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Attachments: Revision2.docx, Table _Utilisation_Juni Ekowati_JBCPP-revision.docx, Main document_Original_Articles-Juni Ekowati-JBCPP-Revision 240221.docx

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Guest Editor, Journal of Basic and Clinical Physiology and Pharmacology

Herewith, we sent revision of main document original article and revision on table 1 & 2.
Revision of the reference also format according to the author guideline.
With those revisions, hopefully the article can be accepted.

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Best Regards

Dr. Juni Ekowati

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Reviewer(s)' Comments to Author:

Reviewer: 1

Comments to the Author
Abstract
1. Give the full word before abbreviating it to the highlighted word (CVD, ADMET)
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3. in abstract you write that the profiling of its active ingredient was presented by GC-MSD. But in the result was GCMS

Introduction, method, discussion:
4.Give the full word before abbreviating for LDL, ROS, ABTS, ADMET, HPLC, GC-MS
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6.In the discussion, add an explanation of the transdermal requirements so that it can be concluded that the research results are for transdermal
7.Add the references used in the text for Extraction method, Phytochemical screening, Polyphenol Assay, and Quercetin content Assay.
8. The title of table 1 should be made in the middle like tables 2 and 3

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Utilization of domestic waste shallot skins as a source of active pharmaceutical ingredients

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Abstract:	Background: Medicinal plants are a source of many compounds that are useful in the pharmaceutical field for novel drug development. Polyphenols and the flavonoid group in plants are known to have several activities, such as relieving CVD. The outer skin of the shallot which is disposed of as waste is known to have an antiplatelet activity which was tested in vitro. To date, there is no study reported on the ADMET profile and physicochemical properties of the active component of the shallot skins. Methods: The extraction of shallot skins was conducted by ultrasonic irradiation using ethanol.. The phytochemical screenings were carried out by TLC and color reaction. The profiling of its active ingredient was presented by GC-MSD, HPLC and spectrophotometry UV-vis. Whereas their physicochemical properties were analyzed by ChemDraw 17.00 program and the ADMET predictions were studied using pkCSM online tool. The MVD program was operated in the docking study on protein P2Y12 (PDB ID 4PXZ). Results: The extract showed the presence of polyphenol, flavonoids, quercetin, natalensine-3,5-dinitrobenzoate; bis[2- (2-fluorophenyl)-6-fluoroquinolin-4-yl]amine, benzo[a]heptalene, N-(trifluoroacetyl) methyl-N-deacethyl-colchicine. The ADMET prediction data displayed that

the compounds in the extract have good absorption so that they can be used in the oral and transdermal routes. Some components in the extract have lower MDS than clopidogrel.
Conclusion: The ultrasonic shallot skin extract can be used as a new source of the active pharmaceutical ingredients and, to have the potency to be developed as an oral or transdermal preparation.

Keywords: ADMET; polyphenol; pharmacy active ingredients; shallot skin; ultrasonic extraction

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Utilization of domestic waste shallot skins as a source of active pharmaceutical ingredients

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Abstract

Background: Medicinal plants are a source of many compounds that are useful in the pharmaceutical field for novel drug development. Polyphenols and the flavonoid group in plants are known to have several activities, such as relieving CVD. The outer skin of the shallot which is disposed of as waste is known to have an antiplatelet activity which was tested *in vitro* assay. To date, there is no study reported on the ADMET profile and physicochemical properties of the active component of the shallot skins.
Methods: The extraction of shallot skins was conducted by ultrasonic irradiation using ethanol.. The phytochemical screenings were carried out by TLC and color reaction. The profiling of its active ingredient was presented by GC-MSD, HPLC and spectrophotometry UV-vis. Whereas their physicochemical properties were analyzed by ChemDraw 17.00 program and the ADMET predictions were studied using pkCSM online tool. The MVD program was operated in the docking study on protein P2Y12 (PDB ID 4PXZ).
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Conclusion: The ultrasonic shallot skin extract can be used as new source of the active pharmaceutical ingredients and to have the potency to be developed as an oral or transdermal preparation.

Keywords: ADMET; poliphenol; pharmacy active ingredients; shallot skin; ultrasonic extraction

Introduction

Cardiac Vascular Disease (CVD), especially coronary heart disease, is a major cause of death worldwide, and patient medical costs continue to increase due to an increase in the number of sufferers [1, 2]. This disease occurs due to impaired blood flow to the myocardium due to platelet aggregation, thrombus, and the accumulation of oxidative damage to LDL by ROS [1,3]. Oxidant stress causes endothelial dysfunction and thrombus formation [4].

Drugs used to treat coronary heart disease are thrombolytic, anti-platelets and. several antioxidant [5, 6]. Although they can treat coronary heart conditions due to thromboembolism, these drugs also have undesirable side effects such as intracranial bleeding, nausea, dyspnea, and it was reported that the patient had resistance to aspirin as an anti-platelet [7,8]. Therefore, alternative therapies are needed to overcome the above problems with mild side effects.

Medicinal plants are a source of many chemical compounds that are useful in the pharmaceutical field for novel drug development, including polyphenols, the flavonoid class. The flavonoid group are known to have several activities, such as antibacterial and antioxidant [9, 10]. One of the natural ingredients that is widely used in daily food is shallots. Shallots have the active compound i.e. polyphenol quercetin as an antibacterial [11]. Not only the tuber part of the shallot, the outer skin of the shallot which is disposed of as waste is also known to have anti-inflammatory [12] and antimicrobial activity [13, 14]. It was also reported that there is antioxidant activity of the ethanolic extract from shallot skins using the ABTS method [15]. Apart from being antibacterial and antioxidant, the activity of shallot extract as an antiplatelet which was tested in vitro has also been reported by Ro, *et al.* [16]. These things indicate that the shallot skins has the potency as an active pharmaceutical ingredient (API).

Beside the activity, prospective drug compounds also need to be investigated regarding their physicochemical properties and pharmacokinetic profile, including absorption, distribution, metabolism, and excretion as well as its toxicity

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(hereinafter referred to as ADMET) to humans [17]. The pharmacokinetic profile of a drug could been influenced by the physicochemical properties [18] Analysis of the physicochemical properties of drugs in the context of oral bioavailability was first reported by Lipinski, *et al.* which is referred to the "Rule of Five" (Ro5). It includes *Molecular Weight* (MW) <500 g/mol; *Donor H-bond* (HBD) < 5; *H-bond acceptor* (HBA) <10; and the calculation of the logarithm of the partition coefficient of 1-octanol / water (cLogP) <5 [19].

However, until now, there no research on the physicochemical and pharmacokinetics (ADMET) of the active ingredients of shallot skins. The effects of administering the extract on the gastrointestinal tract also need to be studied to ensure its safety in oral use. Therefore, this study aims to find out the component of shallot skin, its physicochemical properties prediction, and its pharmacokinetics (ADMET) prediction.

Pharmacokinetic profile analysis (ADMET) *in silico* is able to be conducted with the help of the online pkCSM program [20]. Prediction with the online pkCSM program has advantages over other software such as SwissADME, since there are more pharmacokinetic parameters that can be predicted with the online pkCSM program [21, 22] The greater number of parameters will have an impact on the broader information obtained to support the next drug development process.

Based on the research of Ro et al. [16] which states that shallot skins extract has anti-platelet activity in vitro, this study also evaluated the inhibition mechanism of the P_2Y_{12} receptor by *in silico* test (PDB ID 4pxz). P2Y12 is a main receptor and the distinctive P2 goal for clinically allowed antiplatelet drugs (herein named as P2Y12 inhibitors) [17,23].

Materials and methods

The waste from shallot skins obtained from traditional markets is collected, cleaned, then dried at room temperature, and powdered using a blender. Previously, the species of shallot skin were examined at the Materia Medica Batu institute, and it was found that the shallot species was *Allium cepa* L.

Extraction

The powder then extracted in ethanol using the ultrasonic method. First, 80 grams of shallot skin powder soaked in 500 ml Erlenmeyer with 350 ml 96% ethanol, then performed ultrasonic at full power and temperature at 40°C for 30 minutes. The extraction product is then filtered using a Buchner funnel under vacuum; the filtrate is accumulated in a different Erlenmeyer. Second, the extracted pulp was put back into the Erlenmeyer 500 ml and added with 300 ml of 96% ethanol. The same process then carried out like the previous process. The extracted filtrate collected and carried out at a rotary evaporator. This ultrasonic extraction was repeated 14 times (until the filtrate did not react with $FeCl_3$, this is indicated by the solution remains clear).

Phytochemical screening

Screening of flavonoid content was carried out by TLC method, using stationary phase silica gel GF254, the mobile phase butanol-acetic acid glasial-water (4:1:5) and ammonia vapor was used as color reagent. While the poliphenol group was detected by solution $FeCl_3$ 2%.

Chromatographic profile

Examination of chemical compounds carried out by GC-MS. The sample was weighed 100 mg, dissolved 2 ml of p.a. ethanol, then vortexed for 2 minutes, centrifuged at 3000 rpm for 5 minutes. The filtrate was injected into 0.1 µl GC-MS, under optimum conditions. The instrument used in this study was Agilent 6980N Network GC system with auto sampler with detector Agilent 5973 inert MSD Inlet split 1/100. Run at a temperature of 250°C, 50°C programmed oven for 5 minutes, an increase of 10°C every minute to 280°C for 15 minutes, the rate in the column is 1ml / minute constant, Aux is 250°C, MS Quad 150°C, MS Source 230°C, solvent delay 0 minutes, Wiley library version 7.0, and sample injection volume is 0.1µL

Polyphenol Assay

Polyphenol content test was carried out by spectrophotometric method. A standard solution of Gallic acid was made with a level of 5-25 ppm. Each with a pipette of 1.0 mL put into the vial, added 0.5ml of Folin-Ciocalteu, left for 5 minutes, and then added 2 mL of 10% sodium carbonate solution. After that the absorbance was measured at λ = 770 nm. Sample preparation was carried out by weighing 50 mg of the sample, dissolved in 50 ml of ethanol, then pipetting 1 ml ad 10 ml, the dilution of the sample was piped 1.0 ml and then put into the vial. Furthermore, 0.5 ml of Folin-Ciocalteu was added, the mixture was 5 minutes, then added 2 ml of 10% sodium carbonate solution, the mixture was added 10 minutes before measuring the absorbance (at λ = 765 nm).

Quercetin content Assay

Quercetin content test was carried out by HPLC. Qualitative analysis was performed by comparing the identical retention time of the sample solution chromatogram with the quercetin standard solution chromatogram at the same HPLC conditions. Quercetin standards were made of a standard solution of 50 ppm, pipette 0.6, 0.8, 1, and 1.2 mL, each put into a 5 mL volumetric flask, then diluted with solvent to the mark line, so that the concentrations solutions are 6,8,10 and 12 ppm. The ethanol extract was filtered by a 0.45 µm filter membrane and sonicator for 20 minutes. After that, each solution was injected into the HPLC system at a certain mobile phase and flow rate. The chromatogram is recorded and a calibration curve is made between the area of the peak and the concentration. From the measurement results, the area obtained is recorded, then the levels are calculated using a calibration curve (linear regression equation): $y = a + bx$.

Physicochemical and ADMET prediction

Physicochemical prediction was carried out by ChemDraw version 17.00 , while the ADMET prediction was carried out by the *online* program, pkCSM that can be accessed from http://biosig.unimelb.edu.au/pkcsm/prediction. These test was ran in ASUS A407UA BV032T Intel core i-3 7th-7020U 2.30 GHz, Windows 10 64 bit.

Docking Study

The docking study was carried out using Molegro Virtual Docker program version 5.5. (Molegro ApS). Some of the steps involved in Molecular Docking program were: obtaining the receptor, ligand preparation, method validation, and docking studies. The receptor used in this study was the P_2Y_{12} receptor, which can been downloaded from the Protein Data Bank (http://www.rcsb.org). This P_2Y_{12} receptor has the code for PDB 4PXZ with 6AD_1201 [A] as native ligand. The ligands that

used in this study were the compounds obtained from shallot skins that was known from GC-MS and quercetin test. The ligands structure drew in ChemDraw 2D version 17.00 and copying into ChemDraw3D version 17.00 to get the 3D structure. The conformation was based on minimum energy, which was run from MMFF94, and then saved in sybil.mol2 extension. The docking process, native ligands, namely 6AD_1201[A] for P_2Y_{12} was re-docked to the appropriate binding site. [22]. The results of the docking studies could be detected visually by comparing the structure of the ligands and receptor P_2Y_{12} (6AD_1202[A]) in the binding site. The results were presented as MolDock scores (MDS), which represented the interaction energy between ligand and receptor. The minimum energy denotes the best binding pose between the functional moiety of the ligand and the amino acid residue of the receptor (17).

Results

Extraction

The extraction of shallot skin in 96% ethanol by ultrasonic method produces as much as 13.149 grams of thick extract. The screening phytochemical extract showed that the extract contained flavonoid and poliphenol compounds. The plate TLC showed the black spot, which is product reaction of phenolic moiety with $FeCl_3$. Whereas that plate showed yelloowish spot which showed flavonoid content.

Chromatographic profile

The results of examination of chemical compounds by GC-MS show in Table 1, which show that Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine has the highest percentage. The measurements were also carried out to determine the presence and levels of quercetin and polyphenol (which using Gallic acid as the standard) in the ethanol extract of shallot skins as shown in table 2.

Physicochemical and ADMET Prediction

The *in silico* test was carried out to predict the physicochemical and pharmacokinetic properties of the compounds contained in the shallot skins as shown in table 3. The molecular weight ranges from 204.272 to 495.479. Log P value, which is a lipophilicity parameter, ranges from 1.988 to 8.417. The bond rotation, HBA, and HBD respectively ranges from 0 (Benzo[a]heptalene) until 7 (N-(trifluoroacetyl)methyl-N-deacethyl-Colchicine and Quercetin), from 0 (Benzo[a]heptalene) until 10 (Natalensine, 3,5-dinitrobenzoate), and from 0 (Benzo[a]heptalene) until 5 (Quercetin).

Docking Study

Figure 1 shows P_2Y_{12} (PDB ID: 4PXZ) with the ligand reference: 6AD-1201. The docking study was conducted in cavity 2 Vol 74.752. While Figure 2 shows the interaction between ligands and amino acids at P_2Y_{12} receptors.

Table 4 summarize the results of the docking study of all compounds, ((2R 3S,4R, 5R)-5-(6-amino-2-(methylthio)-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyltrihy-drogen diphosphate against P_2Y_{12} receptor. Clopidogrel, Quercetin and Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine. Quercetin and Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine has the

similarity amino acid with ((2R,3S,4R,5R)-5-(6-amino-2-(methylthio)-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl trihydrogen diphosphate or Clopidogrel.

Discussion

The outer skins of shallot have known to have anti-inflammatory [12], antimicrobial activity [13, 14] and antioxidant activity [15]. Apart from being antibacterial and antioxidant, the activity of shallot extract as an antiplatelet test also tested *in vitro* by Ro, *et al.* [16]. This study was conducted to determine the benefits of domestic waste shallot skins in the provision of raw materials for Active Pharmaceutical Ingredient.

In this study, the extraction of shallot skins carried out by ultrasonic methods, which. the ultrasonic waves were emitted by passing through the medium conducted the waves by inducing vibrational motion of the molecules. The distance between molecules can vary to be closer or farther as the result of the oscillatory motion of the molecules. If the ultrasonic waves in the medium become more intense, a point will be reached where the intramolecular force of the fluid cannot maintain its molecular structure intact. As a result, the molecular structure of the liquid will break down and a cavity is formed [24].

Cavitation is a mechanical activation process that removes the attraction between molecules in the liquid. Once formed, the tiny air bubbles in the cavity will absorb energy from the ultrasonic waves and make the cavity bigger. As the cavity got bigger, the air bubbles inside could no longer absorb ultrasonic energy. Finally, the fluid around the cavity will enter and break the air cavity. The physical properties of the irradiated mixture are crucial for the effectiveness of cavitation, as well as for the proper transfer of acoustic energy to reactants [25, 26, 27].

The ultrasonic profiling using the GC-MS method on shallot skin extract (**table 1**) showed that the content of Natalensine-3,5-dinitrobenzoate was 13.43%; Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine as much as 36.90%, Benzo[a]heptalene as much as 17.43% and *N*-(trifluoroacetyl)methyl-*N*-deacethyl-Colchicine as much as 32.23%.

Jose, *et al.* [28] stated that the phenyl ring and three methoxyl groups of the colchicine derivative contribute additives to the binding strength of colchicine and its analogues with tubulin, which will affect cancer. Kaivan, *et al* [29] stated, the use of colchicine for short-term myocardial infarction would reduce the size of the infarction compared to placebo. From this, it could been seen that shallot skin has great potential as API in the treatment of cancer.

Many foods in a healthy diet contain high levels of natural phenols in fruits, vegetables, cereals, tea and coffee. Fruits such as grapes, apples, pears, cherries, and berries contain up to 200-300 mg of polyphenols per 100 grams of fresh weight (0.2-0.3% w / w) [30]. Literatures reported the biological activities of polyphenols such as antioxidants, antibacterial, antineoplastic, antithrombotic, and vasodilating activities [31].

One example of phenolic compound is ferulic acid, which used as anti-thrombolysis. ADP-induced platelet aggregation test shows that the stronger anti-thrombolysis activity is attributable to its moiety [32, 33]. Therefore, further research on polyphenols as anti-thrombolysis is necessary. In this research, extraction of shallot skin, which carried out ultrasonic, had the amount of polyphenols of 11.14% ± 5.12% w/w in the extract.

In relation to the activity of flavonoids as antiplatelet, structure-activity relationship analysis showed that anti-aggregation activity of flavonoids are highly dependent on the C-ring structure that determines the class of compounds. If double bond is present between C2 and C3, it increases anti-aggregation activity of flavonoids in case of non-methylated

flavonoids. Most active flavonoids possess hydroxyl group at the position 6. Methylation of rings A and B decreases antiplatelet activity [34]. Flavonoid have several mechanisms of action such as change of bilayer function, change in ROS concentrations and oxidative stress, change of intracellular Ca^{2+} concentration, inhibition of enzymes (phospholipase C, cAMP phosphodiesterase, cyclooxygenase, thromboxane A2 synthase) [35]

Quercetin is a commonly consumed flavonoid that found in foods and has demonstrated beneficial properties in numerous studies reporting anticancer, antimicrobial, and antiviral effects. Quercetin intake has been implicated in reduced CVD risk, intake it linked to a reduction in plasma low-density lipoprotein, reduced systolic and diastolic blood pressure, and reduced risk of ischemic heart disease. Quercetin was also reported to possess antiplatelet effects, with an inhibition of platelet aggregation upon in vitro addition as well as ex vivo post-supplementation [36]

The absorption of active ingredients in the gastrointestinal tract is influenced by several factors i.e. the physicochemical properties of the drug, the dosage form used, and the anatomy and physiology of the absorption site [37]. Passive diffusion is influenced by the size and shape of the molecule, the rate of ionization, and the solubility of a drug in fat. Meanwhile, active ingredients that are weakly alkaline will be absorbed at a more alkaline pH, namely in the small intestine [38]

Predicting the solubility of active ingredients in water plays an important role in the absorption process of drugs after oral administration and is a consideration in parenteral drug administration. This is useful in the manipulating and testing process in the drug design and development process and is an important factor in the bioavailability of drugs in the blood [39]. The ADMET profile of a drug is also related to its physicochemical properties [40, 41]. In table 3, there are various parameters of physicochemical properties, it is known that the water solubility of Quercetin is 2.925 x 10^{-4} mol/L; Bis [2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine is 3.577 x 10^{-4} mol/L; *N*-(trifluoroacetyl)methyl-N-deacethyl-Colchicine is 3.781 x 10^{-4} mol/L; Benzo[a]heptalene is 3.691 x 10^{-4} mol/L, and Natalensine, 3,5-dinitrobenzoate is 4,896 x 10^{-4} mol/L.

The greater the solubility of the drug in fat (log P), the higher the absorption of the drug into the body's membrane. However, the drug must still be slightly hydrophilic in order for extracellular fluids to be transported and to be distributed throughout the body [42]. Based on Lipinski's law, log P of the active ingredients in the extract, apart from Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine, all of which meet these requirements. Related to Rule of Five [19], the compound Bis [2- (2-fluorophenyl) -6-fluoroquinolin-4-yl] amine is a compound that meets these criteria because the number of hydrogen bond donors (HBD) of each compound <5 and number of hydrogen bond acceptors (HBA) of each compound <10.

tPSA is a molecular descriptor as a parameter for intestinal absorption and drug penetration into the blood brain barrier [43]. From table 3 It is known that two compounds from shallot skin extract, namely Quercetin and Benzo[a]heptalene, have tPSA values <140Å. So, that compounds meet Veber's law requirements. Caco-2 permeability is an absorption model that uses monolayer Caco-2 cells as an in vitro model predicting the absorption of an orally administered drug. [20]. The compounds in the shallot skins have good permeability apart from 3,5-dinitrobenzoate-Natalensine, this indicates that the compounds in the shallot skins have the potency to be used orally and also have the potential if used through the transdermal route.

The volume of distribution (VDss) is the theoretical volume at which the total drug dose needs to be uniformly distributed to give the same concentration as in blood plasma. The higher the VDss, the more drug is distributed in tissues than in plasma [20]. The compounds in the shallot skins are predicted to have different VDss values so that some of the

shallot skins compounds will survive in the blood vessels and most of them in the tissues, a good antiplatelet compound is expected more distributed in blood vessels than in tissues.

The Central Nervous System (CNS) permeability measures blood-brain permeability (logPS), which compounds with log PS> -2 are considered to penetrate the Central Nervous System (CNS), while compounds with logPS <-3 are unable to penetrate [20]. Of the five test compounds, Quercetin and *N*-(trifluoroacetyl) methyl-*N*-deacethyl-Colchicine had a logPS value <-3 meaning the compound was predicted not to penetrate the central nervous system. Meanwhile, the other three compounds had a logPS value >-2, which means that the test compounds were predicted to penetrate the central nervous system.

CYP450 substrates, namely CYP2D6 and CYP3A4. are important to identify because CYP450 inhibitors can dramatically alter the pharmacokinetics of drugs metabolized by CYP450 [20]. It was found that apart from Quercetin, the test compound became a CYP3A4 substrate, whereas for CYP2D6, the five compounds did not become a substrate for CYP2D6.

Total clearence is related to bioavailability, and it is important to determine the dosage level to reach a steady-state concentration. Total clearances are expressed in logs (ml/min/kg) [20]. The test results showed that the five test compounds had a total clearance value stated in logs (ml / minute / kg) of -0.275 to 0.489.

Toxicity is a pharmacokinetic parameter that is important to determine before designing a drug in order to create a drug that is not only effective and of good quality, but also safe to use. Many compounds can cause hepatotoxicity such as certain drugs, laboratory chemicals and some of herbal medicines [44]. In the shallot skins extract, it is known that Quercetin and Benzo[a]heptalene compounds are not hepatotoxic. Rat Oral Acute Toxicity (LD50) is the amount of compound given at once that can cause the death of 50% of a group of test animals (moles / kg) [20]. The five test compounds have an LD50 value between 1.573 and 3.335.

Prediction of anti-platelet activity was carried out at the P1Y12 receptor, a G1-protein on platelet membrane surface receptors. It stimulated adenylyl cyclase inhibition and intracellular calcium mobilization. [45, 46]. The first generation of P2Y12 receptor inhibitors is the thienopyridine ticlopidine class, which has the side effect of neutropenia. The second generation is the clopidrogel, which is highly metabolized by the CYP450 enzyme [47].

Based on the *in silico* test against the P_2Y_{12} receptor, it is known that, quercetin and Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine has amino acids similar to ((2R, 3S, 4R, 5R)-5-(6-amino-2-(methylthio)-9H-purine-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyltri-hydrogen diphosphate or clopidogrel, which used as standard. Bis [2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine has an MDS value that is close to the standard, whereas quercetin although it has a greater MDS value than the standard, so that its binding ability is smaller, it still does not eliminate the possibility that quercetin can be used against the P_2Y_{12} receptor as antiplatelet. After going through the *in silico* test phase, the shallot skins extract content should be tested *in vivo*. It was concluded that the ultrasonic shallot skin extract can be used as new source of the active pharmaceutical ingredient and are predicted to have the potency as anti platelet in an oral or transdermal preparation.

Conclusions

The ultrasonic shallot skin extract can be used as new source of the active ingredient for drug development and are predicted to have the potency to be developed as an oral or transdermal preparation..

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2. Manuscript Title	Utilization of domestic waste shallot skins as a source of active pharmaceutical ingredients
3. Manuscript Identifying Number (if you know it)	

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1. Given Name (First Name)	Juni
2. Surname (Last Name)	Ekowati
3. Are you the corresponding author?	Yes ☑ No ☐
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	☐		☐	☐		
	☐		☐	☐		
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	☐		☐	☐		
	☐		☐	☐		
	☐		☐	☐		
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	☐		☐	☐		
	☐		☐	☐		
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	☐		☐	☐		
	☐		☐	☐		
	☐		☐	☐		
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	☐		☐	☐		
	☐		☐	☐		
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	☐		☐	☐		
	☐		☐	☐		
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Author contributions

All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests

Authors state no conflict of interest.

Informed consent

Authors state no informed consent

Ethical approval

Authors state no ethical approval

.

Table 1 Examination of chemical compounds by GC-MS

RT	Compound Name	%Normalitas	Qual
28.34	Natalensine, 3,5-dinitrobenzoate	13.43%	30
28.85 dan 33.05	Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine	36.90%	35
29.13	Benzo[a]heptalene	17.43%	95
29.30	N-(trifluoroacetyl)methyl-N-deacethyl-Colchicine	32.23%	35

Table 2 Quercetin and polyphenol content in extract

Content	Quantity in extract Mean %(b/b) ± RPD
Quercetin	4.607 ± 2.431
Polyphenol	11.14 ± 5.12

Table 3 Physicochemical and pharmacokinetic prediction

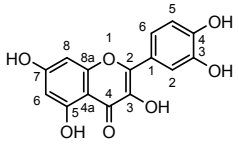
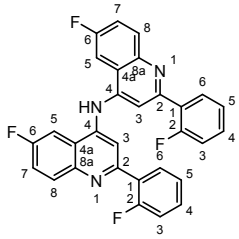
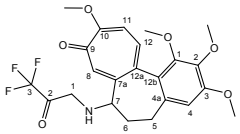
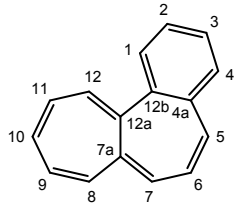
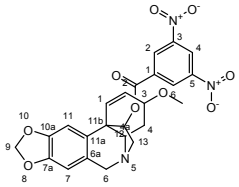
Compound	Structure	MW	Boiling Point (K)	Melting Point (K)	Log P	Bond rotation	HBA	HBD	PSA	Absorption	
										Water solubility	Intestinal absorption
Quercetin		302.238	1135.37	970.62	1.988	1	7	5	122.108	-2.925	77.207
Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine		495.479	1225.17	896.7	8.417	4	3	1	208.617	-3.577	93.87
N-(trifluoroacetyl)methyl-N-deacethyl-Colchicine		467.44	1076.21	754.77	3.4565	7	7	1	187.966	-3.781	93.15
Benzo[a]heptalene		204.272	643	367.64	2.24	0	0	0	94.932	-3.691	99.286
Natalensine, 3,5-dinitrobenzoate		495.444	-	-	2.8678	5	10	0	203.836	-4.896	94.254

Table 3 Physicochemical and pharmacokinetic prediction (continued)

Compound	Absorption		Distribution			Metabolism		Excretion	Toxicity		
	Skin permeability	Caco-2 permeability	VDss (human)	BBB permeability	CNS permeability	CYP2D6 substrate	CYP3A4 substrate	Total clearance	AMES Toxicity	Hepato toxicity	LD50
Quercetin	-2.735	-0.229	1.559	-1.098	-3.065	No	No	0.407	No	No	2.471
Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine	-2.735	1.165	-0.826	0.343	-0.819	No	Yes	-0.275	No	Yes	3.335
N-(trifluoroacetyl)methyl-N-deacethyl-Colchicine	-2.716	1.139	0.833	-1.231	-3.21	No	Yes	0.479	No	Yes	2.992
Benzo[a]heptalene	-1.645	1.539	0.207	0.619	-1.986	No	Yes	0.205	No	No	1.573
Natalensine, 3,5-dinitrobenzoate	-2.737	-0.006	0.235	-0.891	-2.55	No	Yes	0.489	Yes	Yes	2.252

Table 4 Results of the docking of the test ligand at the binding site of P_2Y_{12} receptor

Compound Name	MDS	Amino acid
((2R,3S,4R,5R)-5-(6-amino-2-(methylthio)-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl trihydrogen diphosphate	-157.334	Arg 256, Asn 159, Asn 191, Cys 97, Gln 263, His 187, Lys 179, Lys 280, Ser 101, Tyr 105, Tyr 259, Val 190
Clopidogrel	-128.010	Arg 256, Asn 159, Asn 191, Cys 194, His 187 Met 152, Ser 156, Tyr 105
Quercetin	-116.863	Asn 159, Asn 191, Cys 97, Cys 194, Lys 179, Met 152, Phe 106, Ser 101, Ser 156, Tyr 109
Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine	-157.041	Arg 93, Cys 97, Cys 175, Gln 263, Lys 80, Lys 179, Lys 280, Ser 101, Tyr 105, Tyr 259
N-(trifluoroacetyl)methyl-N-deacethyl-Colchicine	-122.252	Arg 93, Cys 175, Gln 263, Lys 80, Lys 280, Ser 101, Tyr 105, Tyr 259, Val 96, Val 102
Benzo[a]heptalene	-101.726	Cys 194
Natalensine, 3,5-dinitrobenzoate	-150.457	Arg 93, Arg 256, Asn 191, Asp 84, Cys 97, Cys 175, Gln 263, His 187, Leu 284, Lys 80, Lys 179, Lys 280, Phe 104,Ser 105, Tyr 105, Val 96

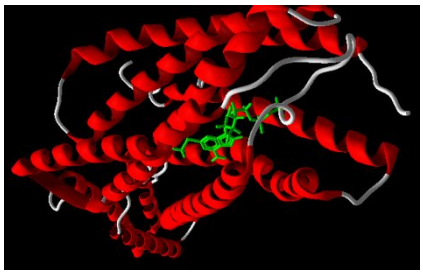


Figure 1 P_2Y_{12} receptor with PDB: ID 4PXZ by which the binding site of the reference ligand and protein will be occupied by the test compounds

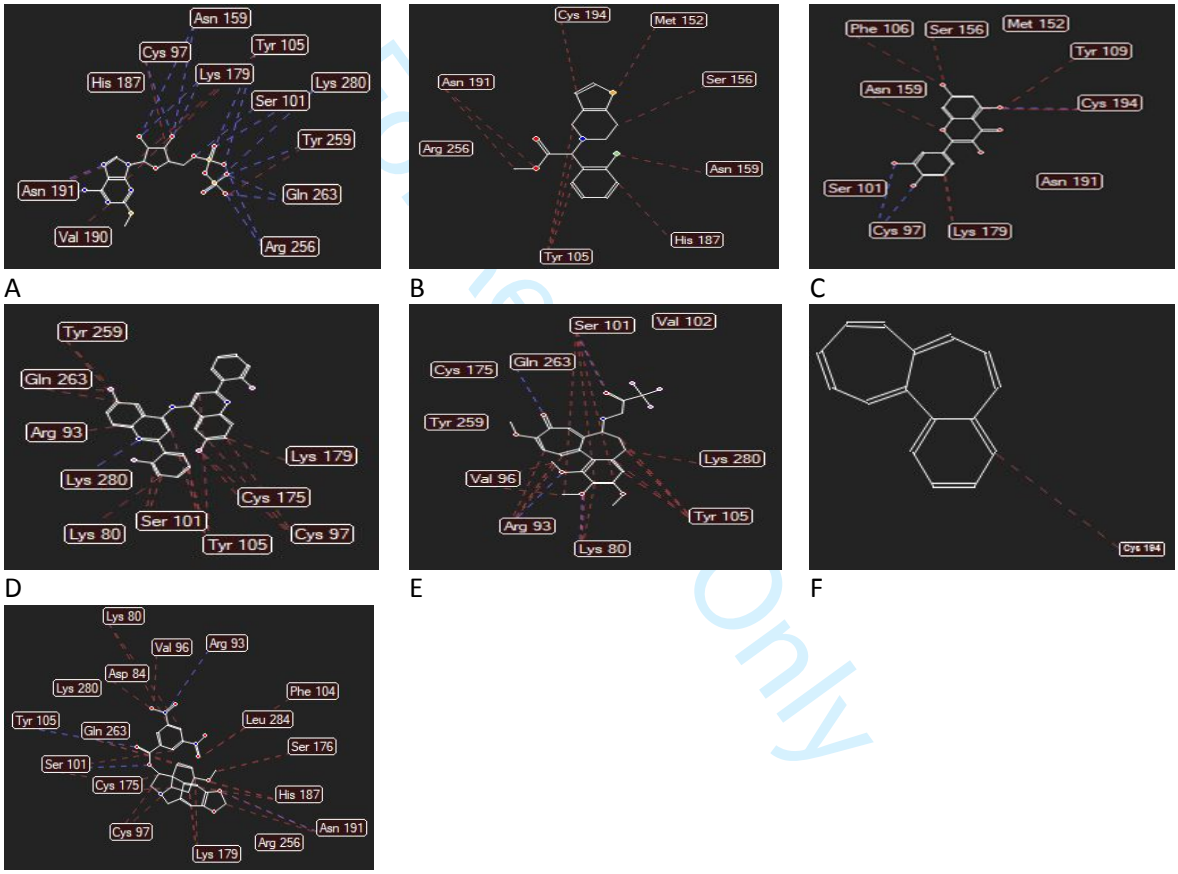


Figure 2 Map of the interaction between shallot skins compound and P_2Y_{12} receptor: (A) ((2R,3S,4R,5R)-5-(6-amino-2-(methylthio)-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl trihydrogen diphosphate, (B) Clopidogrel, (C) Quercetin, (D) Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine, (E) *N*-(trifluoroacetyl)methyl-*N*-deacethyl-Colchicine, (F) Benzo[a]heptalene, and (G) Natalensine, 3,5-dinitrobenzoate

Table 1 Examination of chemical compounds by GC-MS

RT	Compound Name	%Normality	Qual
28.34	Natalensine, 3,5-dinitrobenzoate	13.43%	30
28.85 dan 33.05	Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine	36.90%	35
29.13	Benzo[a]heptalene	17.43%	95
29.30	N-(trifluoroacetyl)methyl-N-deacethyl-Colchicine	32.23%	35

Table 2 Quercetin and polyphenol content in extract

Content	Quantity in extract Mean %(b/b) ± RPD
Quercetin	4.61 ± 2.43
Polyphenol	11.14 ± 5.12

RPD : relative percent difference

Table 3 Physicochemical and pharmacokinetic prediction

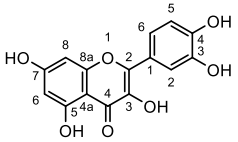
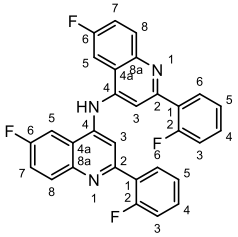
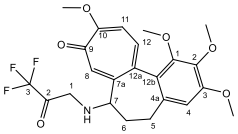
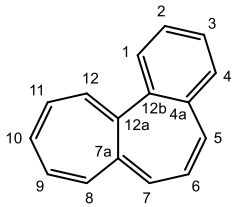
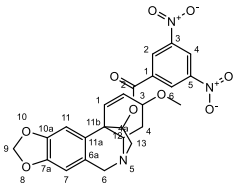
Compound	Structure	MW	Boiling Point (K)	Melting Point (K)	Log P	Bond rotation	HBA	HBD	PSA	Absorption	
										Water solubility	Intestinal absorption
Quercetin		302.238	1135.37	970.62	1.988	1	7	5	122.108	-2.925	77.207
Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine		495.479	1225.17	896.7	8.417	4	3	1	208.617	-3.577	93.87
N-(trifluoroacetyl)methyl-N-deacethyl-Colchicine		467.44	1076.21	754.77	3.4565	7	7	1	187.966	-3.781	93.15
Benzo[a]heptalene		204.272	643	367.64	2.24	0	0	0	94.932	-3.691	99.286
Natalensine, 3,5-dinitrobenzoate		495.444	-	-	2.8678	5	10	0	203.836	-4.896	94.254

Table 3 Physicochemical and pharmacokinetic prediction (continued)

Compound	Absorption		Distribution			Metabolism		Excretion	Toxicity		
	Skin permeability	Caco-2 permeability	VDss (human)	BBB permeability	CNS permeability	CYP2D6 substrate	CYP3A4 substrate	Total clearance	AMES Toxicity	Hepato toxicity	LD50
Quercetin	-2.735	-0.229	1.559	-1.098	-3.065	No	No	0.407	No	No	2.471
Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine	-2.735	1.165	-0.826	0.343	-0.819	No	Yes	-0.275	No	Yes	3.335
N-(trifluoroacetyl)methyl-N-deacethyl-Colchicine	-2.716	1.139	0.833	-1.231	-3.21	No	Yes	0.479	No	Yes	2.992
Benzo[a]heptalene	-1.645	1.539	0.207	0.619	-1.986	No	Yes	0.205	No	No	1.573
Natalensine, 3,5-dinitrobenzoate	-2.737	-0.006	0.235	-0.891	-2.55	No	Yes	0.489	Yes	Yes	2.252

Table 4 Results of the docking of the test ligand at the binding site of P2Y12 receptor

Compound Name	MDS	Amino acid
((2R,3S,4R,5R)-5-(6-amino-2-(methylthio)-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl trihydrogen diphosphate	-157.334	Arg 256, Asn 159, Asn 191, Cys 97, Gln 263, His 187, Lys 179, Lys 280, Ser 101, Tyr 105, Tyr 259, Val 190
Clopidogrel	-128.010	Arg 256, Asn 159, Asn 191, Cys 194, His 187 Met 152, Ser 156, Tyr 105
Quercetin	-116.863	Asn 159, Asn 191, Cys 97, Cys 194, Lys 179, Met 152, Phe 106, Ser 101, Ser 156, Tyr 109
Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine	-157.041	Arg 93, Cys 97, Cys 175, Gln 263, Lys 80, Lys 179, Lys 280, Ser 101, Tyr 105, Tyr 259
N-(trifluoroacetyl)methyl-N-deacethyl-Colchicine	-122.252	Arg 93, Cys 175, Gln 263, Lys 80, Lys 280, Ser 101, Tyr 105, Tyr 259, Val 96, Val 102
Benzo[a]heptalene	-101.726	Cys 194
Natalensine, 3,5-dinitrobenzoate	-150.457	Arg 93, Arg 256, Asn 191, Asp 84, Cys 97, Cys 175, Gln 263, His 187, Leu 284, Lys 80, Lys 179, Lys 280, Phe 104,Ser 105, Tyr 105, Val 96

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Utilization of domestic waste shallot skins as a source of active pharmaceutical ingredients

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Section/Category:	• Cardiovascular-Pulmonary Interactions
Keywords:	ADMET, polyphenol, pharmaceutical active ingredients, shallot skin, ultrasonic extraction
Abstract:	Background: Medicinal plants are a source of many compounds that are useful in the pharmaceutical field for novel drug development. Polyphenols and the flavonoid group in plants are known to have several activities, such as relieving CVD. The outer skin of the shallot which is disposed of as waste is known to have an antiplatelet activity which was tested in vitro. To date, there is no study reported on the ADMET profile and physicochemical properties of the active component of the shallot skins. Methods: The extraction of shallot skins was conducted by ultrasonic irradiation using ethanol.. The phytochemical screenings were carried out by TLC and color reaction. The profiling of its active ingredient was presented by GC-MSD, HPLC and spectrophotometry UV-vis. Whereas their physicochemical properties were analyzed by ChemDraw 17.00 program and the ADMET predictions were studied using pkCSM online tool. The MVD program was operated in the docking study on protein P2Y12 (PDB ID 4PXZ). Results: The extract showed the presence of polyphenol, flavonoids, quercetin, natalensine-3,5-dinitrobenzoate; bis[2- (2-fluorophenyl)-6-fluoroquinolin-4-yl]amine, benzo[a]heptalene, N-(trifluoroacetyl) methyl-N-deacethyl-colchicine. The ADMET prediction data displayed that

the compounds in the extract have good absorption so that they can be used in the oral and transdermal routes. Some components in the extract have lower MDS than clopidogrel.
Conclusion: The ultrasonic shallot skin extract can be used as a new source of the active pharmaceutical ingredients and, to have the potency to be developed as an oral or transdermal preparation.

Keywords: ADMET; polyphenol; pharmacy active ingredients; shallot skin; ultrasonic extraction

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Utilization of domestic waste shallot skins as a source of active pharmaceutical ingredients

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Abstract

Background: Medicinal plants are a source of many compounds that are useful in the pharmaceutical field for novel drug development. Polyphenols and the flavonoid group in plants are known to have several activities, such as relieving CVD. The outer skin of the shallot which is disposed of as waste is known to have an antiplatelet activity which was tested *in vitro* assay. To date, there is no study reported on the ADMET profile and physicochemical properties of the active component of the shallot skins.
Methods: The extraction of shallot skins was conducted by ultrasonic irradiation using ethanol.. The phytochemical screenings were carried out by TLC and color reaction. The profiling of its active ingredient was presented by GC-MSD, HPLC and spectrophotometry UV-vis. Whereas their physicochemical properties were analyzed by ChemDraw 17.00 program and the ADMET predictions were studied using pkCSM online tool. The MVD program was operated in the docking study on protein P2Y12 (PDB ID 4PXZ).
Results: The extract showed the presence of polyphenol, flavonoids, quercetin, natalensine-3,5-dinitrobenzoate; bis[2- (2-fluorophenyl)-6-fluoroquinolin-4-yl]amine, benzo[a]heptalene, *N*-(trifluoroacetyl) methyl-*N*-deacethyl-colchicine. The ADMET prediction data displayed that the compounds in the extract have good absorption so that they can be used in the oral and transdermal routes. Some components in the extract have lower MDS than clopidogrel.
Conclusion: The ultrasonic shallot skin extract can be used as new source of the active pharmaceutical ingredients and to have the potency to be developed as an oral or transdermal preparation.

Keywords: ADMET; poliphenol; pharmacy active ingredients; shallot skin; ultrasonic extraction

Introduction

Cardiac Vascular Disease (CVD), especially coronary heart disease, is a major cause of death worldwide, and patient medical costs continue to increase due to an increase in the number of sufferers [1, 2]. This disease occurs due to impaired blood flow to the myocardium due to platelet aggregation, thrombus, and the accumulation of oxidative damage to LDL by ROS [1,3]. Oxidant stress causes endothelial dysfunction and thrombus formation [4].

Drugs used to treat coronary heart disease are thrombolytic, anti-platelets and. several antioxidant [5, 6]. Although they can treat coronary heart conditions due to thromboembolism, these drugs also have undesirable side effects such as intracranial bleeding, nausea, dyspnea, and it was reported that the patient had resistance to aspirin as an anti-platelet [7,8]. Therefore, alternative therapies are needed to overcome the above problems with mild side effects.

Medicinal plants are a source of many chemical compounds that are useful in the pharmaceutical field for novel drug development, including polyphenols, the flavonoid class. The flavonoid group are known to have several activities, such as antibacterial and antioxidant [9, 10]. One of the natural ingredients that is widely used in daily food is shallots. Shallots have the active compound i.e. polyphenol quercetin as an antibacterial [11]. Not only the tuber part of the shallot, the outer skin of the shallot which is disposed of as waste is also known to have anti-inflammatory [12] and antimicrobial activity [13, 14]. It was also reported that there is antioxidant activity of the ethanolic extract from shallot skins using the ABTS method [15]. Apart from being antibacterial and antioxidant, the activity of shallot extract as an antiplatelet which was tested in vitro has also been reported by Ro, *et al.* [16]. These things indicate that the shallot skins has the potency as an active pharmaceutical ingredient (API).

Beside the activity, prospective drug compounds also need to be investigated regarding their physicochemical properties and pharmacokinetic profile, including absorption, distribution, metabolism, and excretion as well as its toxicity

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(hereinafter referred to as ADMET) to humans [17]. The pharmacokinetic profile of a drug could been influenced by the physicochemical properties [18] Analysis of the physicochemical properties of drugs in the context of oral bioavailability was first reported by Lipinski, *et al.* which is referred to the "Rule of Five" (Ro5). It includes *Molecular Weight* (MW) <500 g/mol; *Donor H-bond* (HBD) < 5; *H-bond acceptor* (HBA) <10; and the calculation of the logarithm of the partition coefficient of 1-octanol / water (cLogP) <5 [19].

However, until now, there no research on the physicochemical and pharmacokinetics (ADMET) of the active ingredients of shallot skins. The effects of administering the extract on the gastrointestinal tract also need to be studied to ensure its safety in oral use. Therefore, this study aims to find out the component of shallot skin, its physicochemical properties prediction, and its pharmacokinetics (ADMET) prediction.

Pharmacokinetic profile analysis (ADMET) *in silico* is able to be conducted with the help of the online pkCSM program [20]. Prediction with the online pkCSM program has advantages over other software such as SwissADME, since there are more pharmacokinetic parameters that can be predicted with the online pkCSM program [21, 22] The greater number of parameters will have an impact on the broader information obtained to support the next drug development process.

Based on the research of Ro et al. [16] which states that shallot skins extract has anti-platelet activity in vitro, this study also evaluated the inhibition mechanism of the P_2Y_{12} receptor by *in silico* test (PDB ID 4pxz). P2Y12 is a main receptor and the distinctive P2 goal for clinically allowed antiplatelet drugs (herein named as P2Y12 inhibitors) [17,23].

Materials and methods

The waste from shallot skins obtained from traditional markets is collected, cleaned, then dried at room temperature, and powdered using a blender. Previously, the species of shallot skin were examined at the Materia Medica Batu institute, and it was found that the shallot species was *Allium cepa* L.

Extraction

The powder then extracted in ethanol using the ultrasonic method. First, 80 grams of shallot skin powder soaked in 500 ml Erlenmeyer with 350 ml 96% ethanol, then performed ultrasonic at full power and temperature at 40°C for 30 minutes. The extraction product is then filtered using a Buchner funnel under vacuum; the filtrate is accumulated in a different Erlenmeyer. Second, the extracted pulp was put back into the Erlenmeyer 500 ml and added with 300 ml of 96% ethanol. The same process then carried out like the previous process. The extracted filtrate collected and carried out at a rotary evaporator. This ultrasonic extraction was repeated 14 times (until the filtrate did not react with $FeCl_3$, this is indicated by the solution remains clear).

Phytochemical screening

Screening of flavonoid content was carried out by TLC method, using stationary phase silica gel GF254, the mobile phase butanol-acetic acid glasial-water (4:1:5) and ammonia vapor was used as color reagent. While the poliphenol group was detected by solution $FeCl_3$ 2%.

Chromatographic profile

Examination of chemical compounds carried out by GC-MS. The sample was weighed 100 mg, dissolved 2 ml of p.a. ethanol, then vortexed for 2 minutes, centrifuged at 3000 rpm for 5 minutes. The filtrate was injected into 0.1 µl GC-MS, under optimum conditions. The instrument used in this study was Agilent 6980N Network GC system with auto sampler with detector Agilent 5973 inert MSD Inlet split 1/100. Run at a temperature of 250°C, 50°C programmed oven for 5 minutes, an increase of 10°C every minute to 280°C for 15 minutes, the rate in the column is 1ml / minute constant, Aux is 250°C, MS Quad 150°C, MS Source 230°C, solvent delay 0 minutes, Wiley library version 7.0, and sample injection volume is 0.1µL

Polyphenol Assay

Polyphenol content test was carried out by spectrophotometric method. A standard solution of Gallic acid was made with a level of 5-25 ppm. Each with a pipette of 1.0 mL put into the vial, added 0.5ml of Folin-Ciocalteu, left for 5 minutes, and then added 2 mL of 10% sodium carbonate solution. After that the absorbance was measured at λ = 770 nm. Sample preparation was carried out by weighing 50 mg of the sample, dissolved in 50 ml of ethanol, then pipetting 1 ml ad 10 ml, the dilution of the sample was piped 1.0 ml and then put into the vial. Furthermore, 0.5 ml of Folin-Ciocalteu was added, the mixture was 5 minutes, then added 2 ml of 10% sodium carbonate solution, the mixture was added 10 minutes before measuring the absorbance (at λ = 765 nm).

Quercetin content Assay

Quercetin content test was carried out by HPLC. Qualitative analysis was performed by comparing the identical retention time of the sample solution chromatogram with the quercetin standard solution chromatogram at the same HPLC conditions. Quercetin standards were made of a standard solution of 50 ppm, pipette 0.6, 0.8, 1, and 1.2 mL, each put into a 5 mL volumetric flask, then diluted with solvent to the mark line, so that the concentrations solutions are 6,8,10 and 12 ppm. The ethanol extract was filtered by a 0.45 µm filter membrane and sonicator for 20 minutes. After that, each solution was injected into the HPLC system at a certain mobile phase and flow rate. The chromatogram is recorded and a calibration curve is made between the area of the peak and the concentration. From the measurement results, the area obtained is recorded, then the levels are calculated using a calibration curve (linear regression equation): $y = a + bx$.

Physicochemical and ADMET prediction

Physicochemical prediction was carried out by ChemDraw version 17.00 , while the ADMET prediction was carried out by the *online* program, pkCSM that can be accessed from http://biosig.unimelb.edu.au/pkcsm/prediction. These test was ran in ASUS A407UA BV032T Intel core i-3 7th-7020U 2.30 GHz, Windows 10 64 bit.

Docking Study

The docking study was carried out using Molegro Virtual Docker program version 5.5. (Molegro ApS). Some of the steps involved in Molecular Docking program were: obtaining the receptor, ligand preparation, method validation, and docking studies. The receptor used in this study was the P_2Y_{12} receptor, which can been downloaded from the Protein Data Bank (http://www.rcsb.org). This P_2Y_{12} receptor has the code for PDB 4PXZ with 6AD_1201 [A] as native ligand. The ligands that

used in this study were the compounds obtained from shallot skins that was known from GC-MS and quercetin test. The ligands structure drew in ChemDraw 2D version 17.00 and copying into ChemDraw3D version 17.00 to get the 3D structure. The conformation was based on minimum energy, which was run from MMFF94, and then saved in sybil.mol2 extension. The docking process, native ligands, namely 6AD_1201[A] for P_2Y_{12} was re-docked to the appropriate binding site. [22]. The results of the docking studies could be detected visually by comparing the structure of the ligands and receptor P_2Y_{12} (6AD_1202[A]) in the binding site. The results were presented as MolDock scores (MDS), which represented the interaction energy between ligand and receptor. The minimum energy denotes the best binding pose between the functional moiety of the ligand and the amino acid residue of the receptor (17).

Results

Extraction

The extraction of shallot skin in 96% ethanol by ultrasonic method produces as much as 13.149 grams of thick extract. The screening phytochemical extract showed that the extract contained flavonoid and poliphenol compounds. The plate TLC showed the black spot, which is product reaction of phenolic moiety with $FeCl_3$. Whereas that plate showed yelloowish spot which showed flavonoid content.

Chromatographic profile

The results of examination of chemical compounds by GC-MS show in Table 1, which show that Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine has the highest percentage. The measurements were also carried out to determine the presence and levels of quercetin and polyphenol (which using Gallic acid as the standard) in the ethanol extract of shallot skins as shown in table 2.

Physicochemical and ADMET Prediction

The *in silico* test was carried out to predict the physicochemical and pharmacokinetic properties of the compounds contained in the shallot skins as shown in table 3. The molecular weight ranges from 204.272 to 495.479. Log P value, which is a lipophilicity parameter, ranges from 1.988 to 8.417. The bond rotation, HBA, and HBD respectively ranges from 0 (Benzo[a]heptalene) until 7 (N-(trifluoroacetyl)methyl-N-deacethyl-Colchicine and Quercetin), from 0 (Benzo[a]heptalene) until 10 (Natalensine, 3,5-dinitrobenzoate), and from 0 (Benzo[a]heptalene) until 5 (Quercetin).

Docking Study

Figure 1 shows P_2Y_{12} (PDB ID: 4PXZ) with the ligand reference: 6AD-1201. The docking study was conducted in cavity 2 Vol 74.752. While Figure 2 shows the interaction between ligands and amino acids at P_2Y_{12} receptors.

Table 4 summarize the results of the docking study of all compounds, ((2R 3S,4R, 5R)-5-(6-amino-2-(methylthio)-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyltrihy-drogen diphosphate against P_2Y_{12} receptor. Clopidogrel, Quercetin and Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine. Quercetin and Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine has the

similarity amino acid with ((2R,3S,4R,5R)-5-(6-amino-2-(methylthio)-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl trihydrogen diphosphate or Clopidogrel.

Discussion

The outer skins of shallot have known to have anti-inflammatory [12], antimicrobial activity [13, 14] and antioxidant activity [15]. Apart from being antibacterial and antioxidant, the activity of shallot extract as an antiplatelet test also tested *in vitro* by Ro, *et al.* [16]. This study was conducted to determine the benefits of domestic waste shallot skins in the provision of raw materials for Active Pharmaceutical Ingredient.

In this study, the extraction of shallot skins carried out by ultrasonic methods, which. the ultrasonic waves were emitted by passing through the medium conducted the waves by inducing vibrational motion of the molecules. The distance between molecules can vary to be closer or farther as the result of the oscillatory motion of the molecules. If the ultrasonic waves in the medium become more intense, a point will be reached where the intramolecular force of the fluid cannot maintain its molecular structure intact. As a result, the molecular structure of the liquid will break down and a cavity is formed [24].

Cavitation is a mechanical activation process that removes the attraction between molecules in the liquid. Once formed, the tiny air bubbles in the cavity will absorb energy from the ultrasonic waves and make the cavity bigger. As the cavity got bigger, the air bubbles inside could no longer absorb ultrasonic energy. Finally, the fluid around the cavity will enter and break the air cavity. The physical properties of the irradiated mixture are crucial for the effectiveness of cavitation, as well as for the proper transfer of acoustic energy to reactants [25, 26, 27].

The ultrasonic profiling using the GC-MS method on shallot skin extract (**table 1**) showed that the content of Natalensine-3,5-dinitrobenzoate was 13.43%; Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine as much as 36.90%, Benzo[a]heptalene as much as 17.43% and *N*-(trifluoroacetyl)methyl-*N*-deacethyl-Colchicine as much as 32.23%.

Jose, *et al.* [28] stated that the phenyl ring and three methoxyl groups of the colchicine derivative contribute additives to the binding strength of colchicine and its analogues with tubulin, which will affect cancer. Kaivan, *et al* [29] stated, the use of colchicine for short-term myocardial infarction would reduce the size of the infarction compared to placebo. From this, it could been seen that shallot skin has great potential as API in the treatment of cancer.

Many foods in a healthy diet contain high levels of natural phenols in fruits, vegetables, cereals, tea and coffee. Fruits such as grapes, apples, pears, cherries, and berries contain up to 200-300 mg of polyphenols per 100 grams of fresh weight (0.2-0.3% w / w) [30]. Literatures reported the biological activities of polyphenols such as antioxidants, antibacterial, antineoplastic, antithrombotic, and vasodilating activities [31].

One example of phenolic compound is ferulic acid, which used as anti-thrombolysis. ADP-induced platelet aggregation test shows that the stronger anti-thrombolysis activity is attributable to its moiety [32, 33]. Therefore, further research on polyphenols as anti-thrombolysis is necessary. In this research, extraction of shallot skin, which carried out ultrasonic, had the amount of polyphenols of 11.14% ± 5.12% w/w in the extract.

In relation to the activity of flavonoids as antiplatelet, structure-activity relationship analysis showed that anti-aggregation activity of flavonoids are highly dependent on the C-ring structure that determines the class of compounds. If double bond is present between C2 and C3, it increases anti-aggregation activity of flavonoids in case of non-methylated

flavonoids. Most active flavonoids possess hydroxyl group at the position 6. Methylation of rings A and B decreases antiplatelet activity [34]. Flavonoid have several mechanisms of action such as change of bilayer function, change in ROS concentrations and oxidative stress, change of intracellular Ca^{2+} concentration, inhibition of enzymes (phospholipase C, cAMP phosphodiesterase, cyclooxygenase, thromboxane A2 synthase) [35]

Quercetin is a commonly consumed flavonoid that found in foods and has demonstrated beneficial properties in numerous studies reporting anticancer, antimicrobial, and antiviral effects. Quercetin intake has been implicated in reduced CVD risk, intake it linked to a reduction in plasma low-density lipoprotein, reduced systolic and diastolic blood pressure, and reduced risk of ischemic heart disease. Quercetin was also reported to possess antiplatelet effects, with an inhibition of platelet aggregation upon in vitro addition as well as ex vivo post-supplementation [36]

The absorption of active ingredients in the gastrointestinal tract is influenced by several factors i.e. the physicochemical properties of the drug, the dosage form used, and the anatomy and physiology of the absorption site [37]. Passive diffusion is influenced by the size and shape of the molecule, the rate of ionization, and the solubility of a drug in fat. Meanwhile, active ingredients that are weakly alkaline will be absorbed at a more alkaline pH, namely in the small intestine [38]

Predicting the solubility of active ingredients in water plays an important role in the absorption process of drugs after oral administration and is a consideration in parenteral drug administration. This is useful in the manipulating and testing process in the drug design and development process and is an important factor in the bioavailability of drugs in the blood [39]. The ADMET profile of a drug is also related to its physicochemical properties [40, 41]. In table 3, there are various parameters of physicochemical properties, it is known that the water solubility of Quercetin is 2.925 x 10^{-4} mol/L; Bis [2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine is 3.577 x 10^{-4} mol/L; *N*-(trifluoroacetyl)methyl-N-deacethyl-Colchicine is 3.781 x 10^{-4} mol/L; Benzo[a]heptalene is 3.691 x 10^{-4} mol/L, and Natalensine, 3,5-dinitrobenzoate is 4,896 x 10^{-4} mol/L.

The greater the solubility of the drug in fat (log P), the higher the absorption of the drug into the body's membrane. However, the drug must still be slightly hydrophilic in order for extracellular fluids to be transported and to be distributed throughout the body [42]. Based on Lipinski's law, log P of the active ingredients in the extract, apart from Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine, all of which meet these requirements. Related to Rule of Five [19], the compound Bis [2- (2-fluorophenyl) -6-fluoroquinolin-4-yl] amine is a compound that meets these criteria because the number of hydrogen bond donors (HBD) of each compound <5 and number of hydrogen bond acceptors (HBA) of each compound <10.

tPSA is a molecular descriptor as a parameter for intestinal absorption and drug penetration into the blood brain barrier [43]. From table 3 It is known that two compounds from shallot skin extract, namely Quercetin and Benzo[a]heptalene, have tPSA values <140Å. So, that compounds meet Veber's law requirements. Caco-2 permeability is an absorption model that uses monolayer Caco-2 cells as an in vitro model predicting the absorption of an orally administered drug. [20]. The compounds in the shallot skins have good permeability apart from 3,5-dinitrobenzoate-Natalensine, this indicates that the compounds in the shallot skins have the potency to be used orally and also have the potential if used through the transdermal route.

The volume of distribution (VDss) is the theoretical volume at which the total drug dose needs to be uniformly distributed to give the same concentration as in blood plasma. The higher the VDss, the more drug is distributed in tissues than in plasma [20]. The compounds in the shallot skins are predicted to have different VDss values so that some of the

shallot skins compounds will survive in the blood vessels and most of them in the tissues, a good antiplatelet compound is expected more distributed in blood vessels than in tissues.

The Central Nervous System (CNS) permeability measures blood-brain permeability (logPS), which compounds with log PS> -2 are considered to penetrate the Central Nervous System (CNS), while compounds with logPS <-3 are unable to penetrate [20]. Of the five test compounds, Quercetin and *N*-(trifluoroacetyl) methyl-*N*-deacethyl-Colchicine had a logPS value <-3 meaning the compound was predicted not to penetrate the central nervous system. Meanwhile, the other three compounds had a logPS value >-2, which means that the test compounds were predicted to penetrate the central nervous system.

CYP450 substrates, namely CYP2D6 and CYP3A4. are important to identify because CYP450 inhibitors can dramatically alter the pharmacokinetics of drugs metabolized by CYP450 [20]. It was found that apart from Quercetin, the test compound became a CYP3A4 substrate, whereas for CYP2D6, the five compounds did not become a substrate for CYP2D6.

Total clearence is related to bioavailability, and it is important to determine the dosage level to reach a steady-state concentration. Total clearances are expressed in logs (ml/min/kg) [20]. The test results showed that the five test compounds had a total clearance value stated in logs (ml / minute / kg) of -0.275 to 0.489.

Toxicity is a pharmacokinetic parameter that is important to determine before designing a drug in order to create a drug that is not only effective and of good quality, but also safe to use. Many compounds can cause hepatotoxicity such as certain drugs, laboratory chemicals and some of herbal medicines [44]. In the shallot skins extract, it is known that Quercetin and Benzo[a]heptalene compounds are not hepatotoxic. Rat Oral Acute Toxicity (LD50) is the amount of compound given at once that can cause the death of 50% of a group of test animals (moles / kg) [20]. The five test compounds have an LD50 value between 1.573 and 3.335.

Prediction of anti-platelet activity was carried out at the P1Y12 receptor, a G1-protein on platelet membrane surface receptors. It stimulated adenylyl cyclase inhibition and intracellular calcium mobilization. [45, 46]. The first generation of P2Y12 receptor inhibitors is the thienopyridine ticlopidine class, which has the side effect of neutropenia. The second generation is the clopidrogel, which is highly metabolized by the CYP450 enzyme [47].

Based on the *in silico* test against the P_2Y_{12} receptor, it is known that, quercetin and Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine has amino acids similar to ((2R, 3S, 4R, 5R)-5-(6-amino-2-(methylthio)-9H-purine-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyltri-hydrogen diphosphate or clopidogrel, which used as standard. Bis [2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine has an MDS value that is close to the standard, whereas quercetin although it has a greater MDS value than the standard, so that its binding ability is smaller, it still does not eliminate the possibility that quercetin can be used against the P_2Y_{12} receptor as antiplatelet. After going through the *in silico* test phase, the shallot skins extract content should be tested *in vivo*. It was concluded that the ultrasonic shallot skin extract can be used as new source of the active pharmaceutical ingredient and are predicted to have the potency as anti platelet in an oral or transdermal preparation.

Conclusions

The ultrasonic shallot skin extract can be used as new source of the active ingredient for drug development and are predicted to have the potency to be developed as an oral or transdermal preparation..

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2. Manuscript Title	Utilization of domestic waste shallot skins as a source of active pharmaceutical ingredients
3. Manuscript Identifying Number (if you know it)	

IDENTIFYING INFORMATION - SUBMITTING AUTHOR

1. Given Name (First Name)	Juni
2. Surname (Last Name)	Ekowati
3. Are you the corresponding author?	Yes ☑ No ☐
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	☐		☐	☐		
	☐		☐	☐		
	☐		☐	☐		
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	☐		☐	☐		
	☐		☐	☐		
	☐		☐	☐		
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	☐		☐	☐		
	☐		☐	☐		

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	☐		☐	☐		
	☐		☐	☐		
	☐		☐	☐		
Payment for manuscript preparation	☐		☐	☐		
	☐		☐	☐		
	☐		☐	☐		
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	☐		☐	☐		
	☐		☐	☐		
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	☐		☐	☐		
	☐		☐	☐		
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	☐		☐	☐		
	☐		☐	☐		
	☐		☐	☐		
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	☐		☐	☐		
Other	☐		☐	☐		
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Author contributions

All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests

Authors state no conflict of interest.

Informed consent

Authors state no informed consent

Ethical approval

Authors state no ethical approval

.

Table 1 Examination of chemical compounds by GC-MS

RT	Compound Name	%Normalitas	Qual
28.34	Natalensine, 3,5-dinitrobenzoate	13.43%	30
28.85 dan 33.05	Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine	36.90%	35
29.13	Benzo[a]heptalene	17.43%	95
29.30	N-(trifluoroacetyl)methyl-N-deacethyl-Colchicine	32.23%	35

Table 2 Quercetin and polyphenol content in extract

Content	Quantity in extract Mean %(b/b) ± RPD
Quercetin	4.607 ± 2.431
Polyphenol	11.14 ± 5.12

Table 3 Physicochemical and pharmacokinetic prediction

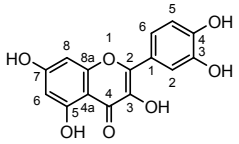
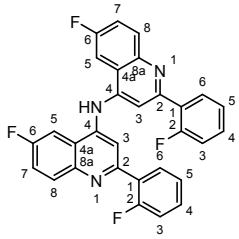
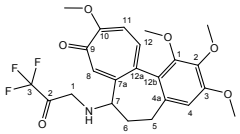
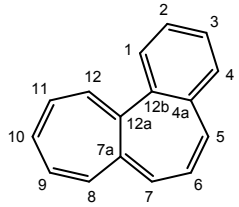
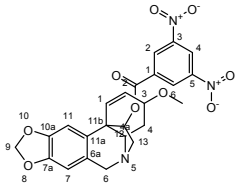
Compound	Structure	MW	Boiling Point (K)	Melting Point (K)	Log P	Bond rotation	HBA	HBD	PSA	Absorption	
										Water solubility	Intestinal absorption
Quercetin		302.238	1135.37	970.62	1.988	1	7	5	122.108	-2.925	77.207
Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine		495.479	1225.17	896.7	8.417	4	3	1	208.617	-3.577	93.87
N-(trifluoroacetyl)methyl-N-deacethyl-Colchicine		467.44	1076.21	754.77	3.4565	7	7	1	187.966	-3.781	93.15
Benzo[a]heptalene		204.272	643	367.64	2.24	0	0	0	94.932	-3.691	99.286
Natalensine, 3,5-dinitrobenzoate		495.444	-	-	2.8678	5	10	0	203.836	-4.896	94.254

Table 3 Physicochemical and pharmacokinetic prediction (continued)

Compound	Absorption		Distribution			Metabolism		Excretion	Toxicity		
	Skin permeability	Caco-2 permeability	VDss (human)	BBB permeability	CNS permeability	CYP2D6 substrate	CYP3A4 substrate	Total clearance	AMES Toxicity	Hepato toxicity	LD50
Quercetin	-2.735	-0.229	1.559	-1.098	-3.065	No	No	0.407	No	No	2.471
Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine	-2.735	1.165	-0.826	0.343	-0.819	No	Yes	-0.275	No	Yes	3.335
N-(trifluoroacetyl)methyl-N-deacethyl-Colchicine	-2.716	1.139	0.833	-1.231	-3.21	No	Yes	0.479	No	Yes	2.992
Benzo[a]heptalene	-1.645	1.539	0.207	0.619	-1.986	No	Yes	0.205	No	No	1.573
Natalensine, 3,5-dinitrobenzoate	-2.737	-0.006	0.235	-0.891	-2.55	No	Yes	0.489	Yes	Yes	2.252

Table 4 Results of the docking of the test ligand at the binding site of P_2Y_{12} receptor

Compound Name	MDS	Amino acid
((2R,3S,4R,5R)-5-(6-amino-2-(methylthio)-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl trihydrogen diphosphate	-157.334	Arg 256, Asn 159, Asn 191, Cys 97, Gln 263, His 187, Lys 179, Lys 280, Ser 101, Tyr 105, Tyr 259, Val 190
Clopidogrel	-128.010	Arg 256, Asn 159, Asn 191, Cys 194, His 187 Met 152, Ser 156, Tyr 105
Quercetin	-116.863	Asn 159, Asn 191, Cys 97, Cys 194, Lys 179, Met 152, Phe 106, Ser 101, Ser 156, Tyr 109
Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine	-157.041	Arg 93, Cys 97, Cys 175, Gln 263, Lys 80, Lys 179, Lys 280, Ser 101, Tyr 105, Tyr 259
N-(trifluoroacetyl)methyl-N-deacethyl-Colchicine	-122.252	Arg 93, Cys 175, Gln 263, Lys 80, Lys 280, Ser 101, Tyr 105, Tyr 259, Val 96, Val 102
Benzo[a]heptalene	-101.726	Cys 194
Natalensine, 3,5-dinitrobenzoate	-150.457	Arg 93, Arg 256, Asn 191, Asp 84, Cys 97, Cys 175, Gln 263, His 187, Leu 284, Lys 80, Lys 179, Lys 280, Phe 104,Ser 105, Tyr 105, Val 96

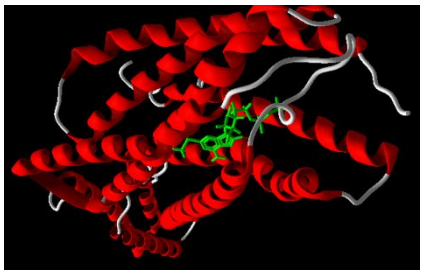


Figure 1 P_2Y_{12} receptor with PDB: ID 4PXZ by which the binding site of the reference ligand and protein will be occupied by the test compounds

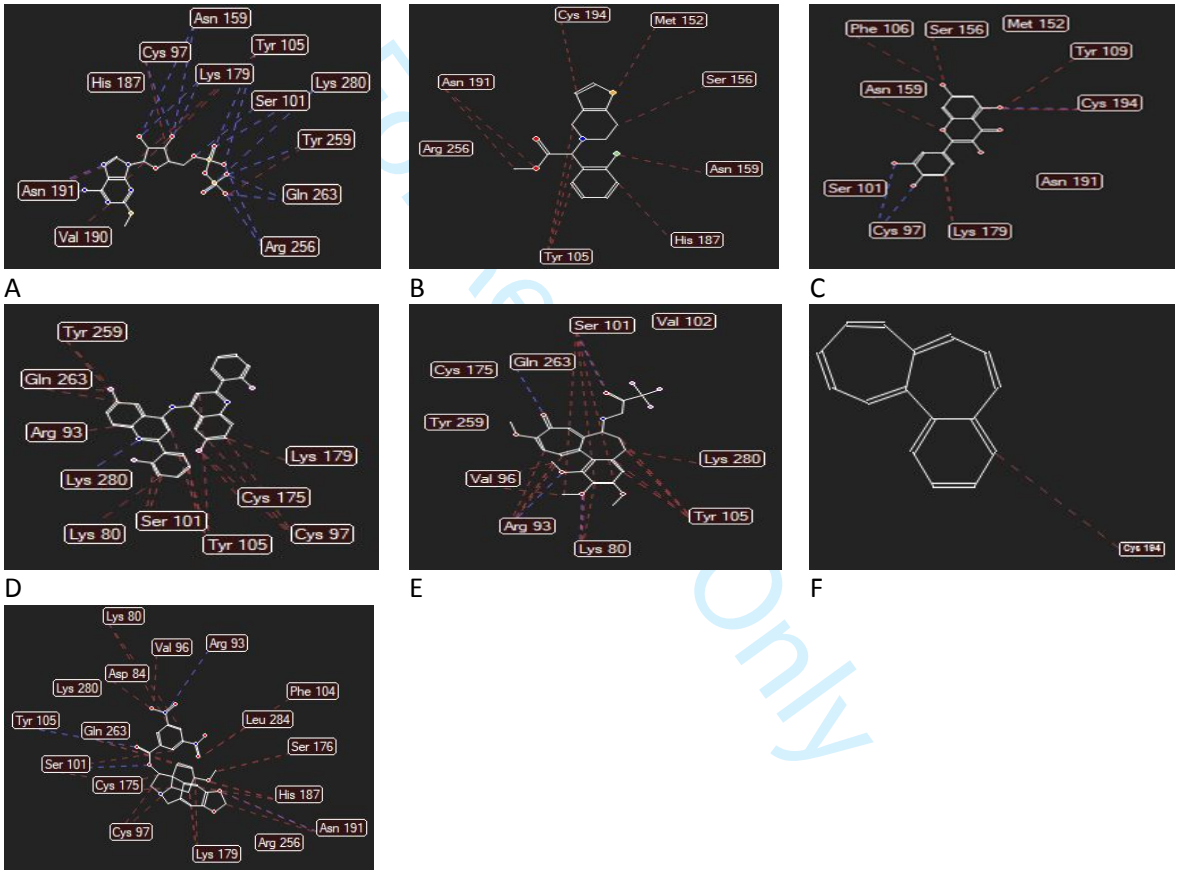


Figure 2 Map of the interaction between shallot skins compound and P_2Y_{12} receptor: (A) ((2R,3S,4R,5R)-5-(6-amino-2-(methylthio)-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl trihydrogen diphosphate, (B) Clopidogrel, (C) Quercetin, (D) Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine, (E) *N*-(trifluoroacetyl)methyl-*N*-deacethyl-Colchicine, (F) Benzo[a]heptalene, and (G) Natalensine, 3,5-dinitrobenzoate

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Subject: JBCPP.2020.0470 - DecisionRevise with Minor Modifications
Attachments: Main-document-Original-Articles-Juni-Ekowati-JBCPP.pdf, Reviewer note on JBCPP.2020.0470.pdf, JBCPP.2020.0470_Proof_hi(3).pdf

15-Jan-2021

Dear Dr. Ekowati:

Thank you again for submitting your manuscript ID JBCPP.2020.0470 entitled "Utilization of domestic waste shallot skins as a source of active pharmaceutical ingredients" to Journal of Basic and Clinical Physiology and Pharmacology (JBCPP). Your manuscript has been reviewed and requires minor modifications prior to acceptance. The comments of the reviewer(s) are included at the bottom of this letter and/or as attached documents. In addition, the similarity index should be less than 30% to be considered published in the journal.

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Once again, thank you for submitting your manuscript to JBCPP. I look forward to receiving your revision.

Kind regards
Dr. Andang Miatmoko
Guest Editor, Journal of Basic and Clinical Physiology and Pharmacology

Reviewer(s)' Comments to Author:

Reviewer: 1

Comments to the Author
Abstract
1. Give the full word before abbreviating it to the highlighted word (CVD, ADMET)
2. Write down the ethanol concentration used for the extraction
3. in abstract you write that the profiling of its active ingredient was presented by GC-MSD. But in the result was GCMS

Introduction, method, discussion:
4.Give the full word before abbreviating for LDL, ROS, ABTS, ADMET, HPLC, GC-MS
5.Add introduction to active ingredient requirements for transdermal.
6.In the discussion, add an explanation of the transdermal requirements so that it can be concluded that the research results are for transdermal
7.Add the references used in the text for Extraction method, Phytochemical screening, Polyphenol Assay, and Quercetin content Assay.
8. The title of table 1 should be made in the middle like tables 2 and 3

Reviewer: 2

Comments to the Author
Please refer to the file attached.

Please also format the reference according to the author guideline.

Revision

Manuscript	Reviewer		Revision
	1	2	
Title: Utilization of domestic waste shallot skins as a source of active pharmaceutical ingredients	The title should be more specific on the aspect investigated in the study, i.e profiling, ADMET prediction.		Shallot skin profilling, computational prediction of physicochemical, ADMET and docking study of its natural components againts P2Y12 receptor
Abstract : Polyphenols and the flavonoid group in plants are known to have several activities, such as relieving CVD. The outer skin of the shallot which is disposed of as waste is known to have an antiplatelet activity which was tested *in vitro* assay. To date, there is no study reported on the ADMET profile and physicochemical properties of the active component of the shallot skins. **Methods:** The extraction of shallot skins was conducted by ultrasonic irradiation using ethanol.. The phytochemical screenings were carried out by TLC and color reaction. The profiling of its active ingredient was presented by GC-MSD, HPLC and spectrophotometry UV-vis. Whereas their physicochemical properties were analyzed by ChemDraw 17.00 program and the ADMET predictions were studied using pkCSM online tool. The MVD program was operated in the docking study on protein P2Y12 (PDB ID 4PXZ). **Results:** The extract showed the presence of polyphenol, flavonoids, quercetin, natalensine-3,5-dinitrobenzoate; bis[2- (2-fluorophenyl)-6-fluoroquinolin-4-yl]amine, benzo[a]heptalene, *N*-(trifluoroacetyl) methyl-*N*-deacethyl-colchicine. The ADMET prediction data displayed that the compounds in the extract have good absorption so that they can be	Abstract : Sentence on the background section : "Polyphenols and the flavonoid group in plants are known to have several activities, such as relieving CVD. The outer skin of the shallot which is disposed of as waste is known to have an antiplatelet activity which was tested in vitro assay. " This sentence might need to be more concise or added with at least 2 examples of disease so it will not be misleading since this study is not only focusing utilization of shallot skin for CVD or antiplatelet . Conclusion : ultrasonic shallot skin extract or ultrasonicated shallot skin extract ? the term should be considered carefully.	Abstract : Abstract 1. Give the full word before abbreviating it to the highlighted word (CVD, ADMET) 2. Write down the ethanol concentration used for the extraction 3. in abstract you write that the profiling of its active ingredient was presented by GC-MSD. But in the result was GCMS	Abstract : Polyphenols and the flavonoid group in plants are known to have several activities, such as relieving Cardio Vascular Disease (CVD), antioxidant and antiinflammatory. The outer skin of the shallot which is disposed of as waste is known to have an antiplatelet activity which was tested *in vitro* assay. To date, there is no study reported on the Absorption, Distribution, Metabolism, Extraction and Toxicity (ADMET) profile and physicochemical properties of the active component of the shallot skins. **Methods:** The extraction of shallot skins was conducted by ultrasonic irradiation using 96% ethanol.. The phytochemical screenings were carried out by TLC and color reaction. The profiling of its active ingredient was presented by GC-MS, HPLC and spectrophotometry UV-vis. Whereas their physicochemical properties were analyzed by ChemDraw 17.00 program and the ADMET predictions were studied using pkCSM online tool. The MVD program was operated in the docking study on protein P2Y12 (PDB ID 4PXZ). **Conclusion:** The ultrasonicated shallot skin extract can be used as additional resource of the active pharmaceutical ingredients and to have the potency to be developed as an oral or transdermal preparation

used in the oral and transdermal routes. Some components in the extract have lower MDS than clopidogrel. **Conclusion:** The ultrasonic shallot skin extract can be used as new source of the active pharmaceutical ingredients and to have the potency to be developed as an oral or transdermal preparation.			
Introduction :			
Cardiac Vascular Disease (CVD), especially coronary heart disease, is a major cause of death worldwide, and patient medical costs continue to increase due to an increase in the number of sufferers [1, 2]. This disease occurs due to impaired blood flow to the myocardium due to platelet aggregation, thrombus, and the accumulation of oxidative damage to LDL by ROS [1,3]. Oxidant stress causes endothelial dysfunction and thrombus formation [4]. Drugs used to treat coronary heart disease are thrombolytic, anti-platelets and. several antioxidant [5, 6]. Although they can treat coronary heart conditions due to thromboembolism, these drugs also have undesirable side effects such as intracranial bleeding, nausea, dyspnea, and it was reported that the patient had resistance to aspirin as an anti-platelet [7,8]. Therefore, alternative therapies are needed to overcome the above problems with mild side effects. Medicinal plants are a source of many chemical compounds that are useful in the pharmaceutical field for novel drug development, including polyphenols, the flavonoid class. The flavonoid group are known to have several activities, such as antibacterial and antioxidant [9, 10]. One of the natural ingredients that is widely used in daily food is	Paragraph 1 and 2 probably are not needed or can be merged together with the 3rd paragraph. This is due to the impression that this study is aimed only for CVD and anti-platelet. Nevertheless, the study covers broader range of activity of the shallot skin.		There are no change in the arrangement of paragraphs 1-3, except for paraphrase of a few sentences because we have adjusted the title of the article to the content, which leads to the use of its natural component as a new source for cardiovascular disease. The paraphrase are : *Cardiac Vascular Disease* (CVD), especially coronary heart disease, greatly contribute to the mortality rate across the globe, and patient medical costs continue to increase due to an increase in the number of sufferers [1, 2]. This disease occurs due to impaired blood flow to the myocardium due to platelet aggregation, thrombus, and the accumulation of oxidative damage to LDL by ROS [1,3]. Oxidant stress causes endothelial dysfunction and thrombus formation [4]. Drugs used to treat coronary heart disease are thrombolytic, anti-platelets and. several antioxidant [5, 6]. Although they can treat coronary heart conditions due to thromboembolism, these drugs also have undesirable side effects such as intracranial bleeding, nausea, dyspnea, and it was reported that the patient had resistance to aspirin as an anti-platelet [7,8]. Therefore, alternative therapies are needed to overcome the above problems with mild side effects. Medicinal plants are a source of many chemical compounds that are useful in the pharmaceutical field for novel drug development, including polyphenols, the

shallots. Shallots have the active compound i.e. polyphenol quercetin as an antibacterial [11]. Not only the tuber part of the shallot, the outer skin of the shallot which is disposed of as waste is also known to have anti-inflammatory [12] and antimicrobial activity [13, 14]. It was also reported that there is antioxidant activity of the ethanolic extract from shallot skins using the ABTS method [15]. Apart from being antibacterial and antioxidant, the activity of shallot extract as an antiplatelet which was tested in vitro has also been reported by Ro, *et al*. [16]. These things indicate that the shallot skins has the potency as an active pharmaceutical ingredient (API). Beside the activity, prospective drug compounds also need to be investigated regarding their physicochemical properties and pharmacokinetic profile, including absorption, distribution, metabolism, and excretion as well as its toxicity (hereinafter referred to as ADMET) to humans [17]. The pharmacokinetic profile of a drug could been influenced by the physicochemical properties [18]. Analysis of the physicochemical properties of drugs in the context of oral bioavailability was first reported by Lipinski, *et al.* which is referred to the "Rule of Five" (Ro5). It includes *Molecular Weight* (MW) <500 g/mol; *Donor H-bond* (HBD) < 5; *H-bond acceptor* (HBA) <10; and the calculation of the logarithm of the partition coefficient of 1-octanol / water (cLogP) <5 [19].			flavonoid class. The flavonoid group are known to have several activities, such as antibacterial and antioxidant [9, 10]. One of the natural ingredients that is widely used in daily food is shallots. Shallots have the active compound i.e. polyphenol quercetin as an antibacterial [11]. Not only the tuber part of the shallot, the outer skin of the shallot which is disposed of as waste is also known to have anti-inflammatory [12] and antimicrobial activity [13, 14]. It was also reported that there is antioxidant activity of the ethanolic extract from shallot skins using the ABTS method [15]. Apart from being antibacterial and antioxidant, the activity of shallot extract as an antiplatelet which was tested in vitro has also been revealed by Ro, *et al.* [16]. These things show that the shallot skins has the potency as an active pharmaceutical ingredient (API). Beside the activity, prospective drug compounds also need to be investigated regarding their physicochemical properties and pharmacokinetic profile, including absorption, distribution, metabolism, and excretion as well as its toxicity (hereinafter referred to as ADMET) to humans [17]. The pharmacokinetic profile of a drug could been influenced by the physicochemical properties [18] Lipnski et al. has formulated several criteria regarding the physical and chemical properties of compounds that can demonstrate its oral bioavalability, consisting of: the ability to accept and donate hydrogen, molecular weight, and log P [19]
Materials and methods The waste from shallot skins obtained from traditional markets is collected, cleaned, then dried at room temperature, and powdered using a blender. Previously, the species of shallot skin were	**Methods** Cleaning process for the shallot waste needs to be detailed, such as was it washed or was it dedusted ?		The waste from shallot skins obtained from traditional markets is collected, washed, then dried at room temperature, and powdered using a blender. Extraction using ethanol p.a (E. Merck).

examined at the Materia Medica Batu institute, and it was found that the shallot species was *Allium cepa* L. Extraction The powder then extracted in ethanol using the ultrasonic method. First, 80 grams of shallot skin powder soaked in 500 ml Erlenmeyer with 350 ml 96% ethanol, then performed ultrasonic at full power and temperature at 40^{o}C for 30 minutes. The extraction product is then filtered using a Buchner funnel under vacuum; the filtrate is accumulated in a different Erlenmeyer. Second, the extracted pulp was put back into the Erlenmeyer 500 ml and added with 300 ml of 96% ethanol. The same process then carried out like the previous process. The extracted filtrate collected and carried out at a rotary evaporator. This ultrasonic extraction was repeated 14 times (until the filtrate did not react with $FeCl_3$, this is indicated by the solution remains clear).	Extraction method : what does it mean by "ultrasonic at full power" ? Materials : reagents used, brand and grades (technical ? p.a ?)		
	Results Table 1 : normalitas or normality? Is there any acceptance criteria for the % obtained ? Table : 2 decimal number for molecular weight should be enough. Explanation on abbreviations used in the table is needed.		Revision for Table 1 & 2 has been done. There are no % criteria acceptance

Juni Ekowati*, Kholidah Febriani, Itsna N. A. Yaqin, Adinda A. Wulandari, Indra H. Mulya, Kholis A. Nofianti, Achmad Syahrani

Shallot skin profilling, computational evaluation of physicochemical properties, ADMET, and molecular docking of its components against P2Y12 receptor

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Abstract

Background: Medicinal plants are a source of many compounds that are useful in the pharmaceutical field for novel drug development. Polyphenols and the flavonoid group in plants are known to have several activities, such as relieving CVD. The outer skin of the shallot which is disposed of as waste is known to have an antiplatelet activity which was tested *in vitro* assay. To date, there is no study reported on the ADMET profile and physicochemical properties of the active component of the shallot skins.
Methods: The extraction of shallot skins was conducted by ultrasonic irradiation using ethanol.. The phytochemical screenings were carried out by TLC and color reaction. The profiling of its active ingredient was presented by GC-MS, HPLC and spectrophotometry UV-vis. Whereas their physicochemical properties were analyzed by ChemDraw 17.00 program and the ADMET predictions were studied using pkCSM online tool. The MVD program was operated in the docking study on protein P2Y12 (PDB ID 4PXZ).
Results: The extract showed the presence of polyphenol, flavonoids, quercetin, natalensine-3,5-dinitrobenzoate; bis[2- (2-fluorophenyl)-6-fluoroquinolin-4-yl]amine, benzo[a]heptalene, *N*-(trifluoroacetyl) methyl-*N*-deacethyl-colchicine. The ADMET prediction data displayed that the compounds in the extract have good absorption so that they can be used in the oral and transdermal routes. Some components in the extract have lower MDS than clopidogrel.
Conclusion: The ultrasonicated shallot skin extract can be used as additional resources of the active pharmaceutical ingredients and to have the potency to be developed as an oral or transdermal preparation.

Keywords: ADMET; polyphenol; pharmaceutical active ingredients; shallot skin; ultrasonic extraction

Introduction

Cardiac Vascular Disease (CVD), especially coronary heart disease, greatly contribute to the mortality rate across the globe, and patient medical costs continue to increase due to an increase in the number of sufferers [1, 2]. This disease occurs due to impaired blood flow to the myocardium due to platelet aggregation, thrombus, and the accumulation of oxidative damage to Low Dendisy Lipid (LDL) by Reactive Oxygen Species (ROS) [1,3]. Oxidant stress causes endothelial dysfunction and thrombus formation [4].

Drugs used to treat coronary heart disease are thrombolytic, anti-platelets and. several antioxidant [5, 6]. Although they can treat coronary heart conditions due to thromboembolism, these drugs also have undesirable side effects such as intracranial bleeding, nausea, dyspnea, and it was reported that the patient had resistance to aspirin as an anti-platelet [7,8]. Therefore, alternative therapies are needed to overcome the above problems with mild side effects.

Medicinal plants are a source of many chemical compounds that are useful in the pharmaceutical field for novel drug development, including polyphenols, the flavonoid class. The flavonoid group are known to have several activities, such as antibacterial and antioxidant [9, 10]. One of the natural ingredients that is widely used in daily food is shallots. Shallots have the active compound i.e. polyphenol quercetin as an antibacterial [11]. Not only the tuber part of the shallot, the outer skin of the shallot which is disposed of as waste is also known to have anti-inflammatory [12] and antimicrobial activity [13, 14]. It was also reported that there is antioxidant activity of the ethanolic extract from shallot skins using the ABTS method [15]. Apart from being antibacterial and antioxidant, the activity of shallot extract as an antiplatelet which was tested in vitro has also been revealed by Ro, *et al.* [16]. These things show that the shallot skins has the potency as an active pharmaceutical ingredient (API).

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Beside the activity, prospective drug compounds also need to be investigated regarding their physicochemical properties and pharmacokinetic profile, including absorption, distribution, metabolism, and excretion as well as its toxicity (hereinafter referred to as ADMET) to humans [17]. The pharmacokinetic profile of a drug could been influenced by the physicochemical properties [18] Lipnski et al. has formulated several criteria regarding the physical and chemical properties of compounds that can demonstrate its oral bioavalability, consisting of: the ability to accept and donate hydrogen, molecular weight, and log P [19].

However, until now, there no research on the physicochemical and pharmacokinetics (ADMET) of the active ingredients of shallot skins. The effects of administering the extract on the gastrointestinal tract also need to be studied to ensure its safety in oral use. Therefore, this study aims to find out the component of shallot skin, its physicochemical properties prediction, and its pharmacokinetics (ADMET) prediction.

Pharmacokinetic profile analysis (ADMET) *in silico* is able to be conducted with the help of the online pkCSM program [20]. Prediction with the online pkCSM program has advantages over other software such as SwissADME, since there are more pharmacokinetic parameters that can be predicted with the online pkCSM program [21, 22] The greater number of parameters will have an impact on the broader information obtained to support the next drug development process.

Based on the research of Ro et al. [16] which states that shallot skins extract has anti-platelet activity in vitro, this study also evaluated the inhibition mechanism of the P2Y12 receptor by *in silico* test (PDB ID 4pxz). P2Y12 is a main receptor and the distinctive P2 goal for clinically allowed antiplatelet drugs (herein named as P2Y12 inhibitors) [17,23].

Materials and methods

The waste from shallot skins obtained from traditional markets is collected, washed, then dried at room temperature, and powdered using a blender. Previously, the species of shallot skin were examined at the Materia Medica Batu institute, and it was found that the shallot species was *Allium cepa* L. Ethanol p.a. (Merck, Germany) was used as solvent of extraction.

Extraction

The powder then extracted in ethanol using the ultrasonic method. First, 80 grams of shallot skin powder soaked in 500 ml Erlenmeyer with 350 ml 96% ethanol, then performed ultrasonic at high power and temperature at 40°C for 30 minutes. The extraction product is then filtered using a Buchner funnel under vacuum; the filtrate is accumulated in a different Erlenmeyer. Second, the extracted pulp was put back into the Erlenmeyer 500 ml and added with 300 ml of 96% ethanol. The same process then carried out like the previous process. The extracted filtrate collected and carried out at a rotary evaporator. This ultrasonic extraction was repeated 14 times (until the filtrate did not react with $FeCl_3$, this is indicated by the solution remains clear).

Phytochemical screening

Screening of flavonoid content was carried out by Thin layer Chromatography (TLC) method, using stationary phase silica gel GF254, the mobile phase butanol-acetic acid glasial-water (4:1:5) and ammonia vapor was used as color reagent. While the poliphenol group was detected by solution $FeCl_3$ 2%.

Chromatographic profile

Examination of chemical compounds carried out by Gas Chromatography – Mass Spectrometry (GC-MS). The sample was weighed 100 mg, dissolved 2 ml of p.a. ethanol, then vortexed for 2 minutes, centrifuged at 3000 rpm for 5 minutes. The filtrate was injected into 0.1 µl GC-MS, under optimum conditions. The instrument used in this study was Agilent 6980N Network GC system with auto sampler with detector Agilent 5973 inert MSD Inlet split 1/100. Run at a temperature of 250°C, 50°C programmed oven for 5 minutes, an increase of 10°C every minute to 280°C for 15 minutes, the rate in the column is 1ml / minute constant, Aux is 250°C, MS Quad 150°C, MS Source 230°C, solvent delay 0 minutes, Wiley library version 7.0, and sample injection volume is 0.1µL

Polyphenol Assay

Polyphenol content test was carried out by spectrophotometric method. A standard solution of Gallic acid was made with a level of 5-25 ppm. Each with a pipette of 1.0 mL put into the vial, added 0.5ml of Folin-Ciocalteu, left for 5 minutes, and then added 2 mL of 10% sodium carbonate solution. After that the absorbance was measured at λ = 770 nm. Sample preparation was carried out by weighing 50 mg of the sample, dissolved in 50 ml of ethanol, then pipetting 1 ml ad 10 ml, the dilution of the sample was piped 1.0 ml and then put into the vial. Furthermore, 0.5 ml of Folin-Ciocalteu was added, the mixture was 5 minutes, then added 2 ml of 10% sodium carbonate solution, the mixture was added 10 minutes before measuring the absorbance (at λ = 765 nm).

Quercetin content Assay

Quercetin content test was carried out by High Pressure Liquid Chromatography (HPLC). Qualitative analysis was performed by comparing the identical retention time of the sample solution chromatogram with the quercetin standard solution chromatogram at the same HPLC conditions. Quercetin standards were made of a standard solution of 50 ppm, pipette 0.6, 0.8, 1, and 1.2 mL, each put into a 5 mL volumetric flask, then diluted with solvent to the mark line, so that the concentrations solutions are 6,8,10 and 12 ppm. The ethanol extract was filtered by a 0.45 µm filter membrane and sonicator for 20 minutes. After that, each solution was injected into the HPLC system at a certain mobile phase and flow rate. The chromatogram is recorded and a calibration curve is made between the area of the peak and the concentration. From the measurement results, the area obtained is recorded, then the levels are calculated using a calibration curve (linear regression equation): y = a + bx.

Physicochemical and ADMET prediction

Physicochemical prediction was carried out by ChemDraw version 17.00 , while the ADMET prediction was carried out by the *online* program, pkCSM that can be accessed from http://biosig.unimelb.edu.au/pkcsm/prediction. These test was ran in ASUS A407UA BV032T Intel core i-3 7th-7020U 2.30 GHz, Windows 10 64 bit.

Docking Study

The docking study was carried out using Molegro Virtual Docker program version 5.5. (Molegro ApS). Some of the steps involved in Molecular Docking program were: obtaining the receptor, ligand preparation, method validation, and docking studies. The

receptor used in this study was the P_2Y_{12} receptor, which can been downloaded from the Protein Data Bank (http://www.rcsb.org). This P_2Y_{12} receptor has the code for PDB 4PXZ with 6AD_1201 [A] as native ligand. The ligands that used in this study were the compounds obtained from shallot skins that was known from GC-MS and quercetin test. The ligands structure drew in ChemDraw 2D version 17.00 and copying into ChemDraw3D version 17.00 to get the 3D structure. The best conformation was determined from MMFF94, and then saved in sybil.mol2 extension. The docking process, native ligands, namely 6AD_1201[A] for P_2Y_{12} was re-docked to the suitable binding site. [22]. The results of the docking studies could be detected visually by comparing the structure of the ligands and receptor P_2Y_{12} (6AD_1202[A]) in the binding site. This resulted in the interaction energy between ligand and receptor was then called as MolDock scores (MDS). The minimum energy denotes the best binding pose between the functional moiety of the ligand and the amino acid residue of the receptor (17).

Results

Extraction

The extraction of shallot skin in 96% ethanol by ultrasonic method produces as much as 13.149 grams of thick extract. The screening phytochemical extract showed that the extract contained flavonoid and poliphenol compounds. The plate TLC showed the black spot, which is product reaction of phenolic moiety with $FeCl_3$. Whereas that plate showed yelloowish spot which showed flavonoid content.

Chromatographic profile

The results of examination of chemical compounds by GC-MS show in Table 1, which show that Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine has the highest percentage. The measurements were also carried out to determine the presence and levels of quercetin and polyphenol (which using Gallic acid as the standard) in the ethanol extract of shallot skins as shown in table 2.

Physicochemical and ADMET Prediction

The *in silico* test was carried out to calculate the physicochemical and pharmacokinetic properties of the compounds contained in the shallot skins as shown in table 3. The molecular weight ranges from 204.272 to 495.479. Log P value, which is a lipophilicity parameter, ranges from 1.988 to 8.417. The bond rotation, HBA, and HBD respectively ranges from 0 (Benzo[a]heptalene) until 7 (N-(trifluoroacetyl)methyl-N-deacethyl-Colchicine and Quercetin), from 0 (Benzo[a]heptalene) until 10 (Natalensine, 3,5-dinitrobenzoate), and from 0 (Benzo[a]heptalene) until 5 (Quercetin).

Docking Study

Figure 1 shows P2Y12 (PDB ID: 4PXZ) with the ligand reference: 6AD-1201. The docking study was carried out in cavity 2 Vol 74.752. While Figure 2 shows the interaction between ligands and amino acids at P_2Y_{12} receptors.

Table 4 revealed the docking results of all tested compounds, ((2R 3S,4R, 5R)-5-(6-amino-2-(methylthio)-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyltrihy-drogen diphosphate against P_2Y_{12} receptor. Clopidogrel, Quercetin and Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine. Quercetin and Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine has the similarity

amino acid with ((2R,3S,4R,5R)-5-(6-amino-2-(methylthio)-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl trihydrogen diphosphate or Clopidogrel.

Discussion

The outer skins of shallot have known to have anti-inflammatory [12], antimicrobial activity [13, 14] and antioxidant activity [15]. Apart from being antibacterial and antioxidant, the activity of shallot extract as an antiplatelet test also tested *in vitro* by Ro, *et al.* [16]. This study was conducted to determine the benefits of domestic waste shallot skins in the provision of raw materials for Active Pharmaceutical Ingredient.

In this study, the extraction of shallot skins carried out by ultrasonic methods, which. the ultrasonic waves were emitted by passing through the medium conducted the waves by inducing vibrational motion of the molecules. The distance between molecules can vary to be closer or farther as the result of the oscillatory motion of the molecules. If the ultrasonic waves in the medium become more intense, a point will be reached where the intramolecular force of the fluid cannot maintain its molecular structure intact. As a result, the molecular structure of the liquid will break down and a cavity is formed [24].

Cavitation is a mechanical activation process that removes the attraction between molecules in the liquid. Once formed, the tiny air bubbles in the cavity will absorb energy from the ultrasonic waves and make the cavity bigger. As the cavity got bigger, the air bubbles inside could no longer absorb ultrasonic energy. Finally, the fluid around the cavity will enter and break the air cavity. The physical characteristic of the irradiated mixture are vital for the cavitation efficiency, and also for the appropriate transfer of acoustic energy to reactants [25, 26, 27].

The ultrasonic profiling using the GC-MS method on shallot skin extract (**table 1**) showed that the content of Natalensine-3,5-dinitrobenzoate was 13.43%; Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine as much as 36.90%, Benzo[a]heptalene as much as 17.43% and *N*-(trifluoroacetyl)methyl-*N*-deacethyl-Colchicine as much as 32.23%.

Jose, *et al.* [28] stated that the phenyl ring and three methoxyl groups of the colchicine derivative contribute additives to the binding strength of colchicine and its analogues with tubulin, which will affect cancer. Kaivan, *et al* [29] stated, the use of colchicine for short-term myocardial infarction would reduce the size of the infarction compared to placebo. From this, it could been seen that shallot skin has great potential as API in the treatment of cancer.

Healthy food contain high levels of natural phenols in fruits, vegetables, cereals, tea and coffee. Fruits such as grapes, apples, pears, cherries, and berries contain up to 200-300 mg of polyphenols per 100 grams of fresh weight (0.2-0.3% w / w) [30]. Literatures reported the biological activities of polyphenols such as antioxidants, antibacterial, antineoplastic, antithrombotic, and vasodilating activities [31].

One example of phenolic compound is ferulic acid, which used as anti-thrombolysis. ADP-induced platelet aggregation test shows that the stronger anti-thrombolysis activity is attributable to its moiety [32, 33]. Therefore, further research on polyphenols as anti-thrombolysis is necessary. In this research, extraction of shallot skin, which carried out ultrasonic, had the amount of polyphenols of 11.14% ± 5.12% w/w in the extract.

In relation to the activity of flavonoids as antiplatelet, structure-activity relationship analysis showed that anti-aggregation activity of flavonoids are highly rely on the C-ring structure that represent the compounds class. If double bond is present between C2 and C3, it increases anti-aggregation activity of flavonoids in case of non-methylated flavonoids. Most active flavonoids possess hydroxyl group at the position 6. Methylation of rings A and B decreases antiplatelet activity [34]. Flavonoid

have several mechanisms of action such as change of bilayer function, change in ROS concentrations and oxidative stress, change of intracellular Ca^{2+} concentration, inhibition of enzymes (phospholipase C, cAMP phosphodiesterase, cyclooxygenase, thromboxane A2 synthase) [35]

Quercetin which is usually found in the food consumed, scientifically reported to have anticancer, antiviral and antimicrobial activity. The use of quercetin is able to decrease CVD risk, LDL (plasma low-density lipoprotein), hypertension, and risk of ischemic heart disease. Its antiplatelet activity also indicated from the ability to inhibit platelet aggregation upon ex vivo post-supplementation and in vitro addition[36]

The absorption of active ingredients in the gastrointestinal tract is affected by the physicochemical characteristic of the drug, the dosage form used, and the anatomy and physiology of the absorption site [37]. Passive diffusion is influenced by the size and shape of the molecule, the rate of ionization, and the solubility of a drug in fat. Meanwhile, active ingredients that are weakly alkaline will be absorbed at a more alkaline pH, namely in the small intestine [38]

Predicting the solubility of active ingredients in water significantly contribute to the drug absorption after oral administration and is a consideration in parenteral drug administration. This is useful in the manipulating and testing process in the drug design and development process and is crucial for the bioavailability of drugs in the blood [39]. The ADMET profile of a drug is also related to its physicochemical properties [40, 41]. In table 3, there are various parameters of physicochemical properties, it is known that the water solubility of Quercetin is 2.925 x 10^{-4} mol/L; Bis [2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine is 3.577 x 10^{-4} mol/L; *N*-(trifluoroacetyl)methyl-N-deacethyl-Colchicine is 3.781 x 10^{-4} mol/L; Benzo[a]heptalene is 3.691 x 10^{-4} mol/L, and Natalensine, 3,5-dinitrobenzoate is 4,896 x 10^{-4} mol/L.

The greater the solubility of the drug in fat (log P), the higher the absorption of the drug into the body's membrane. However, the drug must still be slightly hydrophilic in order for extracellular fluids to be transported and to be distributed throughout the body [42]. Based on Lipinski's law, log P of the active ingredients in the extract, apart from Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine, all of which meet these requirements. Related to Rule of Five [19], the compound Bis [2- (2-fluorophenyl) -6-fluoroquinolin-4-yl] amine is a compound that meets these criteria because the number of hydrogen bond donors (HBD) of each compound <5 and number of hydrogen bond acceptors (HBA) of each compound <10.

tPSA is a molecular descriptor as a parameter for intestinal absorption and drug penetration into the blood brain barrier [43]. From table 3 It is known that two compounds from shallot skin extract, namely Quercetin and Benzo[a]heptalene, have tPSA values <140Å. So, that compounds meet Veber's law requirements. Caco-2 permeability is an absorption model that uses monolayer Caco-2 cells as an in vitro model predicting the absorption of an orally administered drug. [20]. The compounds in the shallot skins have good permeability apart from 3,5-dinitrobenzoate-Natalensine, this indicates that the compounds in the shallot skins have the potency to be used orally and also have the potential if used through the transdermal route.

The volume of distribution (VDss) is the theoretical the volume by which the drug is dissolved in the body. The high Vdss indicates that the majority of the drug is in the tissue [20]. The compounds in the shallot skins are predicted to have different VDss values so that some of the shallot skins compounds will survive in the blood vessels and most of them in the tissues, a good antiplatelet compound is expected more distributed in blood vessels than in tissues.

The drug ability to permeate the Central Nervous System (CNS) was calculated as blood-brain permeability (logPS), which compounds with log PS> -2 are considered to have acess on CNS, while compounds with logPS <-3 are unable to penetrate [20]. Of the five test compounds, Quercetin and *N*-(trifluoroacetyl) methyl-*N*-deacethyl-Colchicine had a logPS value <-3 meaning

the compound was predicted not to permeate the central nervous system. Meanwhile, the other three compounds had a logPS value >-2, which means that the test compounds were predicted to penetrate the central nervous system.

CYP450 substrates, namely CYP2D6 and CYP3A4. are important to identify because CYP450 inhibitors can dramatically alter the pharmacokinetics of drugs metabolized by CYP450 [20]. It was found that apart from Quercetin, the test compound became a CYP3A4 substrate, whereas for CYP2D6, the five compounds did not become a substrate for CYP2D6.

Total clearence is related to bioavailability, and it is important to determine the dosage level to reach a steady-state concentration. Total clearances are expressed in logs (ml/min/kg) [20]. The test results showed that the five test compounds had a total clearance value stated in logs (ml / minute / kg) of -0.275 to 0.489.

Toxicity is a pharmacokinetic parameter that is important to determine before designing a drug in order to create a drug that is not only effective and of good quality, but also safe to use. Many compounds can cause hepatotoxicity such as certain drugs, laboratory chemicals and some of herbal medicines [44]. In the shallot skins extract, it is known that Quercetin and Benzo[a]heptalene compounds are not hepatotoxic. Rat Oral Acute Toxicity (LD50) is the amount of compound given at once that can cause the death of 50% of a group of test animals (moles / kg) [20]. The five test compounds have an LD50 value between 1.573 and 3.335.

Prediction of anti-platelet activity was carried out at the P1Y12 receptor, a G1-protein on platelet membrane surface receptors. It stimulated adenylyl cyclase inhibition and intracellular calcium mobilization. [45, 46]. The first generation of P2Y12 receptor inhibitors is the thienopyridine ticlopidine class, which has the side effect of neutropenia. The second generation is the clopidrogel, which is highly metabolized by the CYP450 enzyme [47].

Based on the *in silico* test against the P_2Y_{12} receptor, it is known that, quercetin and Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine has amino acids similar to ((2R, 3S, 4R, 5R)-5-(6-amino-2-(methylthio)-9H-purine-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyltri-hydrogen diphosphate or clopidogrel, which used as standard. Bis [2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine has an MDS value that is close to the standard, whereas quercetin although it has a greater MDS value than the standard, so that its binding ability is smaller, it still does not eliminate the possibility that quercetin can be used against the P_2Y_{12} receptor as antiplatelet. After going through the *in silico* test phase, the shallot skins extract content should be tested *in vivo*. It was concluded that the ultrasonic shallot skin extract can be used as new source of the active pharmaceutical ingredient and are predicted to have the potency as anti platelet in an oral or transdermal preparation.

Conclusions

The ultrasonic shallot skin extract can be used as new source of the active ingredient for drug development and are predicted to have the potency to be developed as an oral or transdermal preparation..

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Dear Dr. Ekowati:

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Shallot skin profilling, computational evaluation of physicochemical properties, ADMET, and molecular docking of its components against P2Y12 receptor

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Section/Category:	• Cardiovascular Function
Keywords:	ADMET, ultrasonic extraction, shallot skin profilling, cardiovascular disease, P2Y12 receptor, quercetin
Abstract:	Background: Medicinal plants are a source of many compounds that are useful in the pharmaceutical field for novel drug development. Polyphenols and the flavonoid group in plants are known to have several activities, such as relieving CVD. The outer skin of the shallot which is disposed of as waste is known to have an antiplatelet activity which was tested in vitro assay. To date, there is no study reported on the ADMET profile and physicochemical properties of the active component of the shallot skins. Methods: The extraction of shallot skins was conducted by ultrasonic irradiation using ethanol.. The phytochemical screenings were carried out by TLC and color reaction. The profiling of its active ingredient was presented by GC-MS, HPLC and spectrophotometry UV-vis. Whereas their physicochemical properties were analyzed by ChemDraw 17.00 program and the ADMET predictions were studied using pkCSM online tool. The MVD program was operated in the docking study on protein P2Y12 (PDB ID 4PXZ). Results: The extract showed the presence of polyphenol, flavonoids, quercetin, natalensine-3,5-dinitrobenzoate; bis[2- (2-fluorophenyl)-6-fluoroquinolin-4-yl]amine, benzo[a]heptalene, N-(trifluoroacetyl)

methyl-N-deacethyl-colchicine. The ADMET prediction data displayed that the compounds in the extract have good absorption so that they can be used in the oral and transdermal routes. Some components in the extract have lower MDS than clopidogrel.
Conclusion: The ultrasonicated shallot skin extract can be used as additional resources of the active pharmaceutical ingredients and to have the potency to be developed as an oral or transdermal preparation.

Keywords: ADMET; polyphenol; pharmacy active ingredients; shallot skin; ultrasonic extraction

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This section asks for information about the work that you have submitted for publication. The time frame for this reporting is that of the work itself, from the initial conception and planning to the present. The requested information is about resources that you and/or any co-authors received, either directly or indirectly (via your institution), to enable the completion of the work. Checking „No" means that you and any co-authors did the work without receiving any financial support from any third party - that is, the work was supported by funds from the same institution that pays the salary and that institution did not receive third-party funds with which to pay you and/or any co-authors. If you or your institution received funds from a third party to support the work, such as a government granting agency, charitable foundation or commercial sponsor, check „Yes".

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SECTION 1.

IDENTIFYING INFORMATION - SUBMISSION

1. Effective Date (Day-Month-Year) 29-11-2020
2. Manuscript Title Utilization of domestic waste shallot skins as a source of active pharmaceutical ingredients
3. Manuscript Identifying Number (if you know it)

IDENTIFYING INFORMATION - SUBMITTING AUTHOR

1. Given Name (First Name) Juni
2. Surname (Last Name) Ekowati
3. Are you the corresponding author? Yes ☑ No ☐

Corresponding Author's Name Juni Ekowati

IDENTIFYING INFORMATION - CO-AUTHOR

Please add all co-authors of your manuscript. Please ensure that you collected all relevant information of your co-authors correctly, since the submitting author is responsible for the accuracy and completeness of the submitted information.

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Last name	First name	Initials
Febriani	Kholidah	KF
Yaqin	Itsna NA	INAY
Wulandari	Adinda A	AAW
Mulya	Indra H	IHW
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	☐		☐	☐		
	☐		☐	☐		
Consulting fee or honorarium	☐		☐	☐		
	☐		☐	☐		
	☐		☐	☐		
Support for travel to meetings for the study or other purposes	☐		☐	☐		
	☐		☐	☐		
	☐		☐	☐		
Fees for participation in review activities such as data monitoring boards, statistical analysis, end point committees, and the like	☐		☐	☐		
	☐		☐	☐		
	☐		☐	☐		
Payment for writing or reviewing the manuscript	☐		☐	☐		
	☐		☐	☐		
	☐		☐	☐		

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Provision of writing assistance, medicines, equipment, or administrative support	☐		☐	☐		
	☐		☐	☐		
	☐		☐	☐		
Other	☐		☐	☐		
	☐		☐	☐		
	☐		☐	☐		

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RELEVANT FINANCIAL ACTIVITIES OUTSIDE THE SUBMITTED WORK						
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Board Membership	☐		☐	☐		
	☐		☐	☐		
	☐		☐	☐		
	☐		☐	☐		
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	☐		☐	☐		
	☐		☐	☐		
	☐		☐	☐		
Employment	☐		☐	☐		
	☐		☐	☐		
	☐		☐	☐		
	☐		☐	☐		
Expert testimony	☐		☐	☐		
	☐		☐	☐		
	☐		☐	☐		
	☐		☐	☐		

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Grants/grants pending	☐		☐	☐		
	☐		☐	☐		
	☐		☐	☐		
	☐		☐	☐		
Payment for lectures including service on speakers bureaus	☐		☐	☐		
	☐		☐	☐		
	☐		☐	☐		
	☐		☐	☐		
Payment for manuscript preparation	☐		☐	☐		
	☐		☐	☐		
	☐		☐	☐		
	☐		☐	☐		
Patents (planned, pending or issued)	☐		☐	☐		
	☐		☐	☐		
	☐		☐	☐		
	☐		☐	☐		
Royalties	☐		☐	☐		
	☐		☐	☐		
	☐		☐	☐		
	☐		☐	☐		
Payment for development of educational presentations	☐		☐	☐		
	☐		☐	☐		
	☐		☐	☐		
	☐		☐	☐		
Stock/stock options	☐		☐	☐		
	☐		☐	☐		
	☐		☐	☐		
	☐		☐	☐		

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	☐		☐	☐		
	☐		☐	☐		
	☐		☐	☐		
Other	☐		☐	☐		
	☐		☐	☐		
	☐		☐	☐		
	☐		☐	☐		

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Strictlyadhere to the given format.
Statements on Informed consent and Ethical approval may be removed **if not applicable**.

Acknowledgments

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Author contributions

All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests

Authors state no conflict of interest.

Informed consent

Authors state no informed consent

Ethical approval

Authors state no ethical approval

.

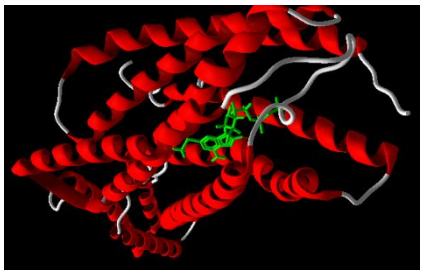


Figure 1 P_2Y_{12} receptor with PDB: ID 4PXZ by which the binding site of the reference ligand and protein will be occupied by the test compounds

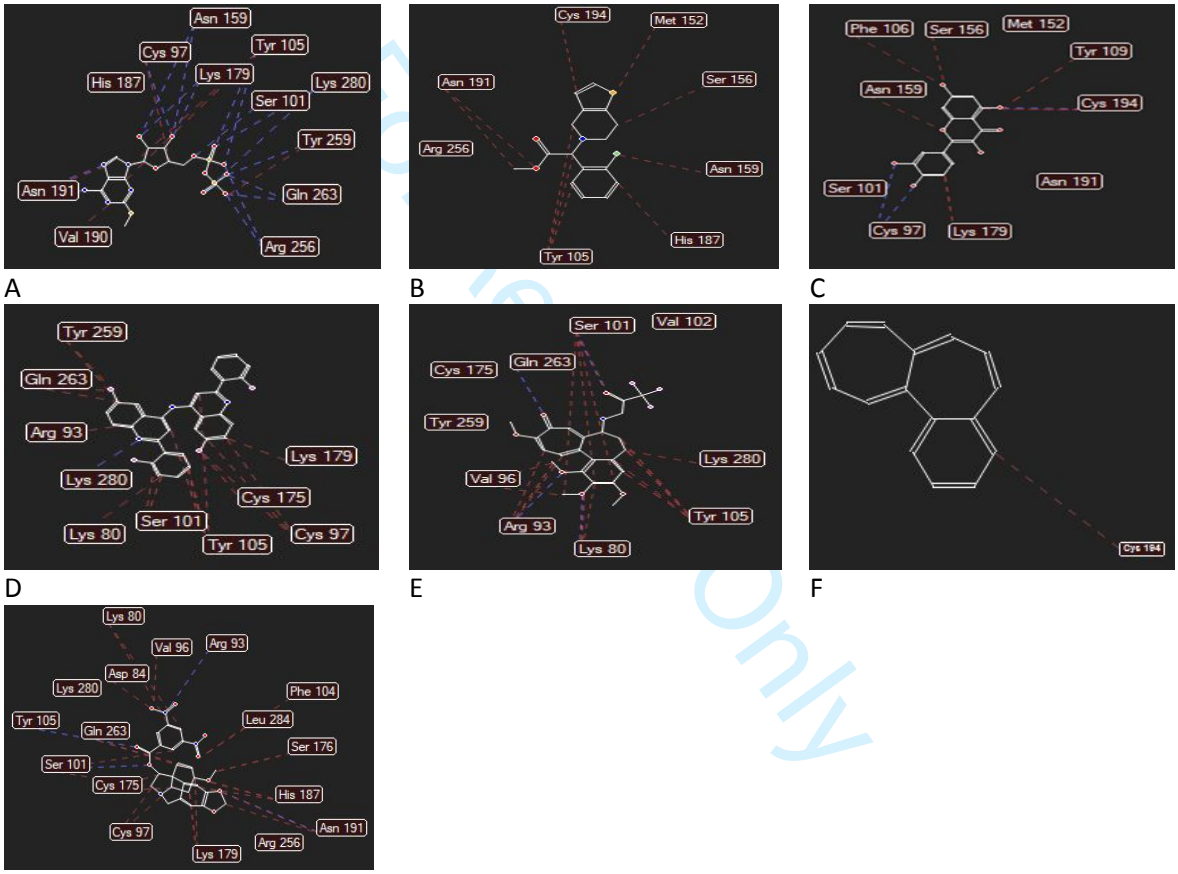


Figure 2 Map of the interaction between shallot skins compound and P_2Y_{12} receptor: (A) ((2R,3S,4R,5R)-5-(6-amino-2-(methylthio)-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl trihydrogen diphosphate, (B) Clopidogrel, (C) Quercetin, (D) Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine, (E) *N*-(trifluoroacetyl)methyl-*N*-deacethyl-Colchicine, (F) Benzo[a]heptalene, and (G) Natalensine, 3,5-dinitrobenzoate

Revision

Manuscript	Reviewer		Revision
	1	2	
Title: Utilization of domestic waste shallot skins as a source of active pharmaceutical ingredients	The title should be more specific on the aspect investigated in the study, i.e profiling, ADMET prediction.		Shallot skin profilling, computational prediction of physicochemical, ADMET and docking study of its natural components againts P2Y12 receptor
Abstract : Polyphenols and the flavonoid group in plants are known to have several activities, such as relieving CVD. The outer skin of the shallot which is disposed of as waste is known to have an antiplatelet activity which was tested *in vitro* assay. To date, there is no study reported on the ADMET profile and physicochemical properties of the active component of the shallot skins. **Methods:** The extraction of shallot skins was conducted by ultrasonic irradiation using ethanol.. The phytochemical screenings were carried out by TLC and color reaction. The profiling of its active ingredient was presented by GC-MSD, HPLC and spectrophotometry UV-vis. Whereas their physicochemical properties were analyzed by ChemDraw 17.00 program and the ADMET predictions were studied using pkCSM online tool. The MVD program was operated in the docking study on protein P2Y12 (PDB ID 4PXZ). **Results:** The extract showed the presence of polyphenol, flavonoids, quercetin, natalensine-3,5-dinitrobenzoate; bis[2- (2-fluorophenyl)-6-fluoroquinolin-4-yl]amine, benzo[a]heptalene, *N*-(trifluoroacetyl) methyl-*N*-deacethyl-colchicine. The ADMET prediction data displayed that the compounds in the extract have good absorption so that they can be	Abstract : Sentence on the background section : "Polyphenols and the flavonoid group in plants are known to have several activities, such as relieving CVD. The outer skin of the shallot which is disposed of as waste is known to have an antiplatelet activity which was tested in vitro assay. " This sentence might need to be more concise or added with at least 2 examples of disease so it will not be misleading since this study is not only focusing utilization of shallot skin for CVD or antiplatelet . Conclusion : ultrasonic shallot skin extract or ultrasonicated shallot skin extract ? the term should be considered carefully.	Abstract : Abstract 1. Give the full word before abbreviating it to the highlighted word (CVD, ADMET) 2. Write down the ethanol concentration used for the extraction 3. in abstract you write that the profiling of its active ingredient was presented by GC-MSD. But in the result was GCMS	Abstract : Polyphenols and the flavonoid group in plants are known to have several activities, such as relieving Cardio Vascular Disease (CVD), antioxidant and antiinflammatory. The outer skin of the shallot which is disposed of as waste is known to have an antiplatelet activity which was tested *in vitro* assay. To date, there is no study reported on the Absorption, Distribution, Metabolism, Extraction and Toxicity (ADMET) profile and physicochemical properties of the active component of the shallot skins. **Methods:** The extraction of shallot skins was conducted by ultrasonic irradiation using 96% ethanol.. The phytochemical screenings were carried out by TLC and color reaction. The profiling of its active ingredient was presented by GC-MS, HPLC and spectrophotometry UV-vis. Whereas their physicochemical properties were analyzed by ChemDraw 17.00 program and the ADMET predictions were studied using pkCSM online tool. The MVD program was operated in the docking study on protein P2Y12 (PDB ID 4PXZ). **Conclusion:** The ultrasonicated shallot skin extract can be used as additional resource of the active pharmaceutical ingredients and to have the potency to be developed as an oral or transdermal preparation

used in the oral and transdermal routes. Some components in the extract have lower MDS than clopidogrel. **Conclusion:** The ultrasonic shallot skin extract can be used as new source of the active pharmaceutical ingredients and to have the potency to be developed as an oral or transdermal preparation.			
Introduction :			
Cardiac Vascular Disease (CVD), especially coronary heart disease, is a major cause of death worldwide, and patient medical costs continue to increase due to an increase in the number of sufferers [1, 2]. This disease occurs due to impaired blood flow to the myocardium due to platelet aggregation, thrombus, and the accumulation of oxidative damage to LDL by ROS [1,3]. Oxidant stress causes endothelial dysfunction and thrombus formation [4]. Drugs used to treat coronary heart disease are thrombolytic, anti-platelets and. several antioxidant [5, 6]. Although they can treat coronary heart conditions due to thromboembolism, these drugs also have undesirable side effects such as intracranial bleeding, nausea, dyspnea, and it was reported that the patient had resistance to aspirin as an anti-platelet [7,8]. Therefore, alternative therapies are needed to overcome the above problems with mild side effects. Medicinal plants are a source of many chemical compounds that are useful in the pharmaceutical field for novel drug development, including polyphenols, the flavonoid class. The flavonoid group are known to have several activities, such as antibacterial and antioxidant [9, 10]. One of the natural ingredients that is widely used in daily food is	Paragraph 1 and 2 probably are not needed or can be merged together with the 3rd paragraph. This is due to the impression that this study is aimed only for CVD and anti-platelet. Nevertheless, the study covers broader range of activity of the shallot skin.		There are no change in the arrangement of paragraphs 1-3, except for paraphrase of a few sentences because we have adjusted the title of the article to the content, which leads to the use of its natural component as a new source for cardiovascular disease. The paraphrase are : *Cardiac Vascular Disease* (CVD), especially coronary heart disease, greatly contribute to the mortality rate across the globe, and patient medical costs continue to increase due to an increase in the number of sufferers [1, 2]. This disease occurs due to impaired blood flow to the myocardium due to platelet aggregation, thrombus, and the accumulation of oxidative damage to LDL by ROS [1,3]. Oxidant stress causes endothelial dysfunction and thrombus formation [4]. Drugs used to treat coronary heart disease are thrombolytic, anti-platelets and. several antioxidant [5, 6]. Although they can treat coronary heart conditions due to thromboembolism, these drugs also have undesirable side effects such as intracranial bleeding, nausea, dyspnea, and it was reported that the patient had resistance to aspirin as an anti-platelet [7,8]. Therefore, alternative therapies are needed to overcome the above problems with mild side effects. Medicinal plants are a source of many chemical compounds that are useful in the pharmaceutical field for novel drug development, including polyphenols, the

shallots. Shallots have the active compound i.e. polyphenol quercetin as an antibacterial [11]. Not only the tuber part of the shallot, the outer skin of the shallot which is disposed of as waste is also known to have anti-inflammatory [12] and antimicrobial activity [13, 14]. It was also reported that there is antioxidant activity of the ethanolic extract from shallot skins using the ABTS method [15]. Apart from being antibacterial and antioxidant, the activity of shallot extract as an antiplatelet which was tested in vitro has also been reported by Ro, *et al*. [16]. These things indicate that the shallot skins has the potency as an active pharmaceutical ingredient (API). Beside the activity, prospective drug compounds also need to be investigated regarding their physicochemical properties and pharmacokinetic profile, including absorption, distribution, metabolism, and excretion as well as its toxicity (hereinafter referred to as ADMET) to humans [17]. The pharmacokinetic profile of a drug could been influenced by the physicochemical properties [18]. Analysis of the physicochemical properties of drugs in the context of oral bioavailability was first reported by Lipinski, *et al*. which is referred to the "Rule of Five" (Ro5). It includes *Molecular Weight* (MW) <500 g/mol; *Donor H-bond* (HBD) < 5; *H-bond acceptor* (HBA) <10; and the calculation of the logarithm of the partition coefficient of 1-octanol / water (cLogP) <5 [19].			flavonoid class. The flavonoid group are known to have several activities, such as antibacterial and antioxidant [9, 10]. One of the natural ingredients that is widely used in daily food is shallots. Shallots have the active compound i.e. polyphenol quercetin as an antibacterial [11]. Not only the tuber part of the shallot, the outer skin of the shallot which is disposed of as waste is also known to have anti-inflammatory [12] and antimicrobial activity [13, 14]. It was also reported that there is antioxidant activity of the ethanolic extract from shallot skins using the ABTS method [15]. Apart from being antibacterial and antioxidant, the activity of shallot extract as an antiplatelet which was tested in vitro has also been revealed by Ro, *et al*. [16]. These things show that the shallot skins has the potency as an active pharmaceutical ingredient (API). Beside the activity, prospective drug compounds also need to be investigated regarding their physicochemical properties and pharmacokinetic profile, including absorption, distribution, metabolism, and excretion as well as its toxicity (hereinafter referred to as ADMET) to humans [17]. The pharmacokinetic profile of a drug could been influenced by the physicochemical properties [18] Lipnski et al. has formulated several criteria regarding the physical and chemical properties of compounds that can demonstrate its oral bioavalability, consisting of: the ability to accept and donate hydrogen, molecular weight, and log P [19]
Materials and methods The waste from shallot skins obtained from traditional markets is collected, cleaned, then dried at room temperature, and powdered using a blender. Previously, the species of shallot skin were	**Methods** Cleaning process for the shallot waste needs to be detailed, such as was it washed or was it dedusted ?		The waste from shallot skins obtained from traditional markets is collected, washed, then dried at room temperature, and powdered using a blender. Extraction using ethanol p.a (E. Merck).

examined at the Materia Medica Batu institute, and it was found that the shallot species was *Allium cepa* L. Extraction The powder then extracted in ethanol using the ultrasonic method. First, 80 grams of shallot skin powder soaked in 500 ml Erlenmeyer with 350 ml 96% ethanol, then performed ultrasonic at full power and temperature at 40^{o}C for 30 minutes. The extraction product is then filtered using a Buchner funnel under vacuum; the filtrate is accumulated in a different Erlenmeyer. Second, the extracted pulp was put back into the Erlenmeyer 500 ml and added with 300 ml of 96% ethanol. The same process then carried out like the previous process. The extracted filtrate collected and carried out at a rotary evaporator. This ultrasonic extraction was repeated 14 times (until the filtrate did not react with $FeCl_3$, this is indicated by the solution remains clear).	Extraction method : what does it mean by "ultrasonic at full power" ? Materials : reagents used, brand and grades (technical ? p.a ?)		
	Results Table 1 : normalitas or normality? Is there any acceptance criteria for the % obtained ? Table : 2 decimal number for molecular weight should be enough. Explanation on abbreviations used in the table is needed.		Revision for Table 1 & 2 has been done. There are no % criteria acceptance

Table 1 Examination of chemical compounds by GC-MS

RT	Compound Name	%Normality	Qual
28.34	Natalensine, 3,5-dinitrobenzoate	13.43%	30
28.85 dan 33.05	Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine	36.90%	35
29.13	Benzo[a]heptalene	17.43%	95
29.30	N-(trifluoroacetyl)methyl-N-deacethyl-Colchicine	32.23%	35

Table 2 Quercetin and polyphenol content in extract

Content	Quantity in extract Mean %(b/b) ± RPD
Quercetin	4.61 ± 2.43
Polyphenol	11.14 ± 5.12

RPD : relative percent difference

Table 3 Physicochemical and pharmacokinetic prediction

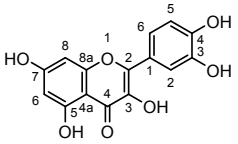
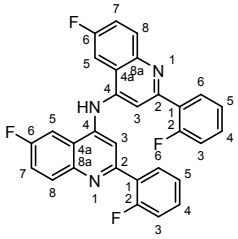
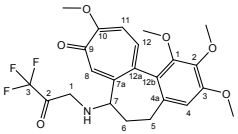
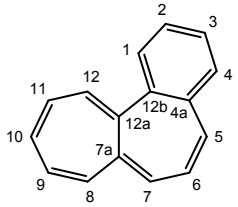
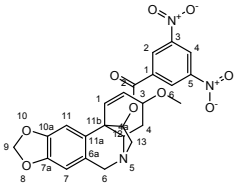
Compound	Structure	MW	Boiling Point (K)	Melting Point (K)	Log P	Bond rotation	HBA	HBD	PSA	Absorption	
										Water solubility	Intestinal absorption
Quercetin		302.238	1135.37	970.62	1.988	1	7	5	122.108	-2.925	77.207
Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine		495.479	1225.17	896.7	8.417	4	3	1	208.617	-3.577	93.87
N-(trifluoroacetyl)methyl-N-deacethyl-Colchicine		467.44	1076.21	754.77	3.4565	7	7	1	187.966	-3.781	93.15
Benzo[a]heptalene		204.272	643	367.64	2.24	0	0	0	94.932	-3.691	99.286
Natalensine, 3,5-dinitrobenzoate		495.444	-	-	2.8678	5	10	0	203.836	-4.896	94.254

Table 3 Physicochemical and pharmacokinetic prediction (continued)

Compound	Absorption		Distribution			Metabolism		Excretion	Toxicity		
	Skin permeability	Caco-2 permeability	VDss (human)	BBB permeability	CNS permeability	CYP2D6 substrate	CYP3A4 substrate	Total clearance	AMES Toxicity	Hepato toxicity	LD50
Quercetin	-2.735	-0.229	1.559	-1.098	-3.065	No	No	0.407	No	No	2.471
Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine	-2.735	1.165	-0.826	0.343	-0.819	No	Yes	-0.275	No	Yes	3.335
N-(trifluoroacetyl)methyl-N-deacethyl-Colchicine	-2.716	1.139	0.833	-1.231	-3.21	No	Yes	0.479	No	Yes	2.992
Benzo[a]heptalene	-1.645	1.539	0.207	0.619	-1.986	No	Yes	0.205	No	No	1.573
Natalensine, 3,5-dinitrobenzoate	-2.737	-0.006	0.235	-0.891	-2.55	No	Yes	0.489	Yes	Yes	2.252

Table 4 Results of the docking of the test ligand at the binding site of P2Y12 receptor

Compound Name	MDS	Amino acid
((2R,3S,4R,5R)-5-(6-amino-2-(methylthio)-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl trihydrogen diphosphate	-157.334	Arg 256, Asn 159, Asn 191, Cys 97, Gln 263, His 187, Lys 179, Lys 280, Ser 101, Tyr 105, Tyr 259, Val 190
Clopidogrel	-128.010	Arg 256, Asn 159, Asn 191, Cys 194, His 187 Met 152, Ser 156, Tyr 105
Quercetin	-116.863	Asn 159, Asn 191, Cys 97, Cys 194, Lys 179, Met 152, Phe 106, Ser 101, Ser 156, Tyr 109
Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine	-157.041	Arg 93, Cys 97, Cys 175, Gln 263, Lys 80, Lys 179, Lys 280, Ser 101, Tyr 105, Tyr 259
N-(trifluoroacetyl)methyl-N-deacethyl-Colchicine	-122.252	Arg 93, Cys 175, Gln 263, Lys 80, Lys 280, Ser 101, Tyr 105, Tyr 259, Val 96, Val 102
Benzo[a]heptalene	-101.726	Cys 194
Natalensine, 3,5-dinitrobenzoate	-150.457	Arg 93, Arg 256, Asn 191, Asp 84, Cys 97, Cys 175, Gln 263, His 187, Leu 284, Lys 80, Lys 179, Lys 280, Phe 104,Ser 105, Tyr 105, Val 96

DE GRUYTER JBCPP. Year; short doi

Juni Ekowati*, Kholidah Febriani, Itsna N. A. Yaqin, Adinda A. Wulandari, Indra H. Mulya, Kholis A. Nofianti, Achmad Syahrani

Shallot skin profilling, computational evaluation of physicochemical properties, ADMET, and molecular docking of its components against P2Y12 receptor

DOI: https://doi.org/xxxxx/xxxxxxxxxx
Received: Month Day, Year; **Accepted**: Month Day, Year

Abstract

Background: Medicinal plants are a source of many compounds that are useful in the pharmaceutical field for novel drug development. Polyphenols and the flavonoid group in plants are known to have several activities, such as relieving CVD. The outer skin of the shallot which is disposed of as waste is known to have an antiplatelet activity which was tested *in vitro* assay. To date, there is no study reported on the ADMET profile and physicochemical properties of the active component of the shallot skins.
Methods: The extraction of shallot skins was conducted by ultrasonic irradiation using ethanol.. The phytochemical screenings were carried out by TLC and color reaction. The profiling of its active ingredient was presented by GC-MS, HPLC and spectrophotometry UV-vis. Whereas their physicochemical properties were analyzed by ChemDraw 17.00 program and the ADMET predictions were studied using pkCSM online tool. The MVD program was operated in the docking study on protein P2Y12 (PDB ID 4PXZ).
Results: The extract showed the presence of polyphenol, flavonoids, quercetin, natalensine-3,5-dinitrobenzoate; bis[2- (2-fluorophenyl)-6-fluoroquinolin-4-yl]amine, benzo[a]heptalene, *N*-(trifluoroacetyl) methyl-*N*-deacethyl-colchicine. The ADMET prediction data displayed that the compounds in the extract have good absorption so that they can be used in the oral and transdermal routes. Some components in the extract have lower MDS than clopidogrel.
Conclusion: The ultrasonicated shallot skin extract can be used as additional resources of the active pharmaceutical ingredients and to have the potency to be developed as an oral or transdermal preparation.

Keywords:shallot skin profilling; ultrasonic extraction; P2Y12 receptor; quercetin; cardiovascular disease , ADMET

Introduction

Cardiac Vascular Disease (CVD), especially coronary heart disease, greatly contribute to the mortality rate across the globe, and patient medical costs continue to increase due to an increase in the number of sufferers [1, 2]. This disease occurs due to impaired blood flow to the myocardium due to platelet aggregation, thrombus, and the accumulation of oxidative damage to Low Dendisy Lipid (LDL) by Reactive Oxygen Species (ROS) [1,3]. Oxidant stress causes endothelial dysfunction and thrombus formation [4].

Drugs used to treat coronary heart disease are thrombolytic, anti-platelets and. several antioxidant [5, 6]. Although they can treat coronary heart conditions due to thromboembolism, these drugs also have undesirable side effects such as intracranial bleeding, nausea, dyspnea, and it was reported that the patient had resistance to aspirin as an anti-platelet [7,8]. Therefore, alternative therapies are needed to overcome the above problems with mild side effects.

Medicinal plants are a source of many chemical compounds that are useful in the pharmaceutical field for novel drug development, including polyphenols, the flavonoid class. The flavonoid group are known to have several activities, such as antibacterial and antioxidant [9, 10]. One of the natural ingredients that is widely used in daily food is shallots. Shallots have the active compound i.e. polyphenol quercetin as an antibacterial [11]. Not only the tuber part of the shallot, the outer skin of the shallot which is disposed of as waste is also known to have anti-inflammatory [12] and antimicrobial activity [13, 14]. It was also reported that there is antioxidant activity of the ethanolic extract from shallot skins using the 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method [15]. Apart from being antibacterial and antioxidant, the activity of shallot extract as an antiplatelet which was tested in vitro has also been revealed by Ro, *et al.* [16]. These things show that the shallot skins has the potency as an active pharmaceutical ingredient (API).

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Beside the activity, prospective drug compounds also need to be investigated regarding their physicochemical properties and pharmacokinetic profile, including absorption, distribution, metabolism, and excretion as well as its toxicity (hereinafter referred to as ADMET) to humans [17]. The pharmacokinetic profile of a drug could been influenced by the physicochemical properties [18] Lipnski et al. has formulated several criteria regarding the physical and chemical properties of compounds that can demonstrate its oral bioavalability, consisting of: the ability to accept and donate hydrogen, molecular weight, and log P [19].

However, until now, there no research on the physicochemical and pharmacokinetics (ADMET) of the active ingredients of shallot skins. The effects of administering the extract on the gastrointestinal tract also need to be studied to ensure its safety in oral use. Therefore, this study aims to find out the component of shallot skin, its physicochemical properties prediction, and its pharmacokinetics (ADMET) prediction.

Pharmacokinetic profile analysis (ADMET) *in silico* is able to be conducted with the help of the online pkCSM program [20]. Prediction with the online pkCSM program has advantages over other software such as SwissADME, since there are more pharmacokinetic parameters that can be predicted with the online pkCSM program [21, 22] The greater number of parameters will have an impact on the broader information obtained to support the next drug development process.

Based on the research of Ro et al. [16] which states that shallot skins extract has anti-platelet activity in vitro, this study also evaluated the inhibition mechanism of the P2Y12 receptor by *in silico* test (PDB ID 4pxz). P2Y12 is a main receptor and the distinctive P2 goal for clinically allowed antiplatelet drugs (herein named as P2Y12 inhibitors) [17,23].

Materials and methods

The waste from shallot skins obtained from traditional markets is collected, washed, then dried at room temperature, and powdered using a blender. Previously, the species of shallot skin were examined at the Materia Medica Batu institute, and it was found that the shallot species was *Allium cepa* L. Ethanol p.a. (Merck, Germany) was used as solvent of extraction.

Extraction

The powder then extracted in ethanol using the ultrasonic method. First, 80 grams of shallot skin powder soaked in 500 ml Erlenmeyer with 350 ml 96% ethanol, then performed ultrasonic at high power and temperature at 40°C for 30 minutes. The extraction product is then filtered using a Buchner funnel under vacuum; the filtrate is accumulated in a different Erlenmeyer. Second, the extracted pulp was put back into the Erlenmeyer 500 ml and added with 300 ml of 96% ethanol. The same process then carried out like the previous process. The extracted filtrate collected and carried out at a rotary evaporator. This ultrasonic extraction was repeated 14 times (until the filtrate did not react with $FeCl_3$, this is indicated by the solution remains clear).

Phytochemical screening

Screening of flavonoid content was carried out by Thin layer Chromatography (TLC) method, using stationary phase silica gel GF254, the mobile phase butanol-acetic acid glasial-water (4:1:5) and ammonia vapor was used as color reagent. While the poliphenol group was detected by solution $FeCl_3$ 2%.

Chromatographic profile

Examination of chemical compounds carried out by Gas Chromatography – Mass Spectrometry (GC-MS). The sample was weighed 100 mg, dissolved 2 ml of p.a. ethanol, then vortexed for 2 minutes, centrifuged at 3000 rpm for 5 minutes. The filtrate was injected into 0.1 µl GC-MS, under optimum conditions. The instrument used in this study was Agilent 6980N Network GC system with auto sampler with detector Agilent 5973 inert MSD Inlet split 1/100. Run at a temperature of 250°C, 50°C programmed oven for 5 minutes, an increase of 10°C every minute to 280°C for 15 minutes, the rate in the column is 1ml / minute constant, Aux is 250°C, MS Quad 150°C, MS Source 230°C, solvent delay 0 minutes, Wiley library version 7.0, and sample injection volume is 0.1µL

Polyphenol Assay

Polyphenol content test was carried out by spectrophotometric method. A standard solution of Gallic acid was made with a level of 5-25 ppm. Each with a pipette of 1.0 mL put into the vial, added 0.5ml of Folin-Ciocalteu, left for 5 minutes, and then added 2 mL of 10% sodium carbonate solution. After that the absorbance was measured at λ = 770 nm. Sample preparation was carried out by weighing 50 mg of the sample, dissolved in 50 ml of ethanol, then pipetting 1 ml ad 10 ml, the dilution of the sample was piped 1.0 ml and then put into the vial. Furthermore, 0.5 ml of Folin-Ciocalteu was added, the mixture was 5 minutes, then added 2 ml of 10% sodium carbonate solution, the mixture was added 10 minutes before measuring the absorbance (at λ = 765 nm).

Quercetin content Assay

Quercetin content test was carried out by High Pressure Liquid Chromatography (HPLC). Qualitative analysis was performed by comparing the identical retention time of the sample solution chromatogram with the quercetin standard solution chromatogram at the same HPLC conditions. Quercetin standards were made of a standard solution of 50 ppm, pipette 0.6, 0.8, 1, and 1.2 mL, each put into a 5 mL volumetric flask, then diluted with solvent to the mark line, so that the concentrations solutions are 6,8,10 and 12 ppm. The ethanol extract was filtered by a 0.45 µm filter membrane and sonicator for 20 minutes. After that, each solution was injected into the HPLC system at a certain mobile phase and flow rate. The chromatogram is recorded and a calibration curve is made between the area of the peak and the concentration. From the measurement results, the area obtained is recorded, then the levels are calculated using a calibration curve (linear regression equation): y = a + bx.

Physicochemical and ADMET prediction

Physicochemical prediction was carried out by ChemDraw version 17.00 , while the ADMET prediction was carried out by the *online* program, pkCSM that can be accessed from http://biosig.unimelb.edu.au/pkcsm/prediction. These test was ran in ASUS A407UA BV032T Intel core i-3 7th-7020U 2.30 GHz, Windows 10 64 bit.

Docking Study

The docking study was carried out using Molegro Virtual Docker program version 5.5. (Molegro ApS). Some of the steps involved in Molecular Docking program were: obtaining the receptor, ligand preparation, method validation, and docking studies. The

receptor used in this study was the P_2Y_{12} receptor, which can been downloaded from the Protein Data Bank (http://www.rcsb.org). This P_2Y_{12} receptor has the code for PDB 4PXZ with 6AD_1201 [A] as native ligand. The ligands that used in this study were the compounds obtained from shallot skins that was known from GC-MS and quercetin test. The ligands structure drew in ChemDraw 2D version 17.00 and copying into ChemDraw3D version 17.00 to get the 3D structure. The best conformation was determined from MMFF94, and then saved in sybil.mol2 extension. The docking process, native ligands, namely 6AD_1201[A] for P_2Y_{12} was re-docked to the suitable binding site. [22]. The results of the docking studies could be detected visually by comparing the structure of the ligands and receptor P_2Y_{12} (6AD_1202[A]) in the binding site. This resulted in the interaction energy between ligand and receptor was then called as MolDock scores (MDS). The minimum energy denotes the best binding pose between the functional moiety of the ligand and the amino acid residue of the receptor (17).

Results

Extraction

The extraction of shallot skin in 96% ethanol by ultrasonic method produces as much as 13.149 grams of thick extract. The screening phytochemical extract showed that the extract contained flavonoid and poliphenol compounds. The plate TLC showed the black spot, which is product reaction of phenolic moiety with $FeCl_3$. Whereas that plate showed yelloowish spot which showed flavonoid content.

Chromatographic profile

The results of examination of chemical compounds by GC-MS show in Table 1, which show that Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine has the highest percentage. The measurements were also carried out to determine the presence and levels of quercetin and polyphenol (which using Gallic acid as the standard) in the ethanol extract of shallot skins as shown in table 2.

Physicochemical and ADMET Prediction

The *in silico* test was carried out to calculate the physicochemical and pharmacokinetic properties of the compounds contained in the shallot skins as shown in table 3. The molecular weight ranges from 204.272 to 495.479. Log P value, which is a lipophilicity parameter, ranges from 1.988 to 8.417. The bond rotation, HBA, and HBD respectively ranges from 0 (Benzo[a]heptalene) until 7 (N-(trifluoroacetyl)methyl-N-deacethyl-Colchicine and Quercetin), from 0 (Benzo[a]heptalene) until 10 (Natalensine, 3,5-dinitrobenzoate), and from 0 (Benzo[a]heptalene) until 5 (Quercetin).

Docking Study

Figure 1 shows P2Y12 (PDB ID: 4PXZ) with the ligand reference: 6AD-1201. The docking study was carried out in cavity 2 Vol 74.752. While Figure 2 shows the interaction between ligands and amino acids at P_2Y_{12} receptors.

Table 4 revealed the docking results of all tested compounds, ((2R 3S,4R, 5R)-5-(6-amino-2-(methylthio)-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyltrihy-drogen diphosphate against P_2Y_{12} receptor. Clopidogrel, Quercetin and Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine. Quercetin and Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine has the similarity

amino acid with ((2R,3S,4R,5R)-5-(6-amino-2-(methylthio)-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl trihydrogen diphosphate or Clopidogrel.

Discussion

The outer skins of shallot have known to have anti-inflammatory [12], antimicrobial activity [13, 14] and antioxidant activity [15]. Apart from being antibacterial and antioxidant, the activity of shallot extract as an antiplatelet test also tested *in vitro* by Ro, *et al.* [16]. This study was conducted to determine the benefits of domestic waste shallot skins in the provision of raw materials for Active Pharmaceutical Ingredient.

In this study, the extraction of shallot skins carried out by ultrasonic methods, which. the ultrasonic waves were emitted by passing through the medium conducted the waves by inducing vibrational motion of the molecules. The distance between molecules can vary to be closer or farther as the result of the oscillatory motion of the molecules. If the ultrasonic waves in the medium become more intense, a point will be reached where the intramolecular force of the fluid cannot maintain its molecular structure intact. As a result, the molecular structure of the liquid will break down and a cavity is formed [24].

Cavitation is a mechanical activation process that removes the attraction between molecules in the liquid. Once formed, the tiny air bubbles in the cavity will absorb energy from the ultrasonic waves and make the cavity bigger. As the cavity got bigger, the air bubbles inside could no longer absorb ultrasonic energy. Finally, the fluid around the cavity will enter and break the air cavity. The physical characteristic of the irradiated mixture are vital for the cavitation efficiency, and also for the appropriate transfer of acoustic energy to reactants [25, 26, 27].

The ultrasonic profiling using the GC-MS method on shallot skin extract (**table 1**) showed that the content of Natalensine-3,5-dinitrobenzoate was 13.43%; Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine as much as 36.90%, Benzo[a]heptalene as much as 17.43% and *N*-(trifluoroacetyl)methyl-*N*-deacethyl-Colchicine as much as 32.23%.

Jose, *et al.* [28] stated that the phenyl ring and three methoxyl groups of the colchicine derivative contribute additives to the binding strength of colchicine and its analogues with tubulin, which will affect cancer. Kaivan, *et al* [29] stated, the use of colchicine for short-term myocardial infarction would reduce the size of the infarction compared to placebo. From this, it could been seen that shallot skin has great potential as API in the treatment of cancer.

Healthy food contain high levels of natural phenols in fruits, vegetables, cereals, tea and coffee. Fruits such as grapes, apples, pears, cherries, and berries contain up to 200-300 mg of polyphenols per 100 grams of fresh weight (0.2-0.3% w / w) [30]. Literatures reported the biological activities of polyphenols such as antioxidants, antibacterial, antineoplastic, antithrombotic, and vasodilating activities [31].

One example of phenolic compound is ferulic acid, which used as anti-thrombolysis. ADP-induced platelet aggregation test shows that the stronger anti-thrombolysis activity is attributable to its moiety [32, 33]. Therefore, further research on polyphenols as anti-thrombolysis is necessary. In this research, extraction of shallot skin, which carried out ultrasonic, had the amount of polyphenols of 11.14% ± 5.12% w/w in the extract.

In relation to the activity of flavonoids as antiplatelet, structure-activity relationship analysis showed that anti-aggregation activity of flavonoids are highly rely on the C-ring structure that represent the compounds class. If double bond is present between C2 and C3, it increases anti-aggregation activity of flavonoids in case of non-methylated flavonoids. Most active flavonoids possess hydroxyl group at the position 6. Methylation of rings A and B decreases antiplatelet activity [34]. Flavonoid

have several mechanisms of action such as change of bilayer function, change in ROS concentrations and oxidative stress, change of intracellular Ca^{2+} concentration, inhibition of enzymes (phospholipase C, cAMP phosphodiesterase, cyclooxygenase, thromboxane A2 synthase) [35]

Quercetin which is usually found in the food consumed, scientifically reported to have anticancer, antiviral and antimicrobial activity. The use of quercetin is able to decrease CVD risk, LDL (plasma low-density lipoprotein), hypertension, and risk of ischemic heart disease. Its antiplatelet activity also indicated from the ability to inhibit platelet aggregation upon ex vivo post-supplementation and in vitro addition[36]

The absorption of active ingredients in the gastrointestinal tract is affected by the physicochemical characteristic of the drug, the dosage form used, and the anatomy and physiology of the absorption site [37]. Passive diffusion is influenced by the size and shape of the molecule, the rate of ionization, and the solubility of a drug in fat. Meanwhile, active ingredients that are weakly alkaline will be absorbed at a more alkaline pH, namely in the small intestine [38]

Predicting the solubility of active ingredients in water significantly contribute to the drug absorption after oral administration and is a consideration in parenteral drug administration. This is useful in the manipulating and testing process in the drug design and development process and is crucial for the bioavailability of drugs in the blood [39]. The ADMET profile of a drug is also related to its physicochemical properties [40, 41]. In table 3, there are various parameters of physicochemical properties, it is known that the water solubility of Quercetin is 2.925 x 10^{-4} mol/L; Bis [2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine is 3.577 x 10^{-4} mol/L; *N*-(trifluoroacetyl)methyl-N-deacethyl-Colchicine is 3.781 x 10^{-4} mol/L; Benzo[a]heptalene is 3.691 x 10^{-4} mol/L, and Natalensine, 3,5-dinitrobenzoate is 4,896 x 10^{-4} mol/L.

The greater the solubility of the drug in fat (log P), the higher the absorption of the drug into the body's membrane. However, the drug must still be slightly hydrophilic in order for extracellular fluids to be transported and to be distributed throughout the body [42]. Based on Lipinski's law, log P of the active ingredients in the extract, apart from Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine, all of which meet these requirements. Related to Rule of Five [19], the compound Bis [2- (2-fluorophenyl) -6-fluoroquinolin-4-yl] amine is a compound that meets these criteria because the number of hydrogen bond donors (HBD) of each compound <5 and number of hydrogen bond acceptors (HBA) of each compound <10.

tPSA is a molecular descriptor as a parameter for intestinal absorption and drug penetration into the blood brain barrier [43]. From table 3 It is known that two compounds from shallot skin extract, namely Quercetin and Benzo[a]heptalene, have tPSA values <140Å. So, that compounds meet Veber's law requirements. Caco-2 permeability is an absorption model that uses monolayer Caco-2 cells as an in vitro model predicting the absorption of an orally administered drug. [20]. The compounds in the shallot skins have good permeability apart from 3,5-dinitrobenzoate-Natalensine, this indicates that the compounds in the shallot skins have the potency to be used orally and also have the potential if used through the transdermal route.

The volume of distribution (VDss) is the theoretical the volume by which the drug is dissolved in the body. The high Vdss indicates that the majority of the drug is in the tissue [20]. The compounds in the shallot skins are predicted to have different VDss values so that some of the shallot skins compounds will survive in the blood vessels and most of them in the tissues, a good antiplatelet compound is expected more distributed in blood vessels than in tissues.

The drug ability to permeate the Central Nervous System (CNS) was calculated as blood-brain permeability (logPS), which compounds with log PS> -2 are considered to have acess on CNS, while compounds with logPS <-3 are unable to penetrate [20]. Of the five test compounds, Quercetin and *N*-(trifluoroacetyl) methyl-*N*-deacethyl-Colchicine had a logPS value <-3 meaning

the compound was predicted not to permeate the central nervous system. Meanwhile, the other three compounds had a logPS value >-2, which means that the test compounds were predicted to penetrate the central nervous system.

CYP450 substrates, namely CYP2D6 and CYP3A4. are important to identify because CYP450 inhibitors can dramatically alter the pharmacokinetics of drugs metabolized by CYP450 [20]. It was found that apart from Quercetin, the test compound became a CYP3A4 substrate, whereas for CYP2D6, the five compounds did not become a substrate for CYP2D6.

Total clearence is related to bioavailability, and it is important to determine the dosage level to reach a steady-state concentration. Total clearances are expressed in logs (ml/min/kg) [20]. The test results showed that the five test compounds had a total clearance value stated in logs (ml / minute / kg) of -0.275 to 0.489.

Toxicity is a pharmacokinetic parameter that is important to determine before designing a drug in order to create a drug that is not only effective and of good quality, but also safe to use. Many compounds can cause hepatotoxicity such as certain drugs, laboratory chemicals and some of herbal medicines [44]. In the shallot skins extract, it is known that Quercetin and Benzo[a]heptalene compounds are not hepatotoxic. Rat Oral Acute Toxicity (LD50) is the amount of compound given at once that can cause the death of 50% of a group of test animals (moles / kg) [20]. The five test compounds have an LD50 value between 1.573 and 3.335.

Prediction of anti-platelet activity was carried out at the P1Y12 receptor, a G1-protein on platelet membrane surface receptors. It stimulated adenylyl cyclase inhibition and intracellular calcium mobilization. [45, 46]. The first generation of P2Y12 receptor inhibitors is the thienopyridine ticlopidine class, which has the side effect of neutropenia. The second generation is the clopidrogel, which is highly metabolized by the CYP450 enzyme [47].

Based on the *in silico* test against the P_2Y_{12} receptor, it is known that, quercetin and Bis[2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine has amino acids similar to ((2R, 3S, 4R, 5R)-5-(6-amino-2-(methylthio)-9H-purine-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyltri-hydrogen diphosphate or clopidogrel, which used as standard. Bis [2-(2-fluorophenyl)-6-fluoroquinolin-4-yl]amine has an MDS value that is close to the standard, whereas quercetin although it has a greater MDS value than the standard, so that its binding ability is smaller, it still does not eliminate the possibility that quercetin can be used against the P_2Y_{12} receptor as antiplatelet. After going through the *in silico* test phase, the shallot skins extract content should be tested *in vivo*. It was concluded that the ultrasonic shallot skin extract can be used as new source of the active pharmaceutical ingredient and are predicted to have the potency as anti platelet in an oral or transdermal preparation.

Conclusions

The ultrasonic shallot skin extract can be used as new source of the active ingredient for drug development and are predicted to have the potency to be developed as an oral or transdermal preparation.

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24-Feb-2021

Dear Dr. Ekowati,

The revsion of your manuscript entitled "Shallot skin profilling, computational evaluation of physicochemical properties, ADMET, and molecular docking of its components against P2Y12 receptor" has been successfully submitted online and is presently being given full consideration for publication in Journal of Basic and Clinical Physiology and Pharmacology (JBCPP).

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Kind regards
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03-Mar-2021

Dear Dr. Ekowati:

I would like to thank you for submitting your manuscript entitled "Shallot skin profilling, computational evaluation of physicochemical properties, ADMET, and molecular docking of its components against P2Y12 receptor" to Journal of Basic and Clinical Physiology and Pharmacology (JBCPP). Your manuscript has been reviewed, and it is a pleasure to accept it for publication in JBCPP. The comments of the reviewer(s) are included at the bottom of this letter.

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Thank you for your fine contribution. On behalf of the Editors of Journal of Basic and Clinical Physiology and Pharmacology we look forward to your continued contributions to the Journal.

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Reviewer(s)' Comments to Author: