagan's active compounds in the treatment of RA.

RESEARCH METHODS

This research was conducted in the Laboratory and Halal Center University of Islam Malang. The study use *in silico* analysis by conducting an approach to some conditions evident in compounds active of phytosterols are found on the leaves pegagan (*Centella asiatica* L) tested influence to the target protein interleukin-6 (IL-6) of RA by virtual prediction.

Analysis Physicochemistry and Toxicity of Active Compound

Identification of physicochemistry characteristic from bioactive compound of pegagan was obtained form Lipinski Rule Database (http://www.scfbioiitd.res.in/software/drugdesign/lipinski.jsp) and program pkCSM Online Tool is known For ADME and Toxicity analysis (http://biosig.unimelb.edu.au/pkcsm/prediction) including SMILES code for each compound active.

Ligand Preparation

Study This using the ligand obtained from the PubChem Web Server. Compound active used in study is compound steroids from acquired pentium from the PubChem Web Server, steroid compounds have compound derivative among them Campesterol (CID173183), β-Sitosterol (CID222284), and Stigmasterol (CID5280794). The 3D structure of (compounds) as sdf file were downloaded from PubChem database. All bioactive compounds were prepared by Discovery Studio (v21.1.0.20298)

Receptor Protein Preparation

The PDB 3D structure of IL-6 (1ALU) was downloaded from RCSB PDB (https://www.rcsb.org/). Protein was prepared in Discovery Studio (v21.1.0.20298) by removing original ligands, chains and water molecules.

Molecular Docking

Docking between targets with the most potent ligands from mechanism interaction biologics that have analyzed. Approach computational ligand-target docking was applied for analyze structure IL-6 complex as a target. Molecular docking analysis was carried out using Biovia Discovery STUDIO (v21.1.0.20298) with PyRx Software. The results of the docking analysis displayed binding affonity and interaction ligand receptors. First thing to do is input target proteins (receptors) and ligands that have been prepared to in application. Furthermore, after the target protein and ligand are inputted then followed by setting the Grid box. Then you can do molecular docking. The results then saved in PDBQT format for proceed to stage furthermore.

Visualization of Docking Results

Visualization of the interaction of Molecular docking results was carried out using the Discovery Studio Virtualizer (v21.1.0.20298). To see the resulting interactions from molecular docking, next done visualization, 2D and 3D using Discovery studio virtualizers (v21.1.0.20298). The visualization results can show the interactions of amino acids in 2 dimensions and 3 dimensions. Furthermore, the interaction is analyzed by the bond formed between the receptor and the ligand.

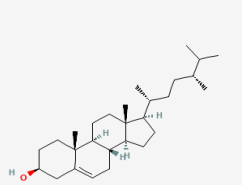
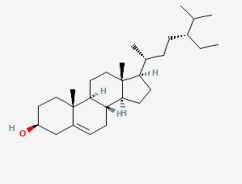
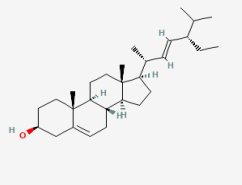
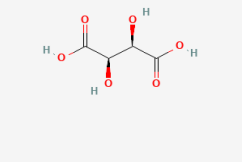
RESULTS AND DISCUSSIONS

Rheumatoid arthritis (RA) is a multifactorial disease, the pathogenesis of which remains heterogeneous. Conventional treatment of RA is associated with serious side effects. Available synthetic metalloproteinase inhibitors, such as collagen peptidomimetics, and non-peptidomimetics have shown unsatisfactory results in clinical trials for the treatment of RA. There is a need to develop drugs that cure the disease with fewer side effects with fewer side effects. Evidence-based interventions shows that medicinal plants are the most potent source of bioactive of bioactive compounds that have the potential to serve as new life-saving life-saving drugs or many diseases [8].

Pegagan has been shown to have anti-inflammatory effects, as observed by the reduction of interleukin1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor α (TNFα), as well as prostaglandin E2 (PGE2) and cyclooxygenase-2 (COX-2). In addition, pegagan has also been shown to reduce inflammation by inhibiting lipoxygenase activity and proteinase activity, thereby inhibiting protein denaturation. This is important because suppression of protein denaturation may improve RA [9].

Based on the results of the Lipinski analysis shown in Table. 1 All test compounds used fulfil Lipinski's rule, i.e. campesterol. β-Sitosterol, Stigmasterol and Tartaric Acid. All the compounds used have heavy molecules not less than 500 g/mol. If a compound owns more molecules than 500 g/mol then influence molecule will diffuse to penetrate the cell membrane. All compounds have less hydrogen acceptors than 10 and less hydrogen donors than 5. The higher the hydrogen bonding capacity, then the higher the energy required for absorption. process. All compounds have A high lipophilicity, greater than 5, will influence the binding to plasma proteins that are carried by the blood throughout the body [10]. Based on Lipinski analysis can be concluded that all compounds used in research are easily absorbed compounds.

Table 1. Lipinski Rule of Five Analysis of phitosterol Compounds in *Centella asiatica*

No	Active Compounds	Molecular Formulas	2D structures	Lipinski's Rule	
				Criteria	Score
1	Campesterol	$C_{28}H_{48}O$		Molecular weight (kDA) Log-P H-bond acceptors H-bond donors Molecular refractivity	400 7.63 1 1 123
2	β-Sitosterol	$C_{29}H_{50}O$		Molecular weight (kDA) Log-P H-bond acceptors H-bond donors Molecular refractivity	414 8.02 1 1 128
3	Stigmasterol	$C_{29}H_{48}O$		Molecular weight (kDA) Log-P H-bond acceptors H-bond donors Molecular refractivity	412 7.80 1 1 128
4	Tartaric Acid	$C_4H_6O_6$		Molecular weight (kDA) Log-P H-bond acceptors H-bond donors Molecular refractivity	144 -4.37 6 0 20

Analysis used in study This is analysis Lipinski and ADMET. This analysis is done for known compound as active as possible made as candidate next drug can be performed clinical trials. On Lipinski's rule, there are a number of a must be noted before performing molecular docking is weight of molecule tested compounds no more than 500 g/mol, H-donor no more than 5 and the H-acceptor no more than 10 medicinal for capable penetrate membrane cell for reach receptor target. Value lipophilicity high (Log-P)<5, and molar refractivity between 40-130. Heavy molecules that are too large will have difficulty penetrating membrane cells because they can interfere with the diffusion process. The more small heavy molecules the drug has, the easier it will diffuse across the cell membrane. Similarly, if the H-donor and H-acceptor values are too high, the more hydrogen bonds formed, the slower the drug will hit the target. Rules that explain that compounds with probabilities as high as drug-like molecules must obey at least two Lipinski rules [10].

Compounds used then done analysis characteristic pharmacology. Predictions characteristic this covers Absorption. Distribution, Metabolism, Excretion, and Toxicity (ADMET) (Table 2). pkCSM online tools are used for test properties of ADME and its toxicity.

Analysis results obtained that compounds used own characteristic good pharmacology that is compound Campesterol. β-Sitosterol, Stigmasterol and Tartaric Acid. Whereas control compound used is tartaric acid has characteristic bad pharmacology. This is because mark absorption. distribution. and metabolism do not meet criteria.

Table 2. Analysis of absorption, distribution, metabolism, excretion, and toxicity (ADMET) of phytosterol compounds in *Centella asiatica*

Part	Criteria	Active Compounds			
		Campesterol	**β-Sitosterol**	**Stigmasterol**	**Tartaric Acid**
Absorption	Intestinal Absorption (%)	94,543	94,464	94.97	3,709
	$CaCO_2$ Permeability (logpapp 10-6 cm/s)	1,223	1,201	1,213	-0.374
Distributions	VDSS (Log L/kg)	0.427	0.193	0.178	-0.857
	BBB Permeability (BB logs)	0.774	0.781	0.771	-0.867
Metabolism	CYP2D6 substrates	No	No	No	No
	CYP2D6 inhibitors	No	No	No	No
Excretion	Total clearances	0.572	0.628	0.618	0.885
	Renal OCT2 substrates	No	No	No	No
Toxicity	LD_{50} (mg/kg)	2.08	2,552	2.54	1,744
	Class	4	4	4	4
	AMES Toxicity	No	No	No	No
	Hepatotoxicity	No	No	No	No

Based on the interstinal absorption data, the test compound has a high own mark absorption which is around 94%. This is in contrast to the control compound which has a mark absorption of 3.709% and is therefore categorised as low. Intestinal absorption is said to be good if own mark absorption >80% and poor if <30%. A high absorption value indicates good absorption in the body and level good absorption in the human intestine [11].

The log-papp value of the tartaric acid compound is -0.374, indicating low permeability in CaCo2 cells, whereas for compounds containing campesterol β-Sitosterol, Stigmasterol and Tartaric Acid have Log-Papp values in the range of 1.201 - 1.223 showing good permeability as the value is > 0.90. This is to predict the absorption of orally administered drugs, permeable CaCO2 monolayer cells are often used as an in vitro model of the intestinal mucosa [11].

The results in Table. 2 state that the VDSS value of the control compound had low value is -0.847 so have a volume of distribution low and experienced distribution evenly with the same concentration as with blood plasma. In contrast to the test compound campesterol. β-Sitosterol, and Stigmasterol, which have VDSS values >3 and therefore have a high volume of distribution. The volume of distribution at steady state (VDSS) is a theoretical volume which states that the total drug dose must be evenly distributed to have a similar concentration to blood plasma. A high VDSS value indicates that more drug is distributed in tissues than in plasma. The volume of distribution is considered low when Log VDSS < -0.15 and high when > 0.45 [12].

Table 2 shows that the compound campesterol, β-Sitosterol, and stigmasterol are included in the good category because their own logBB value > 0.3, it can be predicted that all compounds penetrate the blood-brain barrier in a moderate way. In contrast, the control compound has a low logBB value of <0.3. Ability drug for penetrating barrier blood-brain (blood-brain barrier) is an important parameter that is necessary to help reduce side effects and toxicity or for increasing efficacy active drug pharmacologically is inside the brain. Brain-blood permeability is measured in vivo in animal models as the logBB, which is the ratio of logarithmic concentration in the brain to plasma. Compounds are said to penetrate the blood-brain barrier well if they have a log BB value > 0.3, and may not be well distributed if log BB < -1 [13].

On the Table 2 can be seen that all compound active no hinder not influence CYP2D6 enzymes. so can be predicted that derivative compound that tend to be metabolized by the P450 enzymes involved important in in detoxification body. CYP2D6 is responsible for the metabolism of most drugs and chemical compounds. CYP2D6 is widely distributed in some networks and is the largest found in the liver [14].

Based on Table 2, we can see that all test compounds and compounds control predicted own mark excretion compounds that are not too fast, so compound not cause poisoning in the body. This step is to predict compounds absorbed by the hepatic, biliary and renal as measured by the total excretion (CLtot), which is part of the predicted mark excretion total clearance. Something excretion compound good if heavy the molecule small and hydrophilic If it's heavy molecule possessing height characteristic hydrophobic, so excreted compounds the more small can potentially cause toxicity [15].

From the table it can be seen that all compound active no influence OCT2 substrate. Renal OCT2 (Organic Cation Transporter) substrate is a step beginning in secretion organic

renal glands, therefore it plays a big role important in determining the pharmacokinetics containing medicine. So can be predicted compound derivative no OCT2 substrate, because potential OCT2 substrate increases the effective side when given together with OCT2 inhibitors.

Based on in silico predictions that have been done all compounds own LD50 values ranged between 1.744 - 2.54, meaning compound that is in class 4 (slightly toxic), which means own a little effect toxicity acute. In the AMES mutagenicity test and toxicity in the liver showed all compounds tested active nno showing exists mutagenic compounds as well as toxicity to the liver. The Ames toxicity test is a widely used method for assessing the potency of mutagenic compounds using bacteria. In silico tests can also be performed for oral toxicity to rodents (LD50). The LD50 is the amount of compound that can cause death in 50% of the animals in a group test [16].

Molecular Docking Analysis of Phytosterol Compounds with IL-6

At the stage prediction interaction between phytosterols active compound pegagan of pegagan with an IL-6 based inhibitor analysis molecular docking (Table 3), obtained binding affinity of -5.8 kcal/mol in the compound campesterol and an RMSD value of 12,279 Å. -5.8 kcal/mol for the compound β-sitosterol and RMSD value of 1,775 Å, -6. 4 kcal/mol for the compound stigmasterol and RMSD values of 2.077 Å, and -4.2 kcal/mol for the compounds tartaric acid and RMSD value of 1.541 Å. From the molecular docking data obtained marked highest on stigmasterol, binding affinity value showed more optimal stigmasterol as a compound in RA handler instead of native ligand/control.

Results visualisation of docking results show interaction of internal amino acids 2D and 3D shapes later obtained type bonds that are formed between receptors and ligands (Figure 1).

Following results visualisation results of docking between phytosterols and native ligand with IL-6. Compound active campesterol own type hydrogen bond interactions with bonds acidic amino PRO139, LEU147, LYS150 and type the bond alkyl. Compound active β-Sitosterol own type Hydrogen bond interactions with bonds acid amino LEU 64, LYS66, LEU165, ARG168 and type the bond alkyl. Compound active stigmasterol own type hydrogen bond interactions with bonds sour amino LYS 150, PRO139 and type the bond alkyl. Compound active tartaric acid (native ligand/control) has type hydrogen bond interactions with bonds acidic amino ARG 104, ASP160 and type the bond conventional hydrogen bond.

IL-6 is released in a systemic manner and elicits symptom systemic including fatigue, anaemia and reactions phase acut, IL-6 also induced activation of immunity and triggering improvement activity RA disease. Repair of iron metabolism by IL-6 contributes to anemia associated with chronic inflammation. IL-6 induces hepcidin production, which is a peptide artificial heart, proposed to become centre regulator substance intestinal iron absorption and recycling iron by macrophages. IL-6 also degrades transferrin, which is an iron transporter main delivery iron to bone marrow for erythropoiesis. Because of this, phytosterol compounds in pegagan are predicted to hinder anaemia associated with chronic inflammation. In addition, phytosterol compounds increase insulin resistance and may lower HbA1c levels in diabetic patients with RA. Remember that IL-6 induces serum amyloid A (SAA) protein; prolonged production of IL-6 can increase amyloid A amyloidosis, which is a kidney disease in RA patients. Phytosterol compounds inhibit SAA production and clear amyloid deposits in amyloid A amyloidosis [17].

Table 3. Results of Molecular docking of *Centella asiatica* Phytosterol Compounds with IL -6

No	Name of Compound/Ligand	Binding Affinity (kcal/mol)	Interaction Type
1	Campesterol	-5.8	Hydrogen Bonds: LEU64, LYS66, LEU165, ARG168
2	β-Sitosterol	-5.8	Hydrogen Bonds: PRO139, LEU147, LYS150
3	Stigmasterol	-6.4	Hydrogen Bonds: LYS150. PRO139
4	Tartaric acid (native ligand/control)	-4.2	Hydrogen Bonds: ARG104, ASP160

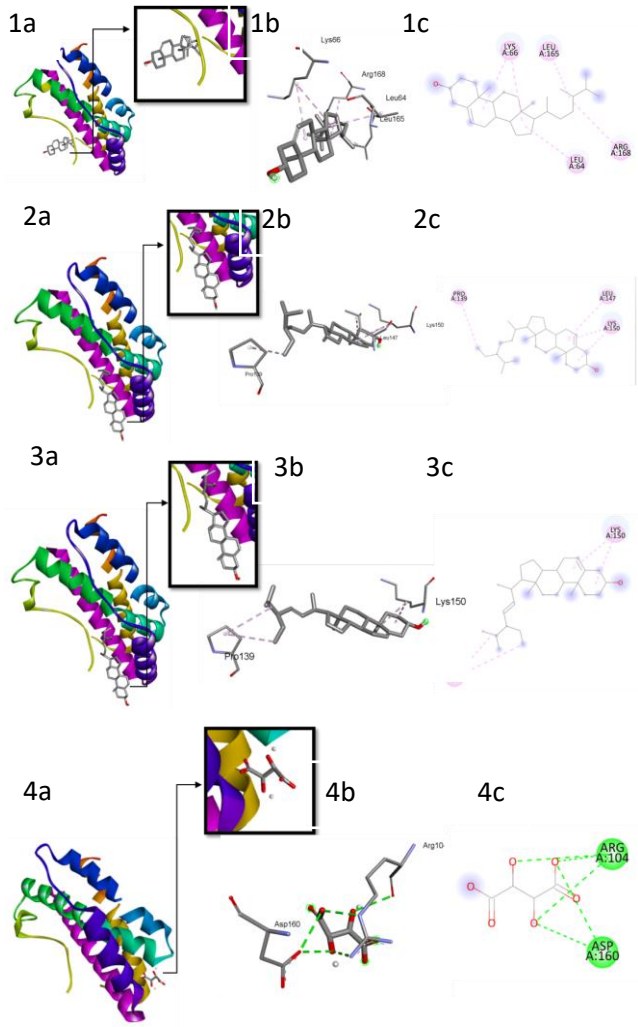


Figure 1. Molecular docking protein simulation results : 1a-c) campesterol; 2a-c) β-Sitosterol; 3a-c) stigmasterol; 4a-c) tartaric acid (native ligand/control)

In addition, IL-6 affects function, psychosomatic symptoms, sleep and fatigue. Because it is a predictable phytosterol compound capable of increasing sleep quality and fatigue. This caused phytosterol compounds can increase the hormone cortisol produced by the body. Symptom sleep disturbance and fatigue to correlate significantly with the decline in human quality of life.

One component of the immune system that plays an important role is cytokine. If the immune system is activated it will cause an increase in cytokine. Very important role cytokines to pathogenicity and progression or severity of RA. RA occurs when T lymphocytes infiltrate the joint, followed by an increase in macrophages and fibroblasts induced by cytokine shedding. Th2 activation produces a number of cytokines: interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6) and interleukin-10 (IL-10) [18].

CONCLUSION

Based on the data, the phytosterol compounds in Pegagan have good physicochemical properties and can be used as a medicine. Molecular docking analysis showed that all the compounds had active values that could exceed the control compound, tartaric acid. The stigmasterol compound is the best compound for RA inhibition compared to other compounds. The stigmasterol compound passes all the tests and has the highest binding affinity. -6.4 kcal/mol and the type of bond formed between the receptor and the ligand, hydrogen bond interaction type with hydrophilic bonds in the amino acids LYS150 (Lysine 150), PRO139 (Proline 139) and alkyl bond type. The phytosterol compounds in pegagan are predicted to increase insulin resistance, reduce HbA1c levels and inhibit the production of SAA, which plays a major role in the development of RA.

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REFERENCES

[1] Masyeni. KMM. Rheumatoid Arthritis. Dalam Rangka Menjalani Kepaniteraan Klinik Madya Di Bagian Ilmu Penyakit Dalam RSUP Sanglah. Udayana University, Bali, **2018**.

[2] Sukmayati. Ni Putu Desi. Gambaran Asuhan Keperawatan Pada Pasien Rheumatoid Arthritis Dengan Pemberian Kompres Hangat Jahe Untuk Menurunkan Nyeri Kronis (Studi Kasus Dilakukan di UPT Kesmas Sukawati 1 Gianyar Tahun 2020). Denpasar Polytechnic, Bali, **2020**.

[3] Nola, F., Putri, GK, Malik, LH, Andriani. N. Isolasi Senyawa Metabolit Sekunder Steroid dan Terpenoid dari 5 Tanaman. *Syntax Idea*. **2021**, 3(7), 1-8.

[4] Sutardi. Kandungan Bahan Aktif Tanaman Pegagan Dan Khasiatnya Untuk Meningkatkan Sistem Imun Tubuh. *Journal R&D Agriculture*. **2016**, 35(3), 121 – 130.

[5] Kartika, W., Lindawati, N.Y., & Nirwana, A.P. Uji Aktivitas Larvasida Ekstrak Herba Pegagan (*Centella asiatica* (L.)) Terhadap Mortalitas Larva *Aedes aegypti* L. *Jurnal Farmasetis*. **2022**, 11(3):251-262.

[6] Maryam, F.. Subehan. Musthainah. L. Isolasi dan Karakterisasi Senyawa Steroid dari Ekstrak Biji Mahoni. *Journal Indonesian Phytopharmacy*. **2020**, 7(2), 6-11.

[7] Mustakim. M., Wulandari. V.F, Khoiriyah. N.M, Mawardi. A., Wulandari. R.M, Fauziah. T.F, Mubarakati. N.J. Investigation Of Terpenoids From Pegagan Based On Network - Target Protein : Study Of Discovery Drug For Alzheimer's Via In Silico. *Journal of Biological Sciences*. **2022**. 9(1), 122-129.

[8] Anjali, P., and Vimalavathini, R. In-silico Molecular Docking of Courmarin and Naphthalene Derivatives from *Pyrenacantha volubilis* with the Pathological Mediators of Rheumatoid Arthritis. *Research J. Pharm and Tech*. **2021**. 14(10):5121-5125.

[9] Lopez, E.A., Zand, N., Ojo, O., Snowden, M.J., and Kochhar, T. A Systematic Review of the Effect of *Centella asiatica* on Wound Healing. *International Journal of Environmental Research and Public Health*. **2022**. 19(3266):1-13

[10] Pratiwi. R.I.A., Rahayu. T. and Mubarakati. N.J. In Silico Screening of *Schleichera oleosa* Phytocompounds as Estrogen Receptors Alpha Inhibitors for Breast Cancer. *JSMARTech*. **2020**, 2(1), 14–21.

[11] Mawardi. A., Wulandari. R.M, Fauziah. T.F, Khoiriyah. N.M, Mustakim. M., Wulandari. V.F, and Mubarakati. N.J. Evaluation Of Physicochemical And Bioactive Toxicity From Mango Seed As A Study In Searching New Drug For Acne In Silico. *International Conference on Multidisciplinary Sciences for Humanity in the Era of Society 5.0. Malang, Indonesia*. **2021**, 5, 488-497.

[12] Maftucha. N., Manalu. RT, Amelia, R., Cordiya. P., & Bupu. R. Potensi Senyawa Turunan Xanton dari Kulit Buah Manggis (*Garcinia mangostana* L.) Sebagai Inhibitor Protein *Mycobacterium tuberculosis*: Studi In Silico. *Pharmaceutical Journal of Indonesia*. **2022**. 7(2), 123–128.

[13] Dwi. DK, Sasongkowati. R. and Haryanto, E. Studi in Silico Sifat Farmakokinetik, Toksisitas, Dan Aktivitas Imunomodulator Brazilein Kayu Secang Terhadap Enzim 3-Chymotrypsin-Like Cysteine Protease Coronavirus. *Journal of Indonesian Medical Laboratory and Science (JoIMedLabS)*. **2020**, 1(1), 76–85.

[14] Hartanti. IPR Utri, AA, Auliya. N. Triadenda. AL, Laelasari. E., Suhandi. C. & Muchtaridi. M.. Molecular Docking Senyawa Xanton, Benzofenon, Dan Triterpenoid Sebagai Antidiabetes Dari Ekstrak Tumbuhan Garcinia Cowa Plant. *Journal of Chemistry*. **2022**, 16(1), 72-80.

[15] Wright, S.H. Molecular and cellular physiology of organic cation transporter 2. *American Journal of Physiology - Renal Physiology*. **2019**, 317(6), 1669–1679.

[16] Cahyani. F.N, Ardiana. N., Khasanah. D.U, Dewi. A.S, & Aiyaningsih. O.R. Pengembangan dan Optimasi Kapsul Mikrosfer Ekstrak Licorice sebagai Bentuk Sediaan Oral Extended Release Kanker Payudara. *Pharmaceutical Journal of Indonesia*. **2021**, 7(1), 63–70.

[17] Ogata, A. Kato, Y., Higa. S., & Yoshizaki. K. IL-6 inhibitor for the treatment of rheumatoid arthritis: A comprehensive review. *Modern Rheumatology*. **2019**, 29(2), 258–267.

[18] Tania, POA, Simamora. D., Parmasari. W. D, & Rahmawati. F. Kadar Interleukin 6 (Il-6) Sebagai Indikator Progresivitas Penyakit Reumatoid Arthritis (RA). *Scientific Medicine*. **2014**, 3, 40–47.