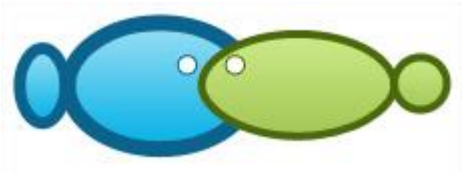

Fatty acid profile and in silico pharmacological study of diatom *Amphora* sp.

[1]Ach. Khumaidi, [2]Feni Iranawati, [3]Mohamad Fadjar, [3]Maftuch, [4]Masruri, [5]Uun Yanuhar, [5]Yuni Kilawati

[1] Department of Aquaculture, Faculty of Science and Technology, University of Ibrahimy, Situbondo, Indonesia; [2] Department of Marine Science, Faculty of Fisheries and Marine Science, University of Brawijaya, Malang, Indonesia; [3] Department of Aquaculture, Faculty of Fisheries and Marine Sciences, University of Brawijaya, Malang, Indonesia; [4] Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Brawijaya, Malang, Indonesia; [5] Department of Water Resources Management, Faculty of Fisheries and Marine Sciences, University of Brawijaya, Malang, Indonesia. Corresponding author: A. Khumaidi, ach.khumaidi@gmail.com

Abstract. *Amphora* sp. is a type of potential microalgae that has a considerably high content of lipid. The current experiment was carried out for the extraction and identification of *Amphora* sp. fatty acids, using n-hexane and petroleum ether as solvents, before studying their pharmacological properties, *in silico*. The results showed that petroleum ether gives a better yield of 3.58%, compared to n-Hexane (2.74%). The GCMS analysis of the sample extracted using petroleum ether shows six peaks corresponding to 6-Dodecanone; Methyl (E)-octadec-11-enoate; Methyl 15-methylhexadecanoate; 2,5-Dimethylcyclohexanol; 2,3-Dimethyl-undec-1-en-3-ol and 3,6,6-Trimethyl-2-norpinanol. Meanwhile, the sample extracted using n-hexane only shows two fatty acid compounds, i.e.: 2,3-Dimethyl-undec-1-en-3-ol and 3,6,6-Trimethyl-2-norpinanol. The absorption, distribution, metabolism, excretion and toxicity (ADMET) analysis showed that fatty acid compounds contained in *Amphora* sp. have potential as drug ingredients, based on the Lipinski's rule of five and on their pharmacokinetic properties. Biological activity based on the prediction of activity spectra for substances (PASS) shows that fatty acids contained in *Amphora* sp. have a considerably good activity as antivirals with a probability activity (Pa) value of 0.592-0.723, but also have several other activities, such as anti-inflammatory, antibacterial, antifungal, antineoplastic, and antioxidant. These results indicate that petroleum ether solvent is more effective than n-Hexane to extract fatty acids in *Amphora* sp., with a high potential as natural antiviral agents, in silico.

Key Words: antiviral, Bacillarophyceae, lipid, microalgae, aquaculture.

Introduction. Microalgae *Amphora* sp. is one of the Bacillariophyceae whose potential utilization is rarely studied (Yi et al 2017). Bacillariophyceae has a key role in recycling carbon and silica in marine waters. The main carbon storage compounds in diatoms are lipids, among which triacylglycerides (TAG) and fatty acids ranging from 15 to 25% of the dry biomass (Mangas-sánchez & Adlercreutz 2015). So far, the use of *Amphora* sp. is limited as a natural feed for fish and shrimp (Brown 2002; Viera et al 2005). According to Chtourou et al (2015), *Amphora* sp. has a crude fat content of 140 mg g^{-1} dry weight. Wiyarno et al (2011) revealed that microalgae lipids are composed of neutral lipids (triglycerides, wax esters, hydrocarbons, free fatty acids, and sterols) and polar lipids (phospholipids, glycolipids, and carotenoids) which have various important biological functions.

Fatty acids have a vital role for human, namely as nutrient content, energy source, secondary metabolites, and signalling molecules that are directly or indirectly involved in physiological processes. According to Proschak et al (2017), besides being an energy source, fatty acids have an important biological role as signalling molecules that regulate various physiological effects in metabolism and inflammation.

The biological role of the extract is determined by the composition of the active compound produced in the extraction process. One of the key factors in the extraction process is the organic solvent selection. The solvent used in the extraction process will affect the composition of the fatty acids from microalgae. Habibi et al (2010) revealed that n-hexane and petroleum ether are the common solvent in the extraction of microalgae. This study provides a comparative analysis of the effectiveness of n-hexane and petroleum ether as the solvents used in extracting *Amphora* sp. This article also discusses the in silico analysis of *Amphora* sp. potential for supplying pharmacological components, with ADMET and PASS.

Material and Method

Amphora sp. Diatom *Amphora* sp. (GenBank: MN592659.1) was obtained from a culture in the BPBAP Situbondo natural feed laboratory. Methods and nutrients used for the culture of *Amphora* sp. follow previous research (Khumaidi et al 2020). Fertilizers used for the culture were KNO_3, NaH_2PO_4, Na_2EDTA, $FeCl_3$, silicate, micro solutions ($ZnCl_2$; $CoC1_2$; $6H_2O$; $(NH_4)6MO_7O_{24}$-$4H_2O$; $CuSO_4$; $5H_2O$), and vitamins (B1 and B12). Each ingredient was mixed and given at a dose of one ml L^{-1} of culture media. The water quality was optimized according to the growth conditions of *Amphora* sp., i.e.: a temperature of 27-31ºC, a pH of 5.5-7.4, a salinity of 26-31 ppt, a light intensity (illuminance) of 2,500-5,000 lux and the appropriate nutrient concentrations (nitrogen, phosphorus, silica, iron and vitamins). *Amphora* sp. was harvested on the 5^{th} day of culture with a density of $230x10^4$ cells cm^{-2}. The collected *Amphora* sp. was dried at the room temperature for three days, before being processed into flour.

Lipid and fatty acid extraction of Amphora sp. Fatty acid extraction was carried out by dissolving *Amphora* sp. flour using organic solvents with ratio of 1:10, the equivalent of 5 g of *Amphora* sp. flour dissolved in two different solvents: n-hexane (Merck, 104367) and petroleum ether (Merck, 101769), each one in a quantity of 50 mL, at a practical grade (p.a.). In the extraction process, a 20 Khz ultrasonic (Branson Digital Aonifier) wave is applied for 15 minutes. After the treatment with ultrasonic waves, filtering was carried out using filter paper (Whatman no. 42) to separate the pulp from the extracted solution. The extraction results are then evaporated by a rotary evaporator vacuum at a temperature of 60ºC (Sharmin et al 2016), a speed of 60 rpm, and a pressure of 200 mBar until there are no more solvents dripping. Furthermore, the yield was calculated using the following equation (Malekzadeh et al 2016):

$$L = \frac{Mi}{Ms}$$

Where:
L - lipid content (%);
M*i* - lipid mass (g);
M*s* - sample mass (g).

Sample preparation for GC/MS. The extracted sample of 1 mL was put into a threaded tube and hydrolyzed with 1 mL of 1 M KOH (in 70% ethanol solvent) at 90ºC, for 1 hour, then acidified with 0.2 mL of HCl 6 M (in water solvent) and added with 1 mL of water and 1 mL of n-hexane. Two separate layers of water and the organic layer will be formed. The organic layer was then allowed to evaporate and methylated with 1 mL of 10% BF_3 (in methanol solvent) at 37ºC, for 20 minutes. The result was then added by 1 mL of water and 1 mL of n-hexane. The organic layer formed was used for GC/MS measurements.

Fatty acid analysis using GC/MS. Fatty Acid analysis was performed using Gas Chromatography-Mass Spectrometer (GC/MS) Shimadzu, QP2010 (Shimadzu Corp, Kyoto, Japan). One microliter sample was injected into the capillary column with Helium (He) as a carrier gas. The temperature of the injector and detector was set at 310°C.

Injection was carried out in split mode (1:30). The initial temperature of the column was set to 80°C and the final temperature reached 310°C. The column flow rate was of 0.45 mL min^{-1} and the pressure measured 10.9 kPa. The aux temperature (GC to MS interface) is set to be 310ºC, while the mass range (ratio of the mass number and the charge number) is set at 20-500 m/z. Identification of fatty acid compounds was performed by comparing the mass spectrum of each compound with the mass compound standard in the literature.

In silico analysis of fatty acid Amphora sp. The analysis was carried out in several stages. First, the PubChem server (https://pubchem.ncbi.nlm.nih.gov/) was accessed to obtain information related to phytochemicals of *Amphora* sp. fatty acids such as synonyms, canonical SMILES (Simplified Molecular Input Line Entry Specification) and 3D structures of bioactive compounds. Fatty acid target compounds were then analysed for the properties of absorption, distribution, metabolism, and excretion (ADME), including analysis of aqueous solubility, Blood Brain Barrier (BBB) properties, plasma protein binding, cytochrome inhibition, intestinal absorption, by accessing http://www.swissadme.ch/ (Biswal & Pazhamalai 2019). Toxicity of fatty acid compounds was analyzed by accessing ProTox II web server (Banerjee et al 2018). The biological activity assessment (such as anti-inflammatory, antifungal, antibacterial, antiviral, antineoplastic, and antioxidant potentials) of *Amphora* sp. fatty acids was carried out by online prediction of activity specifications for substances (PASS) (http://www.way2drug.com/PASSOnline/index.php) (Jamkhande et al 2016).

Results. A solvent is one of the critical components that can determine the extraction results. Table 1 show that n-hexane and petroleum ether can produce relatively low oil yield, namely 0.137 mg g^{-1} (2.74%) with n-hexane and 0.179 mg g^{-1} (3.58%) with petroleum ether. Of the two types of solvents used in this study, petroleum ether produced a higher oil yield than n-hexane. In addition, GC/MS analysis also shows that petroleum ether can extract more fatty acids (Figure 1 and Table 2) compared to n-hexane, which extracts only two types of fatty acids (Figure 2 and Table 3).

Table 1

Lipid yield of *Amphora* sp. extract with n-hexane and petroleum ether solvent

Solvent	*Sample wt. (g)*	*Lipid content (g)*	*% of lipid content*
n-hexane	5±0.00	0.137±0.020	2.74±0.41
Petroleum ether	5±0.00	0.179±0.013	3.58±0.27

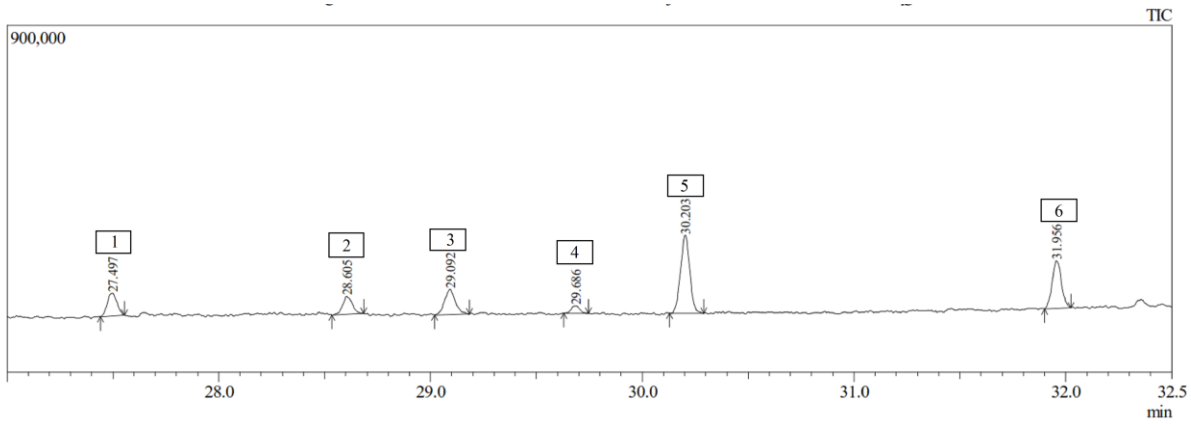

Figure 1. GC/MS screening of fatty acid extracts of *Amphora* sp. with petroleum ether solvent.

Table 2

Profile of fatty acids of *Amphora* sp. extract with petroleum ether solvent

No.	*Retention time (min.)*	*Area (%)*	*Similarity index (SI) %*	*Compound*	*Biological activity*
1	27.500	8.073	90	6-Dodecanone	Flavor and fragrance agent
2	28.608	2.852	88	Methyl (E)-octadec-11-enoate	Absorption and distribution in human plasma and lipoprotein lipids
3	29.093	9.389	90	Methyl 15-methylhexadecanoate	Drug agent
4	29682	3.259	86	2,5-Dimethylcyclohexanol	No activity report
5	30.202	55.512	89	2,3-Dimethyl-undec-1-en-3-ol	No activity report
6	31.956	20.915	85	3,6,6-Trimethyl-2-norpinanol	Antifungal, antibacterial, antioxidant, antidiabetic, anticancer

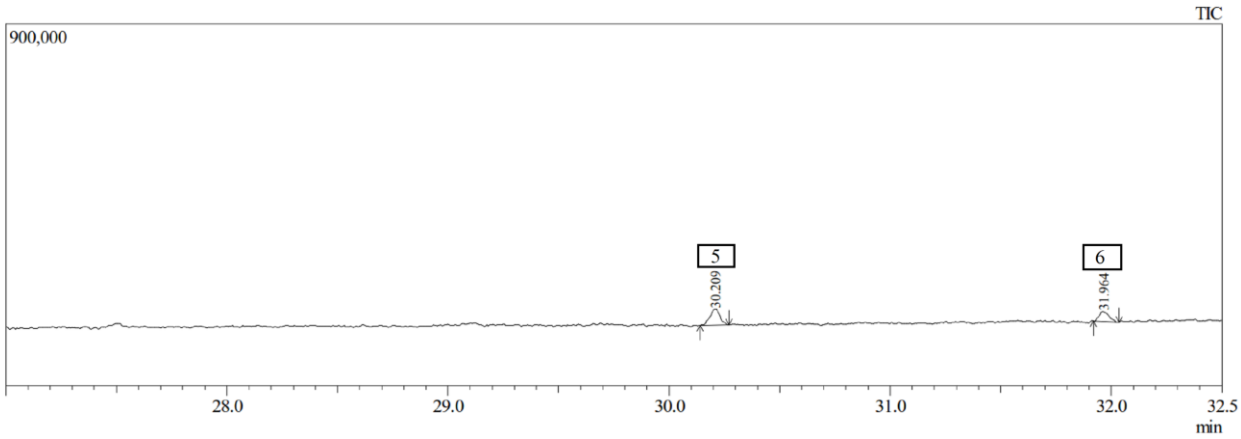

Figure 2. GC/MS screening of fatty acid extracts of *Amphora* sp. with n-hexane solvent.

Table 3

Profile fatty acids of *Amphora* sp. extract with n-hexane solvent

No.	*Retention time (min.)*	*Area (%)*	*Similarity index (SI) %*	*Compound*	*Biological activity*
1	30.210	71.536	89	2,3-Dimethyl-undec-1-en-3-ol	No activity report
2	31.967	28.464	85	(1R,2R,3R,5S)-3,6,6-Trimethylbicyclo[3.1.1] heptan-2-ol	Antifungal, antibacterial, antioxidant, antidiabetic, anticancer

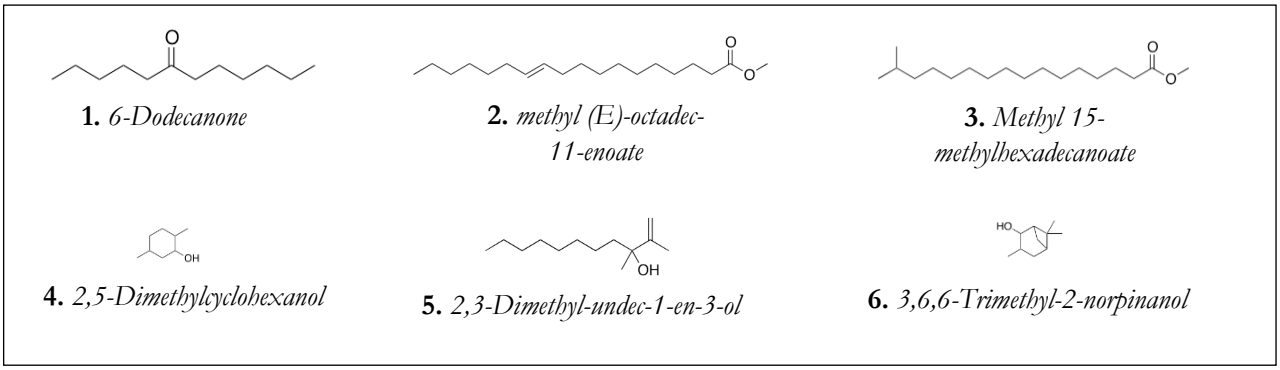

Figure 3. Structure of fatty acid of *Amphora* sp.

ADME and PASS analysis of fatty acid Amphora sp. Analysis of the potentially active compounds contained in amphora sp. is aimed to see whether the compound can reach the targeted location in sufficient concentration and can last for a sufficiently long time in order to function as expected.

Several fatty acids extracted from *Amphora* sp. were analyzed by the Lipinski's rule. The results are shown in Table 4. Six fatty acid compounds have a molecular weight (MW) ranging from 154.25 to 296.5 g mol^{-1}, 0-1 hydrogen bond acceptors (HBA), 0-2 hydrogen bond donors (HBD), 0-16 rotatable bonds, a Topological Polar Surface Area (TPSA) of 17.2-26.3 (Å), and an implicit log P (iLOGP) of 2.2-4.9. The predicted results of the toxicity of *Amphora* sp. fatty acid compounds have 50% Lethal Dose (LD_{50}) values ranging from 340-5,000 mg/kg in rodents. It can classify in the toxicity class IV and V (Table 5).

Pharmacokinetics (PK) is a mathematical description of the ADME process rate and the concentration-time relationship. Many pharmacologically active compounds are selected which then fail to develop due to several factors such as poor bioavailability, high cleansing, low solubility, and difficulty in the formulation. Prediction of pharmacokinetic analysis of *Amphora* sp. fatty acids is shown in Table 6. As shown in Table 5, all of fatty acids *Amphora* sp. has a high ability in intestinal absorption. And four out of six compounds have a blood-brain-barrier permeation ability, except for two compounds, i.e. methyl (E)-octadec-11-enoate and methyl 15-methylhexadecanoate.

Predictions for assessing the biological activity were carried out by online application (http://www.pharmaexpert.ru/passonline/), and the estimated probability value (Pa) is presented in Figure 4. Six of the active compounds contained in *Amphora* sp. have an anti-inflammatory, antifungal, antibacterial, antiviral, antineoplastic and antioxidant activity. However, the antioxidant activity values of these six compounds are relatively low, with Pa values less than 0.3.

Table 4

ADME properties of fatty acid *Amphora* sp.

Compound	*Canonical SMILES*	*Formula*	*MW (g moL^{-1})*	*HBA*	*HBD*	*RB*	*TPSA (Å)*	*Log P(iLOGP)*
6-Dodecanone	CCCCCCC(=O)CCCCC	C12H24O	184.32	1	0	9	17.2	3.3
Methyl (E)-octadec-11-enoate	CCCCCCC=CCCCCCCCCCC(=O)OC	C19H36O2	296.5	0	2	16	26.3	4.79
Methyl 15-methylhexadecanoate	CC(C)CCCCCCCCCCCCCC(=O)OC	C18H36O2	284.5	0	2	15	26.3	4.9
2,5-Dimethylcyclohexanol	CC1CCC(C(C1)O)C	C8H16O	128.21	1	1	0	20.2	2.2
2,3-Dimethyl-undec-1-en-3-ol	CCCCCCCCC(C)(C(=C)C)O	C13H26O	198.34	1	1	8	20.2	3.55
3,6,6-Trimethyl-2-norpinanol	CC1CC2CC(C1O)C2(C)C	C10H18O	154.25	1	1	0	20.2	2.35

MW-molecular weight; TPSA-topology polar surface area; HBA-hydrogen bond acceptor; HBD-hydrogen bound donor; RB-rotatable bonds; iLOGP-partition coefficient.

Table 5

Toxicity fatty acid *Amphora* sp. prediction results from ProTox II

Molecule	*Predicted Toxicity*							
	LD50 (mg kg^{-1})	*Toxicity class*	*Prediction accuracy*	*Hepatotoxicity*	*Carcinogenicity*	*Immuno-toxicity*	*Mutagenicity*	*Cytotoxicity*
6-Dodecanone	730	4	100	Active	Active	Inactive	Inactive	Inactive
methyl (E)-octadec-11-enoate	3000	5	70.97	Active	Active	Inactive	Inactive	Inactive
Methyl 15-methylhexadecanoate	5000	5	100	Active	Active	Inactive	Inactive	Inactive
2,5-Dimethylcyclohexanol	940	4	100	Inactive	Inactive	Inactive	Inactive	Inactive
2,3-Dimethyl-undec-1-en-3-ol	340	4	100	Inactive	Active	Inactive	Inactive	Inactive
3,6,6-Trimethyl-2-norpinanol	2050	5	100	Inactive	Inactive	Inactive	Inactive	Inactive

Table 6

Pharmacokinetics properties of fatty acid *Amphora* sp.

Compound	*GI absorption*	*BBB permeant*	*Pgp substrate*	*CYP1A2 inhibitor*	*CYP2C19 inhibitor*	*CYP2C9 inhibitor*	*CYP2D6 inhibitor*	*CYP3A4 inhibitor*	*log Kp (cm s^{-1})*
6-Dodecanone	High	Yes	No	Yes	No	No	No	No	-4.36
methyl (E)-octadec-11-enoate	High	No	No	Yes	No	No	No	No	-2.82
Methyl 15-methylhexadecanoate	High	No	No	Yes	No	No	No	No	-2.62
2,5-Dimethylcyclohexanol	High	Yes	No	No	No	No	No	No	-5.32
2,3-Dimethyl-undec-1-en-3-ol	High	Yes	No	Yes	No	No	No	No	-3.97
3,6,6-Trimethyl-2-norpinanol	High	Yes	No	No	No	No	No	No	-5.56

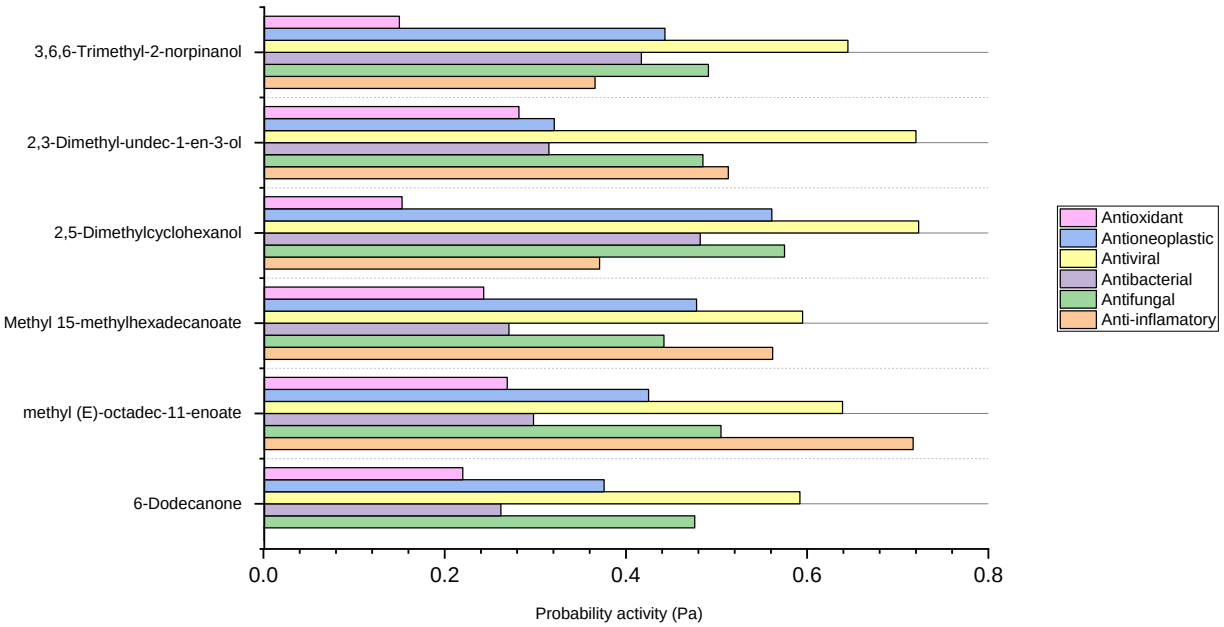

Figure 4. PASS prediction of fatty acid *Amphora* sp.

Discussion. Solvents are an important factor in the lipid extraction process. The two solvents used in the extraction of *Amphora* sp. were compared in this study. Based on the yield of extraction and GC-MS analysis, petroleum ether is considered more effective than n-hexane. *Amphora* sp. extraction results using petroleum ether show lipid concentrations of 0.179 mg g^{-1} or 3.58% of the dry weight. This percentage is higher than the lipid concentrations in the microalgae *Chlorella vulgaris*, with a yield of 3.18% extracted by Jay et al (2018). According to Abdel et al (2015), petroleum ether is a non-polar solvent that has a fairly good ability to extract fatty acid compounds. Hamdi et al (2018) also revealed that petroleum ether is a suitable solvent for extracting fatty acids in *Haplophyllum tuberculatum* and producing fatty acids that have anti-inflammatory and analgesic activity.

GC/MS analyses showed that *Amphora* sp. contains fatty acids which have an important biological role. 6-Dodecanone with a ketone group can be used as "flavor and aroma" (Malik et al 2016). 3,6,6-Trimethyl-2-norpinanol has the chemical properties of monoterpenes (Chaudhary et al 2019). According to Kozio et al (2014), monoterpene is a significant component of essential lipids that are widely used for human health, as they have antifungal, antibacterial, antioxidant, anti-diabetic, anticancer, and several other essential properties. Besides, 3,6,6-Trimethyl-2-norpinanol is also one of the compounds showing antibacterial activity in the methanol extract of *Enteromorpha* sp., which was tested on *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* (Ayşe et al 2019). Methyl 15-methylhexadecanoate or methyl-isoheptadecanoate has a good potential to be used as medicinal ingredients. Lawal et al (2016) showed that the Methyl isoheptadecanoate, one of the most substantial parts in the Nigerian bee (*Apis mellifera*), is an active pharmaceutical agent.

Predictions of the potential as medicinal ingredients have recently been developed using in silico method (Wang et al 2015), by analysing the rate of absorption, distribution, metabolism, and excretion (ADME) through computer models available at the following website, http://www.swissadme.ch (Daina et al 2017). ADME parameters referred to Lipinsky et al (2012) or so-called Lipinski's Rule of Five are used to determine the range of physicochemical features of the chemical compounds with high probabilities of functioning as oral drugs (drug likeness). 6-Dodecanone, methyl (E) -octadec-11-enoate, Methyl 15-methylhexadecanoate, 2,5-Dimethylcyclohexanol, 2,3-Dimethyl-undec-1-en-3-ol and 3,6,6-Trimethyl-2-norpinanol are excellentcandidates for drugs development. These estimations are based on MW values<500 g mol^{-1}, HBA≤10, HBD≤5,

TPSA≤140Å, and iLOGP<5 (Lipinski et al 2012; Sehgal et al 2016; Mohaiminul 2017). Compounds that have a molecular weight exceeding 500 g moL^{-1} are characterized by: weak bioavailability, low fractions absorbed, high bound fraction and weak cleaning of kidneys (Lipinski et al 2012). TPSA is an envelope surface of a molecule that emerges from a polar atom, such as oxygen, nitrogen or hydrogen, that is bound to oxygen or nitrogen atoms. Clark (2008) and Hassan & Lenz (2005) revealed that bioactive compounds which have a TPSA value of ≥140Å would be difficult to enter the cell. Conversely, if the bioactive compound has a TPSA value of ≤140Å, the compound will easily enter the cell (Aristyani et al 2018).

The toxicity value fatty acid compounds *Amphora* sp. is 340-5,000 mg kg^{-1} (Table 5). Supandi & Merdekawati (2018) stated that the higher the LD50 value, the lower the toxicity value. Table 5 shows that three compounds can potentially cause hepatotoxicity and carcinogenicity, namely 6-Dodecanone, methyl (E)-octadec-11-enoate, and Methyl 15-methylhexadecanoate. Besides, 2,3-Dimethyl-undec-1-en-3-ol compounds are also predicted to have carcinogenic potential. Based on this toxicity prediction, there are two Amphora sp fatty acid compounds, namely 2,5-Dimethylcyclohexanol and 3,6,6-Trimethyl-2-norpinanol, which can be followed up as health drug agents, because it has a low toxicity value and does not have the potential to cause hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity.

The nature or character of biological compounds can be predicted using PASS (prediction of activity spectra for substances) method that can be accessed via online tools. PASS software can predict more than 300 pharmacological effects and biochemical mechanisms, based on the structural formula of a substance, and can be used efficiently to find new targets or mechanisms for several ligands. Also, it can be used to discover new ligands for several biological targets (Lagunin et al 2000). In Figure 4, it appears that the six active compounds of *Amphora* sp. fatty acid tend to have antiviral activity with a fairly high Pa value for each active compound, namely: 6-Dodecanone, methyl (E)-octadec-11-enoate, Methyl 15-methylhexadecanoate, 2,5-Dimethylcyclohexanol, 2,3-Dimethyl-undec-1-en-3-ol, and 3,6,6-Trimethyl-2-norpinanol. Pa>0.7 indicates that the compound is very likely to show an activity similar to other known drug agents, while 0.5<Pa<0.7 indicates that the substance tends to show an activity which may not be similar to other known drugs (Lagunin et al 2000). Apart from being an antiviral, the methyl (E)-octadec-11-enoate has anti-inflammatory (Pa value of 0.595) and antifungal (Pa of 0.505) activity. The Methyl 15-methylhexadecanoate is an anti-inflammatory, the 2,5-Dimethylcyclohexanol is an antifungal and antineoplastic, and the 2,3-Dimethyl-undec-1-en-3-ol has an anti-inflammatory activity.

The cytochrome p450 (CYP) enzyme is one of the enzymes involved in xenobiotic metabolism (Kirchmair et al 2015), and has a crucial role in drug elimination through metabolic biotransformation (Di 2014). Many researchers analysed five major CYP isoforms, namely CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4 (Huang et al 2008). In Table 5 it is shown that four active compounds have an inhibiting action on CYP1A2, i.e.: 6-Dodecanone, methyl (E)-octadec-11-enoate, Methyl 15-methylhexadecanoate and 2,3-Dimethyl-undec-1-en-3-ol. These results indicate that some active compounds from *Amphora* sp. can inhibit CYP1A2. However, all compounds do not have inhibitor character in the other four major CYP isoforms which suggest that they have sufficient activity in clearing the accumulation of drugs and their metabolites (Kirchmair et al 2015).

These results indicate that the medicinal ingredients from *Amphora* sp. fatty acids have antiviral, anti-inflammatory, antibacterial, antifungal, antineoplastic and antioxidant properties, reinforcing the evidences related to the excellent potential of the marine microalgae lipids in the health sector. Previously, the important role in the inhibition of nitric oxide played by the mono-galactosyl-diacyl-glycerols and by the di-galactosyl-diacyl-glycerols, lipids produced from marine microalgae, had been demonstrated (Banskota et al 2013a), together with their anti-inflammatory properties (Banskota et al 2013b). The glycolipid sulfoquinovosyl-diacylglycerol, extracted from red and brown algae, has a strong antiviral activity against the herpes simplex viruses of types 1 and 2, as shown by De Souza et al (2012) and Plouguerné et al (2013). Besides, the methyl-hexadecanoate compound is one of the potential fatty acids extracted from *Porphyridium*

cruentum diatoms, which has antibacterial activity in vitro (Kusmiyati & Agustini 2007). Based on these results, the potential use of *Amphora* sp. can be further developed and these results can be used as a basis for testing at a later stage, either in vitro or in vivo.

Conclusions. Petroleum ether appears to be more effective than n-hexane in extracting *Amphora* sp. fatty acids by producing more fatty acid compounds. The analysis of *Amphora* sp. fatty acid compounds in silico, using the ADMET and PASS prediction methods, meets the Lipinski's rule of five. It is predicted that active compounds in *Amphora* sp. have important biological activities such as anti-inflammatory, antifungal, antibacterial, antineoplastic and antioxidant. Further biological activity can be performed through in vitro and in vivo tests.

References

Abdel R. G., Aboutabl E., Hussein A., Said A., 2015 The composition of the lipoidal matter of the seeds of *Pleiogynium timorense* (DC.) Leenh. Egyptian Pharmaceutical Journal 14(1):65-68.

Aristyani S., Nur M. I., Widyarti S., Sumitro S. B., Sciences N., Sciences N., 2018 In silico study of active compounds ADMET profiling in *Curcuma xanthorrhiza* Roxb and Tamarindus indica as Tuberculosis Treatment. Jurnal Jamu Indonesia 3(3):101–108.

Ayşe E., Eliuz E., Börekçi N. S., Ayas D., 2019 The antimicrobial activity of *Enteromorpha* sp. methanolic extract and gelatin film solution against on some pathogens. Marine Science and Technology Bulletin 8(2):58–63.

Banerjee P., Eckert A. O., Schrey A. K., Preissner R., 2018 ProTox-II: A webserver for the prediction of toxicity of chemicals. Nucleic Acids Research 46(1):257–263.

Banskota A. H., Stefanova R., Gallant P., McGinn P. J., 2013a Mono- and digalactosyldiacylglycerols: Potent nitric oxide inhibitors from the marine microalga *Nannochloropsis granulata*. Journal of Applied Phycology 25(2):349–357.

Banskota A. H., Stefanova R., Sperker S., McGinn P. J., 2013b New diacylglyceryltrimethylhomoserines from the marine microalga *Nannochloropsis granulata* and their nitric oxide inhibitory activity. Journal of Applied Phycology 25(5):1513–1521.

Biswal A. R., Pazhamalai V., 2019 Molecular docking analysis of bioactive compounds of *Acacia concinna* against fungal protein. Journal of Pharmaceutical Science and Research 11(4):1216–1222.

Brown M. R., 2002 Nutritional value and use of microalgae in aquaculture. Memorias del VI Simposium Internacional de Nutrición Acuícola, pp. 281–291.

Chaudhary P., Ahmad S., Khan N. A., 2019 Isolation and characterization of volatile oil by Gc-Ms analysis and screening of in vitro antioxidant potential of hydroalcoholic extracts of some indigenous plants of northern India in rodents. Biomedical & Pharmacology Journal 12:1867–1880.

Chtourou H., Dahmen I., Jebali A., 2015 Characterization of *Amphora* sp. a newly isolated diatom wild strain, potentially usable for biodiesel production. Bioprocess and Biosystems Engineering. https://doi.org/10.1007/s00449-015-1379-6.

Clark D. E., 2008 What has virtual screening ever done for drug discovery? Expert Opinion on Drug Discovery 3(8):841–851.

Daina A., Michielin O., Zoete V., 2017 SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Scientific Reports 7(3):1–13.

De Souza L. M., Sassaki G. L., Romanos M. T. V., Barreto-Bergter E., 2012 Structural characterization and anti-HSV-1 and HSV-2 activity of glycolipids from the marine algae *Osmundaria obtusiloba* isolated from Southeastern Brazilian coast. Marine Drugs 10(4):918–931.

Di L., 2014 The role of drug metabolizing enzymes in clearance. Expert Opinion on Drug Metabolism & Toxicology 10(3):379–393.

Habibi R., Fachriyah E., Kusrini D., 2010 [Synthesis of biodiesel from microalgae oil *Nannochloropsis* sp]. Jurnal Kimia Sains Dan Aplikasi 13(1):30–35. [In Indonesian].

Hamdi A., Majouli K., Abdelhamid A., Belghith H., Chraief I., Bouraoui A., Heyden Y. V., 2018 Pharmacological activities of the organic extracts and chemical fatty acids composition of the petroleum ether extract from *Haplophyllum tuberculatum* leaves. Journal of Ethnopharmacology 24(216):97–103.
Hassan P., Lenz G. R., 2005 Medicinal chemical properties of successful central nervous system drugs. NeuroRx: The Journal of the American Society for Experimental 2:541–553.
Huang S., Strong J. M., Zhang L., 2008 New era in drug interaction evaluation: US Food and drug administration update on CYP enzymes, transporters, initial drug interaction guidance. The Journal of Clinical Pharmacology 48(6):662–670.
Jamkhande P. G., Pathan S. K., Wadher S. J., 2016 In silico PASS analysis and determination of antimycobacterial, antifungal, and antioxidant efficacies of maslinic acid in an extract rich in pentacyclic triterpenoids. International Journal of Mycobacteriology 5(4):417–425.
Jay M. I., Kawaroe M., Effendi H., 2018 Lipid and fatty acid composition microalgae *Chlorella vulgaris* using photobioreactor and open pond. IOP Conference Series: Earth and Environmental Science 141:012015. doi: 10.1088/1755-1315/141/1/012015
Khumaidi A., Iranawati F., Kilawati Y., Yanuhar U., Fadjar M., 2020 Morphology, molecular, and nutritional value of *Amphora* sp. from coastal water of the grouper cultivation center (Situbondo, Indonesia). Ecology, Environment and Conservation 26(2):943–949.
Kirchmair J., Göller A. H., Lang D., Kunze J., Testa B., Wilson I. D., Schneider G., 2015 Predicting drug metabolism: experiment and/or computation? Nature Publishing Group 14(6):387–404.
Kozio A., Stryjewska A., Librowski T., Sa K., Gawe M., 2014 An overview of the pharmacological properties and potential applications of natural monoterpenes. Mini-Reviews in Medicinal Chemistry 14(11):1156–1168.
Kusmiyati, Agustini N. W. S., 2007 [Antibacterial activity assay from *Porphyridium cruentum* microalgae]. Biodiversitas 8(1):48–53. [In Indonesian].
Lagunin A., Stepanchikova A., Filimonov D., Poroikov V., 2000 PASS : prediction of activity spectra for biologically active substances. Bioinformatics Applications Note 16(8):747–748.
Lawal B., Shittu O. K., Abubakar A. N., Olalekan I. A., Jimoh A. M., Abdulazeez A. K., 2016 Drug leads agents from methanol extract of Nigerian bee (*Apis mellifera*) propolis. Journal of Intercultural Ethnopharmacology 5(1):43–48.
Lipinski C. A., Lombardo F., Dominy B. W., Feeney P. J., 2012 Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Advanced Drug Delivery Reviews 64:4–17.
Malekzadeh M., Najafabadi H. A., Hakim M., Feilizadeh M., Vossoughi M., Rashtchian D., 2016 Experimental study and thermodynamic modeling for determining the effect of non-polar solvent (hexane)/polar solvent (methanol) ratio and moisture content on the lipid extraction efficiency from *Chlorella vulgaris*. Bioresource Technology 201:304–311.
Malik B., Pirzadah T. B., Tahir I., Abdin M. Z., Rehman R. U., 2016 Phytochemical studies on *Cichorium intybus* L.(chicory) from Kashmir Himalaya using GC-MS. Journal of Pharmacy Research 10(11):715–726.
Mangas-sánchez J., Adlercreutz P., 2015 Highly efficient enzymatic biodiesel production promoted by particle-induced emulsification. Biotechnology for Biofuels 8(58):1–8.
Mohaiminul I. M., 2017 In silico PASS prediction, molecular docking and ADME/T analysis of natural compounds possessing thrombolytic effect. Department of Pharmacy, State Unviersity of Bangladesh. doi: 10.13140/RG.2.2.17169.84325
Plouguerné E., De Souza L. M., Sassaki G. L., Cavalcanti J. F., Romanos M. T. V., Da Gama B. A. P., Barreto-Bergter E., 2013 Antiviral sulfoquinovosyldiacylglycerols (SQDGs) from the Brazilian brown seaweed *Sargassum vulgare*. Marine Drugs 11(11):4628–4640.
Proschak E., Heitel P., Kalinowsky L., Merk D., 2017 Opportunities and challenges for

fatty acid mimetics in drug discovery. Journal of Medicinal Chemistry 60:5235-5266.
Sehgal S. A., Mannan S., Ali S., 2016 Pharmacoinformatic and molecular docking studies reveal potential novel antidepressants against neurodegenerative disorders by targeting HSPB8. Drug Design, Development and Therapy 10:1605–1618.
Sharmin T., Hasan C. M. M., Aftabuddin S., Rahman M. A., Khan M., 2016 Growth, fatty acid, and lipid composition of marine microalgae *skeletonema costatum* available in Bangladesh coast: Consideration as biodiesel feedstock. Journal of Marine Biology 832847. doi: 10.1155/2016/6832847
Supandi Y., Merdekawati F., 2018 In silico study of pyrazolylaminoquinazoline toxicity by lazar, protox, and admet predictor. Journal of Applied Pharmaceutical Science 8(9):119–129.
Viera M. P., Pincehetti G. J. L., Vicose G. C., Suarez S., Haroun, Ricardo J., Izquierdo M. S., 2005 Suitability of three red macroalgae as a feed for the abalone *Haliotis tuberculata* coccinea reeve suitability of three red macroalgae as a feed for the abalone *Haliotis tuberculata coccinea* Reeve. Aquaculture 248(5):75–78.
Wang Y., Xing J., Xu Y., Zhou N., Peng J., Xiong Z., Liu X., 2015 In silico ADME/T modelling for rational drug design. Quarterly Reviews of Biophysics 48(4):488–515.
Wiyarno B., Yunus R. M., Mel M., 2011 Extraction of algae oil from *Nannochloropsis* sp.: A study of soxhlet and ultrasonic-assicted extractions. Journal of Applied Sciences 11(21):3607–3612.
Yi Z., Xu M., Di X., Brynjolfsson S., Fu W., 2017 Exploring valuable lipids in diatoms. Frontiers in Marine Science 4(1):1–10.
*** PubChem server, https://pubchem.ncbi.nlm.nih.gov/
*** Swiss Institute of Bioinformatics, Swiss Adme, http://www.swissadme.ch/
*** Way2Drug Predictive Services, Cell Line Cytotoxicity Predictor, Prediction of Activity Spectra for Substances, http://www.way2drug.com/PASSOnline/index.php
*** PASS Online, http://www.pharmaexpert.ru/passonline/

Received: 30 April 2020. Accepted: 31 July 2020. Published online: 07 August 2020.
Authors:
Ach. Khumaidi, University of Ibrahimy, Faculty of Science and Technology, Department of Aquaculture, Situbondo, Jl. Raya Banyuwangi Situbondo 68374, East Java, Indonesia, e-mail: ach.khumaidi@gmail.com
Feni Iranawati, University of Brawijaya, Faculty of Fisheries and Marine Science, Department of Marine Science, Malang, Jl. Veteran 16 Malang 65145, East Java, Indonesia, e-mail: fe_iranawati@ub.ac.id
Mohamad Fadjar, University of Brawijaya, Faculty of Fisheries and Marine Sciences, Department of Aquaculture, Malang, Jl. Veteran 16 Malang 65145, East Java, Indonesia, e-mail: f4dj4r_02@ub.ac.id
Maftuch, University of Brawijaya, Faculty of Fisheries and Marine Sciences, Department of Aquaculture, Jl. Veteran 16 Malang, 65145 Malang, East Java, Indonesia, e-mail: maftuch@ub.ac.id
Masruri, University of Brawijaya, Faculty of Mathematics and Natural Sciences, Department of Chemistry, Malang, Jl. Veteran 16 Malang, 65145 Malang, East Java, Indonesia, e-mail: masruri@ub.ac.id
Uun Yanuhar, University of Brawijaya, Faculty of Fisheries and Marine Sciences, Department of Water Resources Management, Jl. Veteran 16 Malang, 65145 Malang, East Java, Indonesia, e-mail: doktoruun@ub.ac.id
Yuni Kilawati, University of Brawijaya, Faculty of Fisheries and Marine Sciences, Department of Water Resources Management, Jl. Veteran 16 Malang, 65145 Malang, East Java, Indonesia, e-mail: yuniqla@ub.ac.id

How to cite this article:
Khumaidi A., Iranawati F., Fadjar M., Maftuch, Masruri, Yanuhar U., Kilawati Y., 2020 Fatty acid profile and in silico pharmacological study of diatom *Amphora* sp. AACL Bioflux 13(4):2050-2060.