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In vitro acetylcholinesterase inhibitory activities of fractions and iso-agelasine C isolated from the marine sponge *Agelas nakamura*

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ABSTRACT: The marine sponges with their unique and wide range of biodiversity, producing unusual metabolites emerge as a good candidate for new therapeutic agents, including as acetylcholinesterase (AChE) inhibitor. The aims of this study are to evaluate the potency of fractions and isolated compound from *Agelas nakamura* as AChE inhibitor, as well as to determine the structure of the isolated compound. The bioassay-guided isolation protocol was carried out in this study. The AChE inhibitory assay was carried out based on the modified Ellman's method. The structure of the isolated compound was determined by NMR spectroscopy and mass spectrometry. A diterpene alkaloid was isolated from the active fraction of *A. nakamura*. This compound exhibited a molecular ion at m/z 422.3280 [M]⁺ in mass spectrometry. The UV profile of the isolated compound showed a strong peak at 269 nm, and the specific rotation value is $[\alpha]_D^{20} +28,0$ (MeOH, c 0.25). These data together with the ¹H and ¹³C NMR spectroscopy data are similar to that reported for iso-agelasine C. The isolated iso-agelasine C gave moderate inhibition against AChE enzyme with an IC₅₀ value of 30.68 ± 0.92 µg/mL, which was lower compared to the extract and fractions. The stronger AChE inhibitory activity in the extract and fractions is possibly due to the synergistic activity of compounds present in the extract and fractions of *A. nakamura*. Iso-agelasine C could serve as a lead for the development of diterpene alkaloid as an AChE inhibitor.

KEYWORDS: Alzheimer's disease; acetylcholinesterase inhibitor; marine sponge; *Agelas nakamura*; iso-agelasine C.

1. INTRODUCTION

Alzheimer's disease (AD) is a disease that causes degeneration of the cells in the brain and accounted for 60-80% of dementia cases in the world. AD is highly prioritized by WHO in its World Mental Health Gap program. From 2000 to 2018, it was reported that there was a 146.2% increase in mortality due to Alzheimer's disease [1]. The number of AD patients increases as the population of elderly people grows. It was predicted that there is a new case of Alzheimer's every 33 seconds [1,2]. Although the pathogenesis of AD is complicated and not fully established, two major hypotheses namely amyloid cascade and cholinergic are currently under consideration regarding the molecular mechanism. Acetylcholine (ACh) is a neurotransmitter that plays an essential role in cognitive function and memory loss [3,4]. In AD patients, there is ACh deficiency due to neuronal loss in the brain. One of the strategies to increase the amount of ACh in the brain is by inhibiting the acetylcholinesterase (AChE) enzyme. The principal role of AChE inhibitor is to prevent rapid hydrolysis of

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ACh to choline and ethanoic acid in the synapses [5]. Therefore, AChE inhibitor is one of the therapeutic strategies in Alzheimer's disease treatment.

Currently, there are three AChE inhibitors approved by the FDA to be used for the symptomatic treatment of AD, namely donepezil, rivastigmine, and galantamine. The use of AChE inhibitors appears to be beneficial for the treatment of mild to moderate AD patients. There is improvement in the behavioral and psychiatric symptoms [3]. Donepezil is a synthetic compound, while rivastigmine is a derivative developed from the natural compound physostigmine [6], whereas galantamine is an alkaloid derived from several plants from the Amaryllidaceae family, such as *Galanthus* spp., *Leucojum* spp., and *Narcissus* sp. [7,8]. Most of the natural AChE inhibitors reported to date belong to the alkaloid group, although there are other classes of compounds that have also been reported as AChE inhibitors [9,10].

The oceans with their unique and wide range of biodiversity emerge as a potential source of unusual metabolites and serve as a good candidate for new therapeutic agents, including AChE inhibitors [5]. Marine sponges from the genus *Agelas* (class Demospongiae, order Agelasida, family Agelasidae) represent rich structure diversities with promising biological activities such as alkaloids, terpenoids, meroterpenoids, carotenoids, glycosphingolipids, and fatty acids [11]. In the previous study, we have investigated the potency of fifteen marine sponges collected from Indonesian water as acetylcholinesterase inhibitors [12]. The results showed that the methanolic extract of *Agelas nakamura* showed strong inhibition against AChE with an IC_{50} value of 1.05 g/mL. The objectives of the current study are to isolate the active compound in *A. nakamura* as an AChE inhibitor by using a bioassay-guided isolation technique as well as to determine the structure of the isolated compound.

2. RESULTS and DISCUSSION

2.1. Fractionation and AChE inhibitory assay of fractions

The methanolic extract was fractionated by a liquid-liquid partition with n-hexane, ethyl acetate, and n-butanol. The fractions obtained were then subjected to the AChE inhibitory assay. The results as can be seen in Figure 1 showed that the ethyl acetate and the n-butanol fractions gave 85.1% and 92.4% inhibition, respectively, whereas the n-hexane fraction was relatively inactive with inhibition of less than 10%. Considering the weight of the samples, further fractionation was conducted on the ethyl acetate fraction.

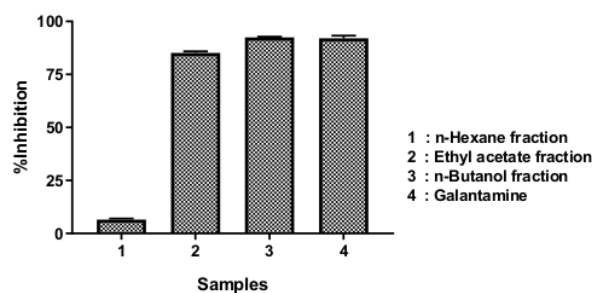


Figure 1. Inhibition of n-hexane, ethyl acetate, n-butanol fractions (100 $\mu\text{g/mL}$), and galantamine (36.8 $\mu\text{g/mL}$) against AChE

Vacuum liquid chromatography was employed for fractionation of the ethyl acetate fraction by using a combination of dichloromethane and methanol in order of increasing polarity to obtain 11 fractions. These were then subjected to the AChE inhibitory screening. The results are presented in Figure 2 which showed that 5 fractions, namely VLC 2 - VLC 6 gave strong activity with 56%-80% inhibition at 100 $\mu\text{g/mL}$ against AChE, whereas the other fractions showed less than 20% inhibition. To further investigate the potency of the fractions, the IC_{50} values were determined for fractions VLC 2 - VLC 6. The results as can be seen in Table 1 indicated that fractions VLC 2 and VLC 3 had better AChE inhibitory activity compared to the other fractions with IC_{50} values of 5.31 $\mu\text{g/mL}$ and 3.84 $\mu\text{g/mL}$, respectively. A t-test analysis was carried out to determine whether the IC_{50} values of fractions VLC 2 and VLC 3 are statistically different. The result suggested that the IC_{50} values of VLC 2 and VLC 3 were not significantly different with a p value of 0.15. Therefore, further purification procedures were carried out on these two fractions.

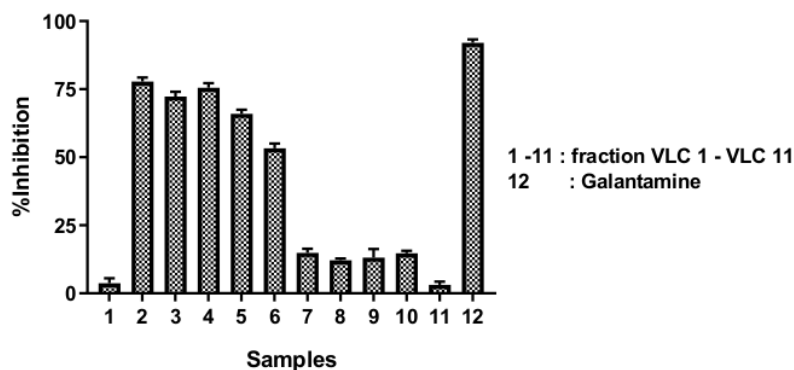


Figure 2. Inhibitory activity of VLC fractions (100 µg/mL) and galantamine (36.8 µg/mL) against AChE

Table 1. IC₅₀ of selected VLC subfractions of *A. nakamurai* and galantamine against AChE

Sample	Weight (g)	IC ₅₀ (µg/mL)
Subfraction 2	0.3709	5.31 ± 0.22
Subfraction 3	2.4139	3.84 ± 0.57
Subfraction 4	0.4279	15.95 ± 0.99
Subfraction 5	0.0193	30.81 ± 3.29
Subfraction 6	0.0225	26.08 ± 1.99
Galantamine	-	0.45 ± 0.13

Data presented as mean ± standard error of the mean (SEM) of three independent experiments, each done in triplicate

2.2 Purification and identification of metabolite

Purification of the fractions VLC 2 and VLC 3 were carried out by using HPLC with a C-18 semipreparative column, employing gradient elution of acetonitrile and water containing 1% TFA. A major peak at retention time 44.107 min was collected (Figure 3) and obtained a white amorphous solid, then was subsequently subjected to structural analysis. The TLC analysis of the isolated compound yielded an orange spot with Dragendorff's reagent, and a purplish red spot with anisaldehyde-sulphuric acid spray reagent at the same retention factor (Rf) value. These results suggested the presence of a terpene alkaloid. The mass spectrum of the isolated compound exhibited a molecular ion at m/z [M]⁺ 422.3280, corresponding to the molecular formula C₂₆H₄₀N₅. The ¹H and ¹³C NMR data (Table 2) indicated the presence of 9-methyladenine moiety from aromatic signals at δ_H 9.54, δ_C 141.3 and δ_H 8.43, δ_C 156.0; one N-methyl at δ_H 3.85 (s), δ_C 32.0; and an amino group at δ_H 7.90. There are two alkenes observed at δ_H 5.38, δ_C 115.4, δ_C 147.2 and δ_H 5.29, δ_C 117.2, δ_C 146.1. The spectra also showed evidence for one methyl doublet at δ_H 0.80, δ_C 16.9, and four methyl singlets at δ_H 1.76, δ_C 17.3; δ_H 0.97, δ_C 23.5; δ_H 0.81, δ_C 28.3, and δ_H 0.82; δ_C 28.0. The presence of signals at δ_H 1.01-1.95 range indicated a terpene moiety. These spectral features resembled iso-agelasine C (Figure 4), which was first reported in 2017 by Chu et al. from *Agelas nakamurai* collected from the South China Sea. The relative configuration of iso-agelasine C has been determined by NOESY experiments, ECD calculation as well as comparison with related congeners of iso-agelasine C [13]. The specific rotation of our compound was measured and obtained $[\alpha]_D^{20} = +28.0$ (MeOH, *c* 0.25), comparable to the reported iso-agelasine C $[\alpha]_D^{20} = +22.1$ (MeOH, *c* 0.1) [13].

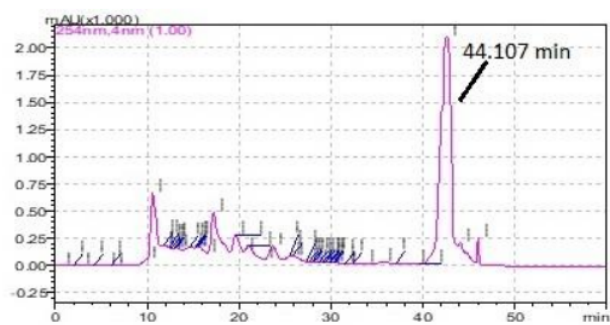


Figure 3. HPLC profile of VLC 2 fraction showing isolated compound at 44.107 min (Detection UV 254 nm)

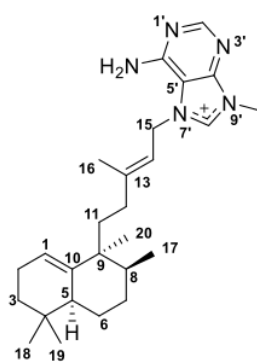


Figure 4. Structure of iso-agelasine C

Table 2. ¹H- and ¹³C-NMR data of isolated compound (400 and 125 MHz, resp.) in DMSO-*d*₆*

Position	δ ppm (H)	δ ppm (C)
1	5.29 (br. t, <i>J</i> = 3.8)	117.2
2	1.95 (m)	23.3
3	1.01; 1.33 (m)	31.3
4	-	31.5
5	1.54 (m)	43.6
6	1.07; 1.74 (m)	30.3
7	1.45; 1.50 (m)	31.5
8	1.26 (m)	44.7
9	-	42.7
10	-	146.1
11	1.04; 1.16 (m)	29.5
12	1.76; 1.83 (m)	34.2
13	-	147.2
14	5.38 (t, <i>J</i> = 6.8)	115.4
15	5.13 (br. d, <i>J</i> = 6.8)	47.6
16	1.76 (s)	17.3
17	0.80 (d, <i>J</i> = 6.8)	16.9
18	0.81 (s)	28.3
19	0.82 (s)	28.0
20	0.97 (s)	23.5
2'	8.43 (s)	156.0
4'	-	149.5
5'	-	109.8
6'	-	152.9
8'	9.54 (s)	141.3
NH ₂ -C(6')	7.90 (br. s)	-
MeN-C(9')	3.85 (s)	32.0

*DMSO-*d*₆ peak was referenced at 2.5 ppm for ¹H NMR and 40 ppm for ¹³C NMR; coupling constant (*J*) are reported in Hz

2.3. AChE inhibitory activity of the isolated compound

The isolated compound demonstrated AChE inhibitory activity with an IC₅₀ value of 30.68 ± 0.92 μg/mL. This compound inhibited the AChE enzyme in a dose-dependent manner (Figure 5), and this was also applied for the fractions and extract of *A. nakamurai*. Based on this data it can be seen that the isolated compound gave lower activity compared to the fraction and extract of *A. nakamurai*. The higher inhibition against AChE in the fractions and extract may be attributed to the synergistic effect of iso-agelasine C with other compounds presents in the fractions and extract.

Iso-agelasine C is a diterpene alkaloid with structural features consisting of a 8-*epi-ent*-halim-1(10)-enes in the diterpene moiety and 9-methyladenine in the alkaloid moiety [14,15]. Halimane purine type of compounds have been reported from Agelasidae sponges, such as *Agelas* sp., *Agelas mauritiana*, and *Agelas nakamurai* [14,15]. Several bioactivities have been documented from halimane purines. Agelasine C isolated from *Agelas* sp. and *Agelas citrina* showed antifungal activity [16]. *Epi*-agelasine C obtained from *A. mauritiana* exhibited antifouling activity against *Ulva conglobata* [17]. *A. nakamurai* has been investigated cytotoxic and antimicrobial activities of iso-agelasine C together with other diterpene alkaloids. Iso-agelasine C demonstrated a weak cytotoxic activity against HCT-116, K562, and HL-60 cell lines. This compound also showed antimicrobial activity against *Candida albicans* and *Proteus bacillus vulgaris* [13]. To the best of our knowledge, this is the first report of iso-agelasine C as an AChE inhibitor. Although there is no report on the cholinesterase inhibitory activity of diterpene alkaloids from marine sources, several diterpene alkaloids isolated from plants such as heterophylline-A and heterophylline-B isolated from the root of *Aconitum heterophyllum* have shown selective inhibition against butyrylcholinesterase (BChE) compared to AChE [18].

Investigation of the same plant resulted in the isolation of other diterpene alkaloids with promising cholinesterase inhibitory activities [19]. Focusing on the possible contribution of halimane moiety of iso-agelasine C in the AChE inhibitory activity, a literature search showed that a halimane diterpenoid such as limbatolide B showed less AChE inhibition compared to clerodane diterpenoid limbatolide A [20]. Based on this finding, it can be suggested that modification of the structure of iso-agelasine C especially on the halimane moiety can be carried out to increase the potency of this compound as an AChE inhibitor. A study on enzyme selectivity can be carried out by comparing inhibition with BChE.

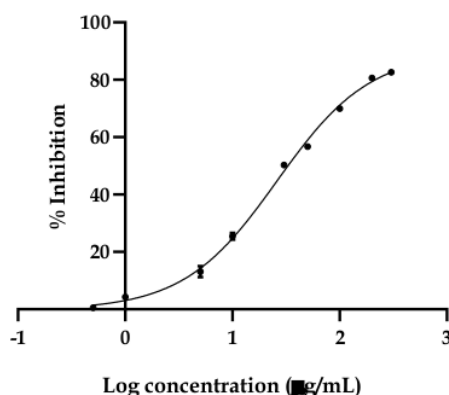


Figure 5. Dose-dependent response of iso-agelasine C against AChE

3. CONCLUSION

Bioassay-guided protocol applied to *A. nakamura* led to the isolation of iso-agelasine C, which showed moderate inhibitory activity against the AChE enzyme. This compound could serve as a lead for the development of halimane alkaloid compounds as AChE inhibitors.

4. MATERIALS AND METHODS

4.1. Chemical and reagents

All analytical grade solvents such as methanol, n-hexane, dichloromethane, and HPLC grade solvents acetonitrile and methanol were purchased from Merck. Silica gel 60 with 40-63 µm particle size, and TLC plates silica gel 60 F₂₅₄, were also obtained from Merck.

The reagent for the AChE assay i.e. acetylcholinesterase from electric eel (AChE type VI-S), acetylthiocholine iodide (ATCI), 5,5'-dithiobis[2-nitrobenzoic acid] (DTNB), bovine serum albumin, galantamine tris buffer, and tris-HCl were purchased from Sigma.

4.2. Sponge collection

Agelas nakamura was collected from Tabuhan Island dive site, Banyuwangi, East Java, Indonesia using SCUBA at a depth of 10-20 m on 23 April 2017. The sponge was frozen at -20°C until extraction. A voucher specimen (23-04-17-07) was made in 80% aqueous ethanol and deposited at the Institute of Tropical Diseases Universitas Airlangga. Identification of the sponges was conducted by Dr. Tri Aryono Hadi of Research Center of Oceanography, Indonesian Institute of Sciences, Jakarta, Indonesia.

4.3. General experimental procedures

HPLC was performed in Shimadzu LC-20AD UV-2200A. The ¹H- and ¹³C-NMR spectra were recorded on JEOL NMR spectrometers operating at 400 MHz (100 MHz for ¹³C). The chemical shifts values are reported in ppm. The optical rotation was determined with an Autopol IV automatic polarimeter (Rudolph Research Analytical). HR-ESIMS were recorded on LC-MS instrument ACQUITY UPLC H-Class System (waters, USA)

fitted with Waters® C-18 column (1.8 µm, 2.1 x 100 mm) connected to HR-ESIMS Xevo G2-S QTOF (Waters, USA), and the data were analysed on Masslynx V4.1.

4.4. Isolation procedures

Sponge material was cut into small pieces, freeze-dried, and powdered. Dried sponge powder (77 g) was extracted with methanol (3 x 450 mL) by using the maceration method to obtain a crude methanolic extract (6.7 g). The extract was fractionated between aqueous methanol and n-hexane (150 mL), the n-hexane layer was collected, and the aqueous layer was further fractionated with ethyl acetate (200 mL), followed by fractionation with n-butanol (100 mL) using the same procedure. Fractionation with each solvent was conducted 3 times. Each of the fractions was then concentrated in a rotary evaporator, and yielded n-hexane (0.8 g), ethyl acetate (4.6 g), and n-butanol (0.7 g) fractions. The ethyl acetate fraction was subjected to vacuum liquid chromatography using silica gel 60 (particle size 40-63 µm) eluted with a combination of dichloromethane and methanol in order of increasing polarity to give 11 subfractions (VLC 1- VLC 11). A portion of subfraction VLC 2 was further purified by HPLC using a semipreparative Waters® column (µBondapak C-18 7.8 x 300 mm). The sample was eluted with gradient elution of 70-100% acetonitrile in water containing 0.1% TFA for 30 min, then eluted with 100% methanol for 30 min, flow rate 1.5 mL/min, detection UV 254 nm. Four fractions were collected including a major peak at retention time (RT) 44 min (VLC 2.4). Repeated HPLC was carried out for VLC 2.4 using the same procedure as already described above to yield iso-agelasine C (19 mg). The same HPLC purification procedure was applied to fraction VLC 3 and yielded iso-agelasine C (26.3 mg)

Iso-Agelasine C was obtained as a white amorphous solid, $[\alpha]_D^{20} = +28.0$ (MeOH, *c* 0.25), UV (MeOH) λ_{\max} 269 nm; HR-ESIMS *m/z* [M+H]⁺ 422.3280, C₂₆H₄₀N₅ calcd. 422.3284, ¹H and ¹³C-NMR see table 2.

4.5. Microplate assay for AChE activity determination

The assay was conducted based on the modified Ellman's method [12,21-25]. Samples were prepared in methanol at a concentration of 10 mg/mL, and were diluted with water to obtain a 1 mg/mL concentration. Further dilution of samples in the microplate well to a final test concentration of 100 µg/mL. Sample solutions (25 µL) were added to a 96-well microplate, followed by the addition of 25 µL ATCI (1.5 mM), 125 µL DTNB (3 mM), and finally 50 µL Tris buffer was added. The enzyme *EeAChE* 25 µL of 0.22 U/mL was then added. The solutions were shaken for 30 s in a microplate reader (Bio-Tek Instrument, USA) before measurement. The absorbance was measured at 405 nm every 5 s for 2 min. Measurement of the absorbance was carried out before and after the addition of the enzyme. The absorbance after addition of the enzyme was subtracted with absorbance before addition of the enzyme, and was then used for calculation of the percentage of inhibition. Galantamine was used as a positive control, and 10% methanol was used as a control. For the measurement of IC₅₀, serial concentrations of the samples (0.5 - 300 µg/mL) were prepared. Experiments were carried out three times, each done in triplicates. The percentage of enzyme inhibition was calculated by using the equation (Eq. 1) below:

$$\%Inhibition = \frac{\text{mean velocity of control} - \text{mean velocity of sample}}{\text{mean velocity of control}} \times 100$$

Equation 1. Calculation of inhibitory activity

4.6. Data analysis

The IC₅₀ values as well as the unpaired t-test analyses were carried out by the Prism software (Graph Pad 8 Inc, San Diego, USA). The IC₅₀ values were obtained by plotting the percentage of inhibition as ordinate and log of concentration as an axis.

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REFERENCES

- [1] Alzheimer's Association. 2020 Alzheimer's disease facts and figures. *Alzheimer's Dement.* 2020; 16(3): 391-460. [\[CrossRef\]](#)
- [2] Alzheimer's Association. 2015 Alzheimer's disease facts and figures. *Alzheimer's Dement.* 2015;11(3):332-384. [\[CrossRef\]](#)
- [3] Konrath EL, Passos CDS, Klein-Júnior LC, Henriques AT. Alkaloids as a source of potential anticholinesterase inhibitors for the treatment of Alzheimer's disease. *J Pharm Pharmacol.* 2013; 65(12): 1701-1725. [\[CrossRef\]](#)
- [4] Breijyeh Z, Karaman R. Comprehensive review on Alzheimer's disease: causes and treatment. *Molecules.* 2020; 25(24): 5789. [\[CrossRef\]](#)
- [5] Dev K, Maurya R. Marine-derived anti-Alzheimer's agents of promise. In: Brahmachari G. (Ed). *Neuroprotective Natural Products, Clinical Aspects and Mode of Action.* Wiley-VCH., Weinheim, 2017, pp. 153-184.
- [6] Mathew M, Subramanian S. *In vitro* screening for anti-cholinesterase and antioxidant activity of methanolic extracts of ayurvedic medicinal plants used for cognitive disorders. *Plos one.* 2014; 9(1): e86804. [\[CrossRef\]](#)
- [7] Hostettmann K, Borloz A, Urbain A, Marston A. Natural product inhibitors of acetylcholinesterase. *Curr Org Chem.* 2006; 10(8): 825-847. [\[CrossRef\]](#)
- [8] Heinrich M, Teoh HL. Galanthamine from snowdrop - the development of a modern drug against Alzheimer's disease from local Caucasian knowledge. *J Ethnopharmacol.* 2004; 92(2-3): 147-162. [\[CrossRef\]](#)
- [9] Ahmed F, Ghalib R, Sasikala P, Mueen AK. Cholinesterase inhibitors from botanicals. *Pharmacogn Rev.* 2013; 7(14): 121-130. [\[CrossRef\]](#)
- [10] Murray A, Faraoni M, Castro M, Alza N, Cavallaro V. Natural AChE inhibitors from plants and their contribution to Alzheimer's disease therapy. *Curr Neuropharmacol.* 2013; 11(4): 388-413. [\[CrossRef\]](#)
- [11] Zhang H, Dong M, Chen J, Wang H, Tenney K, Crews P. Bioactive secondary metabolites from the marine sponge genus *Agelas*. *Mar Drugs.* 2017; 15(11): 351. [\[CrossRef\]](#)
- [12] Suciati, Rabgay K, Fachrunniza Y, Saesong T, Hadi TA, Wahyuni TS, Widyawaruyanti A, Ingkaninan K. Enzyme inhibitory activities of marine sponges against cholinesterase and 5 α -reductase. *Malays Appl Biol.* 2019; 48(3): 77-83.
- [13] Chu M, Tang X, Qin G, Sun Y, Li L, Li P, Li G. Pyrrole derivatives and diterpene alkaloids from the South China Sea sponge *Agelas nakamurai*. *Chem Biodivers.* 2017; 14(7). e1600446. [\[CrossRef\]](#)
- [14] Roncero AM, Tobal IE, Moro RF, Díez D, Marcos IS. Halimane diterpenoids: sources, structures, nomenclature and biological activities. *Nat Prod Rep.* 2018; 35(9): 955-991. [\[CrossRef\]](#)
- [15] García PA, Valles E, Díez D, Castro MÁ. Marine Alkylpurines: A promising group of bioactive marine natural products. *Mar Drugs.* 2018; 16(1): 6. [\[CrossRef\]](#)
- [16] Stout EP, Yu LC, Molinski TF. Antifungal diterpene alkaloids from the Caribbean sponge *Agelas citrina*: Unified configurational assignments of agelasidines and agelasines. *Eur J Org Chem.* 2012; 2012(27): 5131-5135. [\[CrossRef\]](#)
- [17] Hattori T, Adachi K, Shizuri Y. New agelasine compound from the marine sponge *Agelas mauritiana* as an antifouling substance against macroalgae. *J Nat Prod.* 1997; 60(4): 411-413. [\[CrossRef\]](#)
- [18] Nisar M, Ahmad M, Wadood N, Lodhi MA, Shaheen F, Choudhary MI. New diterpenoid alkaloids from *Aconitum heterophyllum* Wall: Selective butyrylcholinesterase inhibitors. *J Enzyme Inhib Med Chem.* 2009; 24(1):47-51. [\[CrossRef\]](#)
- [19] Ahmad H, Ahmad S, Shah SAA, Latif A, Ali M, Khan FA, Tahir MN, Shaheen F, Wadood A, Ahmad M. Antioxidant and anticholinesterase potential of diterpenoid alkaloids from *Aconitum heterophyllum*. *Bioorg Med Chem.* 2017; 25(13): 3368-3376. [\[CrossRef\]](#)
- [20] Ahmad VU, Khan A, Farooq U, Kousar F, Khan SS, Nawaz SA, Abbasi MA, Choudhary MI. Three new cholinesterase-inhibiting cis-clerodane diterpenoids from *Ostostegia limbata*. *Chem Pharm Bull.* 2005; 53(4): 378-381. [\[CrossRef\]](#)
- [21] Ellman GL, Courtney KD, Andres V Jr, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol.* 1961; 7(2): 88-95. [\[CrossRef\]](#)
- [22] Ingkaninan K, Temkitthawon P, Chuenchom K, Yuyaem T, Thongnoi W. Screening for acetylcholinesterase inhibitory activity in plants used in Thai traditional rejuvenating and neurotonic remedies. *J Ethnopharmacol.* 2003; 89(2-3): 261-264. [\[CrossRef\]](#)
- [23] Sirimangkalakitti N, Olatunji OJ, Changwichit K, Saesong T, Chamni S, Chanvorachote P, Ingkaninan K, Plubrukam A, Suwanborirux K. Bromotyrosine alkaloids with acetylcholinesterase inhibitory activity from the thai sponge *Acanthodendrilla* sp. *Nat Prod Commun.* 2015; 10(11): 1945-1949. [\[CrossRef\]](#)
- [24] Suciati, Laili ER, Poerwantoro D, Hapsari AP, Gifanda LZ, Rabgay K, Ekasari W, Ingkaninan K. Evaluation of cholinesterase inhibitory activity of six Indonesian *Cassia* species. *J Res Pharm.* 2020; 24(4): 472-478. [\[CrossRef\]](#)
- [25] Suciati, Poerwantoro D, Widyawaruyanti A, Ingkaninan K. Acetylcholinesterase inhibitory activity of extract and fractions from the root of *Rauwolfia serpentina*(L.) Bth.ex Kurz. *J Basic Clin Physiol Pharmacol.* 2021; 32(4): 313-317. [\[CrossRef\]](#)

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