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β-Patchoulene: Conversion from Patchouli Alcohol by Acid Catalysts and its In silico Anti-inflammatory Study

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Abstract – *β*-Patchoulene (*β*-PAE) is a tricyclic sesquiterpene which performed many potential bioactivities and can be found in patchouli oil but in very low concentration. This study aimed to obtained *β*-PAE in high concentration by conversion of patchouli alcohol (PA) in patchouli oil under acid catalyzed reaction. Patchouli oil was fractinated by vacuum distillation at 96 kPa to get the fraction with the highest PA content. H_2SO_4 and $ZnCl_2$ were used respectively as homogeneous and heterogeneous acid catalysts in the conversion reaction of the selected fraction. Patchouli oil, the fractions and the products were analysed by using GC-MS and FTIR instruments. Moreover, the interaction of *β*-PAE to COX-2 protein was studied to understand the antiinflammation activity of *β*-PAE. The results showed that patchouli oil contains 25.3% of PA. The selected fraction which has the highest PA content (70.3%) was distilled at 151 - 152 ºC. The application of $ZnCl_2$ catalyst in conversion reaction did not succeed. In contrast, H_2SO_4 as a catalyst in acetic acid solvent succeeded in converting the overall fraction of PA to *β*-PAE. Furthermore, the molecular docking study of *β*-PAE against COX-2 enzyme showed van der Waals and alkyl-alkyl stacking interactions on ten amino acid residues.
Keywords – *β*-patchoulene, molecular docking, patchouli oil, patchouli alcohol, sulphuric acid catalyst, conversion mechanism

Introduction

Patchouli oil is one of the mainstay export meridians of the Indonesian state. About 90% of patchouli oil on the world market comes from Indonesia.[1,2] This oil is obtained from patchouli plants (*Pogostemon cablin* Benth) using hydrodistillation,[3] microwave hydrodistillation, or microwave air-hydrodistillation method.[4]

The quality of patchouli oil is very dependent on the concentration of patchouli alcohol (PA) contained.[5] The total PA content in patchouli plants ranges from 21.36% to 34.0%[1], which is distributed in leaf tissue (37.5-51.0%), stems (28.2-42.0%), and roots (14.6-35.1%).[6,7] The PA content of Indonesian patchouli oil is about 30.0%, India 23.7%, China 21.2%, and Malaysia 34.9%.[2,8,9]

Several factors affect PA content in patchouli oil, including varieties,[10] plant age,[11] types of plant tissue,[12] the treatment of plants tissue post-harvest,[13] distillation processes,[14,15] and growing areas.[16,17] Within Indonesia, the concentration of PA in Aceh patchouli oil from Java is lower than from Sumatra.[2] Patchouli farmers from South Sulawesi, especially in Bone-Bone Village, Baraka District, Enrekang Regency, also cultivates Aceh patchouli, but its PA content has never been released.

Patchouli oil ethnobotany shows that it has been widely used to eliminate moisture, reduce fever, outer syndrome, stop vomiting, and stimulate appetite.[18] At the industry level, PA is often used as a basis for the perfume and cosmetics industry.[3,12,19,20] Some compounds isolated from the patchouli plant showed remarkable bioactivities, including antimicrobial, cytotoxic, antiemetic, analgesic, anti-mutagenic, anti-inflammatory, and other essential activities.[21–24] This fact indicates that besides PA, many patchouli oil constituents need to be isolated and utilized for the health sector.

Beta-Patchoulene (*β*-PAE) is one of the main and active constituents of patchouli oil,[25] which has been investigated for its potencies in the field of health science.[14] *β*-PAE acts as an anti-inflammatory,[26] antioxidant,[27–29] antiulcer,[30] and antibacterial agent.[31,32] A recent report showed that *β*-PAE is neuroprotective in ischemic stroke.[33] For its antiinflammatory activity, *β*-PAE has been proven in some reports with the action of

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reducing COX-2 overexpression during inflammation process.[26,28,34] On the other hand, β-PAE can be used as gastroprotector due to its activity in increasing COX-2 level during the use of anti-inflammatory drug such as indomethacin.[35] Thus, β-PAE showed the potency to normalize the function of COX-2 enzyme in the body. As a consequence, a more abundant source of β-PAE is required.

Patchouli oil contains PA of 30.0%, while β-PAE is only 3.2%.[8] Nevertheless, the primary component of PA in patchouli oil can be thought of as beneficial because the compound can be converted to β-PAE using a hydrochloric acid catalyst with a yield of 60.6%.[30]

In this study, the conversion of PA to β-PAE was carried out using two kinds of an acid catalyst, namely Lewis acid ($ZnCl_2$) and BrÖnsted acid (H_2SO_4). Before being used as a precursor, patchouli oil is distilled mainly using distillation fractionation of pressure reduction[36] to get the fraction with the highest PA concentration. The mechanism for the conversion reaction of PA to β-PAE is proposed. In addition, the *in silico* study of anti-inflammatory activity of β-PAE and PA is discussed from the interaction of β-PAE and PA against COX-2 enzyme in order to know how the binding affinity of β-PAE with COX-2, which is responsible on the inflammation process.

Experimental

Materials and Methods – 98% sulfuric acid, zinc chloride, diethyl ether, acetone, ammonium chloride, sodium bicarbonate, and pH paper were purchased from Merck®. Patchouli oil was obtained from Bone-Bone Village, Baraka District, Enrekang Regency, South Sulawesi, Indonesia.

The IR spectra of compounds were recorded with a Shimadzu® 8400s FTIR spectrometer, and the chromatograms and mass spectra were recorded with a Shimadzu® QP-2010 Gas Chromatography-Mass Spectrometer (GC-MS) Ultra.

Distillation of Patchouli Oil – Patchouli oil of 50 g was put into a 250 mL three-neck flask. The temperature in the flask is set up at 200 °C using a thermometer set. The sample was distilled using fractionation distillation at 96 kPa, and the distillate at the constant temperature is collected. The PA content of the patchouli oil sample and distillation fractions were measured using GC-MS. The fraction with the highest PA content is used as a precursor in the conversion phase of PA to β-PAE.

Conversion of PA to β-PAE – Ten mmol (2.22 g) of PA compound was put into a 50 mL round bottom flask, and then one drop of acetic acid and acid catalyst (H_2SO_4 or $ZnCl_2$) was added, respectively. The mixture was stirred with a magnetic stirrer and was refluxed at 100-110 °C for 6 hours. Then, it was cooled to room temperature, put in a separating funnel, and washed with 10 mL of distilled water. The organic layer was collected and extracted with 10 mL of diethyl ether three times. The combined organic layers were washed sequentially with 10% $NaHCO_3$ solution, distilled water, and saturated NaCl solution, then evaporated to remove diethyl ether solvent. Both residue obtained from H_2SO_4 and $ZnCl_2$ catalysts were analyzed by FTIR and GC-MS spectrometers.

GC-MS Analysis – The GC-MS analysis of the PO, all fractions, and the converted products were carried out on a Shimadzu QP-2010 Gas Chromatograph Mass Spectrometer (GC-MS) Ultra, which equipped with Autosampler AOC-20i and SH-Rxi-5Sil MS Column (30 m × 0.25 mm × 0.25 μm) was used to find the chemical composition of the PO and the distillates. The column temperature program was set up as follows: an injection temperature of 250 °C; splitless mode; a column oven temperature of 70 °C at the beginning and held for 2 min, then ramped to 200 °C at the rate of 10 °C/min and end temperature of 280 °C and held for 9 min at the rate 5 °C/min; a MS ion source temperature of 200 °C, and an interface temperature of 280 °C. The identification of most of the oil components was carried out by comparing the mass spectra data with Wiley 8 library information (SI > 95%).

Molecular Docking Study – Molecular docking investigations were conducted using AutoDock4 program with the help of AutoDockTools,[37] Chimera for preparation step,[38] and Discovery Studio Visualizer to visualize the docking result.[39]

Selection and preparation of protein enzyme – Molecular docking analysis of β-PAE in COX-2 was done by using PDB ID 6COX. This PDB file was downloaded from the protein data bank website. All residue and ligands were removed and prepared to dock by selecting the DockPrep menu in Chimera and saving it as .pdb format file.

Preparation of ligands – β-PAE and PA were drawn in the 3D format using Avogadro software,[40] then optimized by employing AM1-BCC semiempirical method in Chimera and save as .pdb format file.

Blind Docking Protocols – In this molecular docking step, blind docking was conducted due to the unknown binding site of the ligand in the active site of COX-2 protein enzyme. This step was divided into two stages, firstly the ligand was placed in any place of the COX-2 surface. Docking protocol was set up to use a big grid size

box 126 × 126 × 126 Å^3 and spacing 0.638 Å to cover the entire surface of protein enzyme, and then ligand occupied the most preferred position. The Lamarckian Genetic Algorithm (LGA)[41] was arranged to produce 100 conformations and run for the maximum number of evaluations 25000000. All of the conformations were analyzed one by one to know the most favourable position of *β*-PAE in COX-2. The resulted good position was chosen as a ligand input file for the next docking stage. Secondly, the chosen conformation of the ligand was docked into the specific site of COX-2 with coordinate XYZ was 29.428 7.988 32.412, the grid box was set 40 × 40 × 40 Å^3 and spacing 0.375 Å. The algorithm also arranged to run from 25000000 as a maximum number of evaluations and produced 20 conformations. All conformation resulted was analyzed, and the best conformation was choosen based on the lowest binding energy value. Visualization of interaction was depicted by using Discovery Studio Visualizer program.

Result and Discussion

The percentage of PA in patchouli oil varies from country to country,[8] and also varies according to species, plant tissue,[20] and growing regions.[2] In Indonesia's territory, the PA content in patchouli oil of *Pogostemon cablin* Benth varies from one province to another.[2,42] Patchouli oil analysis results from Bone-Bone Village Baraka District, Enrekang Regency, South Sulawesi, Indonesia using GC-MS showed a PA composition of 25.3% (Fig. 1A).

This percentage is still below the level of patchouli PA composition of a commercial sample, which is 28.5%[43] and also the average value of PA content of Indonesian patchouli oil, which is 32.0-33.1%,[2] but still higher than in patchouli oil from India.[44] In addition to PA, Bone-Bone Village patchouli oil also contained nine other

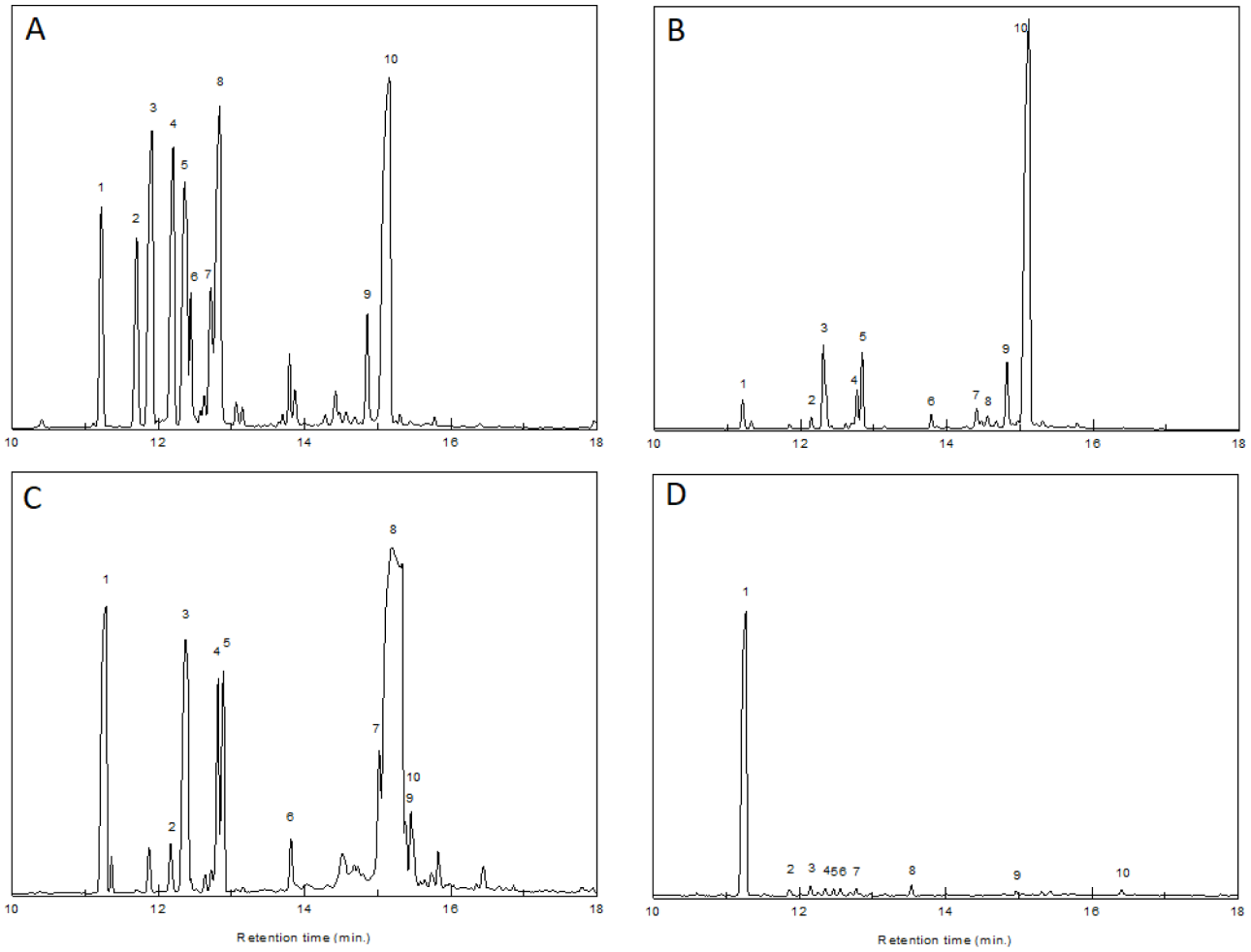
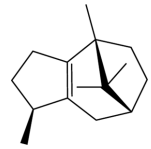
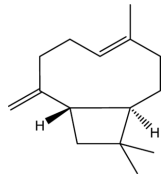
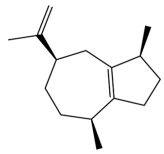
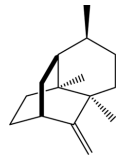
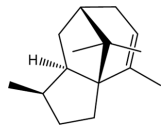
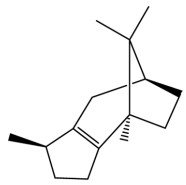
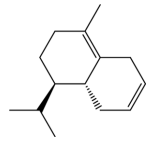
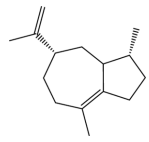
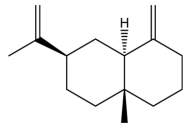
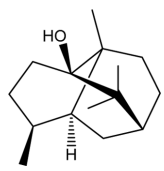


Fig. 1. Chromatograms of patchouli oil from Bone-Bone Village (A), fraction IV of patchouli oil (B), conversion product with $ZnCl_2$ catalyst (C), and conversion product with H_2SO_4 catalyst (D).

Table 1. Content Compounds of Patchouli Oil from Bone-Bone Village

Peak Num.	Compounds Name	RT (Min.)	Mol. Mass	Percentage (%)	Molecular Structure
1.	β-Patchoulene	11.218	204	7.48	
2.	Caryophyllene	11.702	204	6.32	
3.	α-Guaiene	11.912	204	13.77	
4.	Seychellene	12.201	204	10.81	
5.	α-Patchoulene	12.358	204	11.64	
6.	Patchoulene	12.442	204	2.51	
7.	Aciphyllene	12.716	204	4.55	
8.	α-Bulnesene	12.838	204	14.95	
9.	β-Selinene	14.857	204	2.69	
10.	Patchouli alcohol	15.158	222	25.30	

compounds, which are the main constituents, including *β*-PAE, with a percentage of 7.5% (Table 1).

The presence of other compounds in patchouli oil allows side reactions in the conversion of PA to *β*-PAE. The method to get patchouli oil with a high PA level is through the vacuum distillation method.[45,46] The application of this method on patchouli oil from Bone-Bone Village obtained four fractions with different percentage of PA (Table 2).

According to the PA content (Table 2), the most appropriate fraction used as a precursor for the conversion of PA to *β*-PAE is fraction IV, containing 70.3% of PA and 2.4% of *β*-PAE. The chromatogram of this fraction (Fig. 1B) still shows the presence of compounds other than PA (peak 10) and *β*-PAE (peak 1), but their concentration is lower than the PA so that the possible side reactions will not affect the conversion reaction.

The conversion reaction of PA to *β*-PAE was carried out using two types of acid catalysts, namely H_2SO_4 as homogeneous catalyst and $ZnCl_2$ as heterogeneous catalyst. According to the 'Green Chemistry' view, heterogeneous catalysts are far better than homogeneous catalysts. However, based on the results of the conversion, the $ZnCl_2$ catalyst was far from superior. Fig. 1C still shows the existence of a patchouli alcohol peak (peak 10) beside the *β*-PAE peak (peak 1), but Fig. 1D only shows the *β*-PAE peak.

The good result from H_2SO_4 compared to $ZnCl_2$ as a catalyst in the conversion of PA to a β-PAE reaction was also appeared in their FTIR spectra (Fig. 2). The spectra of conversion product from the $ZnCl_2$ catalyst still showed the broad absorption band of O-H groups in the range of

Table 2. Results of Patchouli Oil Distillation from Bone-Bone Village

Fraction Num.	Temperature (°C)	Weight (g)	PA Content (%)	*β*-PAE content (%)
Raw patchouli oil	-	50.00	25.30	7.48
I	141-142	5.57	9.76	11.89
II	145-148	26.68	10.86	8.20
III	149-150	5.16	50.71	4.06
IV	151-152	5.09	70.34	2.38
Refinat	-	2.23	-	-

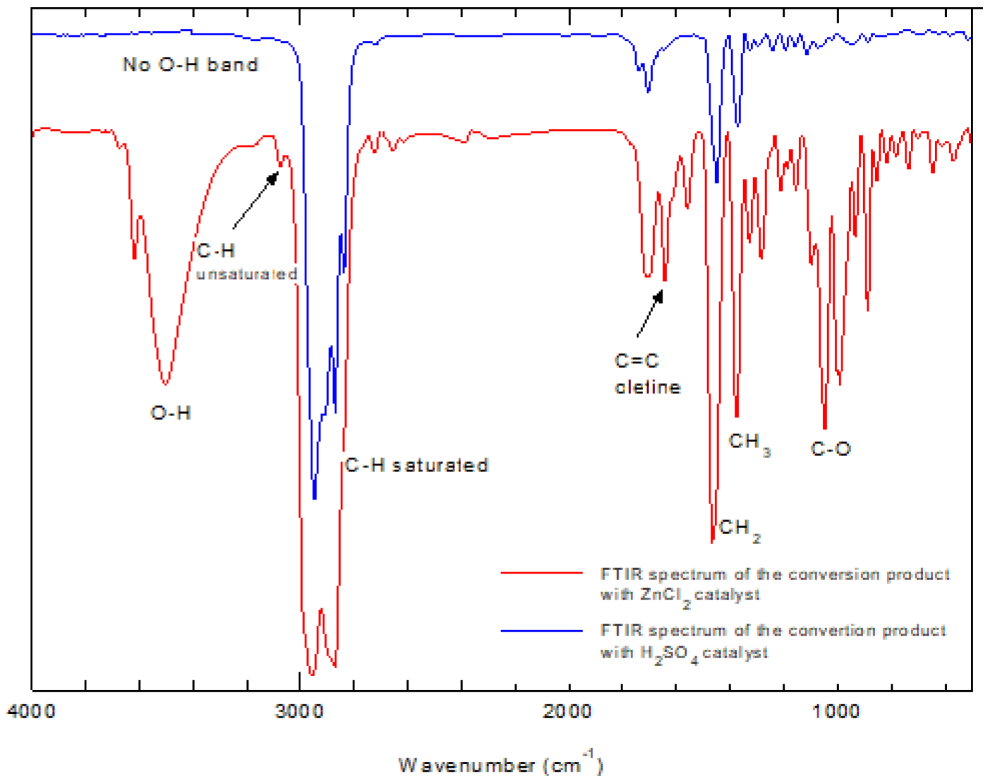


Fig. 2. FTIR spectra of the conversion reaction product with $ZnCl_2$ and H_2SO_4 catalysts.

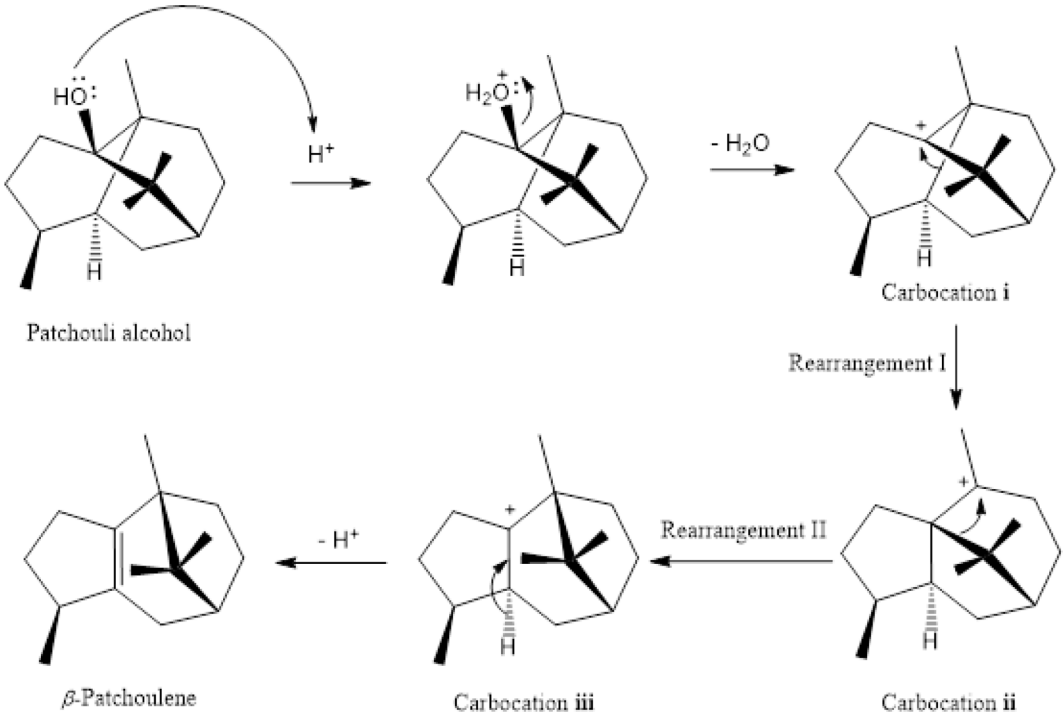


Fig. 3. The mechanism of conversion reaction of PA to *β*-PAE.

wave number 3200-3600 cm^{-1}; while in the spectra of the conversion product from H_2SO_4 catalyst, the band was no longer appear.

Both facts show that all PA in the reaction mixture has been converted to *β*-PAE and it is proven that the performance of the H_2SO_4 catalyst is much better than the $ZnCl_2$ catalyst in the conversion reaction of PA to *β*-PAE. The conversion reaction with H_2SO_4 catalyst succeeded in increasing the concentration of *β*-PAE from 2.5% to 89.6%. The results of our conversion are superior to the conversion that has been done before.[30]

In this paper, the mechanism of the conversion reaction of PA to *β*-PAE has been proposed. The reaction started with the release of water to give carbocation, which subsequently underwent rearrangement twice and followed by the formation of double bonds (Fig. 3). The occurrence of both rearrangements was based on different reasons. The first rearrangement was driven by the stability of carbocation where carbocation **ii** was more stable than carbocation **i** because the effects of hyperconjugation; whereas the second one occurred because of the stability of the fused ring system where the 5-6-5 member ring system (carbocation **iii**) is more stable than the 5-5-6 ring system (carbocation **ii**).

β-PAE is a tricyclic sesquiterpene that has been used as an anti-inflammation drug.[34,47] The mechanism of the anti-inflammatory process described that *β*-PAE expressed the anti-inflammatory mediators such as cyclooxygenase-2 (COX-2) enzyme.[26] However, molecular docking analysis of this compound has never been reported. In this research, blind docking was conducted to know the binding affinity of *β*-PAE in the COX-2 enzyme. Fig. 4 visualized the 2D interaction of *β*-PAE against COX-2, there were ten amino acid residues involved in the binding affinity and resulted in the binding energy of *β*-PAE against COX-2 enzyme of about -7.35 kcal/mol. This low binding energy described the contribution of van der Waals and alky-alkyl stacking. Since there was no functional group in the structure of *β*-PAE then the interaction involved only van der Waals alkyl-alkyl stacking. In order to know how great activity of *β*-PAE in COX-2 protein, we also compared the docking result against PA. Fig. 5 showed interaction of PA in COX-2 protein. There were three kinds of interactions that is hydrogen bond, van der Waals and alkyl-alkyl stacking. Binding energy of PA was also lower than *β*-PAE about -8.04 kcal/mol, this finding was due to the more interactions resulted in PA since the presence of hydroxyl functional group in PA that made hydrogen bond with Arg44 and Cys41 residues. These results support the previous study about *β*-PAE and PA which have anti-inflammation effect by reducing COX-2 protein expression.[30] The pretreatment of β-PAE before

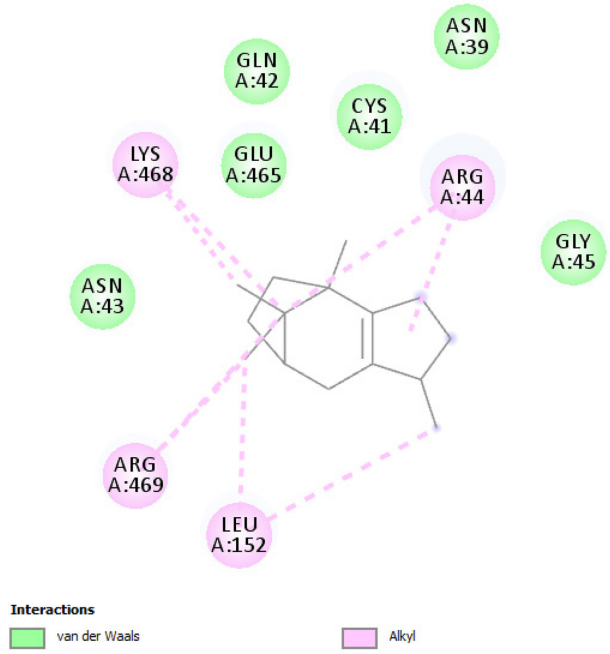


Fig. 4. 2D interaction of β-PAE in COX-2 enzyme.

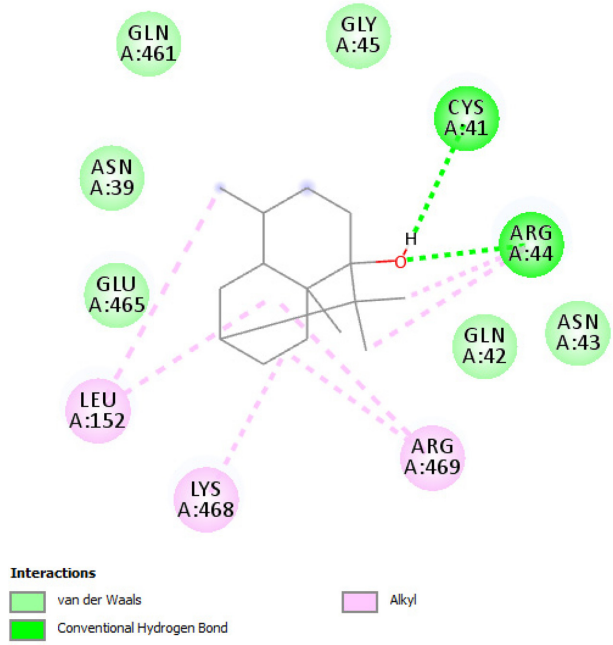


Fig. 5. 2D interaction of PA in COX-2 enzyme.

the pro-inflammatory mediators treatment decreased COX-2 protein level significantly.[26,28,34]

In conclusion, the PA content in patchouli oil from Bone-Bone Village is 25.3%. The application of distillation to patchouli oil at 96 kPa of pressure obtained a fraction at 151-152 ºC with PA concentration of 70.3%. All PA in the fraction have been successfully converted into *β*-PAE using H_2SO_4 catalyst in an acetic acid solvent, whereas the $ZnCl_2$ catalyst was not entirely successful. In addition, the molecular docking study of *β*-PAE against COX-2 enzyme performed low binding energy due to van der Waals and alkyl-alkyl stacking interaction with ten amino acid residues.

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