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Characterization of anti-dengue and cytotoxic activity of protein hydrolysates from the exophytic bacteria of brown algae *Sargassum* sp

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ABSTRACT

The protein of exophytic bacteria *Enterobacter agglomerans* SB 5 (1) derived from *Sargassum* sp. was extracted and hydrolyzed by an enzyme to obtain bioactive peptides. The bioactivity of peptides was tested after being hydrolyzed with pepsin. Pepsin peptide fraction <3 kDa showed the activity of antidengue against Vero cells with the percentage of inhibition and CC_{50} value of 91% and 129 µg/ml, respectively. This fraction was toxic to shrimp larvae in the Lethal Concentration 50 (LC_{50}) value of 1.77 µg/ml. Cytotoxic activity of pepsin peptide fraction <3 kDa against cervical cancer cells showed the LC_{50} value of 0.749 µg/ml. Protein precursors were identified using liquid chromatography-tandem mass spectrometry by searching for Peptides Mass Fingerprint. Sequence analysis of the peptide showed abundant leucine amino acid residue. This peptide sequence has never been reported so far, including its potential biological function as a new antidengue and anticancer agent.

INTRODUCTION

The incidence of dengue and cancer has increased sharply over the past decade, and an estimate of 390 million dengue virus infections per year is indicated by a model (Bhatt *et al*., 2013). Until now, there have been no specific and effective treatments for dengue virus infection and cancer diseases. Interest in the study of the physiological activity of algae and their derivatives has been growing lately, leading to the exploration of millions of novel compounds with biological properties from various marine microsymbiont organisms (Yasuhara-Bell and Lu, 2010). Indonesia has a coastline of 81,000 km, second only to Canada, and comprises 17,000 islands. Such conditions make it an appropriate place for algae cultivation (Wouthuyzen *et al*., 2016).

Marine algae are classified into three groups, namely, Chlorophyceae, Rhodophyceae, and Phaeophyceae. *Sargassum* sp. which belongs to the group Phaeophyceae (brown algae) is widely cultivated in Indonesian waters. Based on the literature review, *Sargassum* sp. is often utilized as a producer of carrageenan (Manuel *et al*., 2015). Even though the proteins of the exophytic bacteria derived from *Sargassum* sp. repeatedly showed a unique biological activity such as antibacterial, antifungal, anticancer, and antidengue (Ahmad *et al*. 2019; Koishi *et al*., 2012; Rodrigues *et al*., 2009; Sugrani *et al*., 2019a), only a few studies have utilized the exophytic bacteria derived from *Sargassum* sp. Furthermore, Schmidt *et al*. (2010) demonstrated and reported that many peptides could inhibit dengue virus replication and infection. In recent years, some studies also released and reported that many types of peptides were used as antidengue agents (Chew *et al*., 2017; Hrobowski *et al*., 2005). Most recently, our group research also reported that many protein hydrolysates from different sources of microsymbionts marine algae could inhibit the pathogenic bacteria and cancer cells line (HeLa) (Asmi *et al*., 2020; Sugrani *et al*., 2020).

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In our previous study, we examined the potential for the antidengue activity of proteins from the exophytic bacteria *Enterobacter agglomerans* SB 5 (1) derived from *Sargassum* sp. Potential protein fraction on antidengue activity was demonstrated in the 60%–80% saturation of ammonium sulfate fraction with the inhibition percentage and also CC_{50} value of 70% and 260.37 µg/ml, respectively (Ahmad *et al*., 2019). Nevertheless, the bioactivity results of antidengue and cytotoxic activity studies have not exhibited the maximum results. According to Wang and Zhang (2016), protein activity will increase from protein hydrolysis. Besides hydrolysis, low molecular weights are also related to the activity of protein hydrolysate. In this study, the protein of exophytic bacteria derived from *Sargassum* sp. would be fractioned, identified, and characterized by its bioactive protein hydrolysate with low molecular weight and its potential as a new antidengue and anticancer agents.

MATERIALS AND METHOD

Materials

This study used the exophytic bacteria *E. agglomerans* SB 5(1) from *Sargassum* sp. obtained from Biochemistry Laboratory, Hasanuddin University, Indonesia, egg of *Artemia salina*, Leach., HeLa cells, Vero cells, dengue virus serotype-2 (DENV-2), Viral ToxGlo™ Assay kit, Dulbecco's modified eagle medium (DMEM) media, 1% (*w/v*) L-glutamine, Bovine Serum Albumin (BSA), Lowry reagents, ammonium sulfate, cellophane bags, and sterile seawater.

Preparation of protein fraction

The cell cultures of the exophytic bacteria *E. agglomerans* SB 5 (1) were prepared according to the method described by Ahmad *et al*. (2019), with a few modifications. Tris–HCl buffer (20 mM, pH 8.2) was added to 30 g of cell pellet and then homogenized. It was then centrifuged at 4,000 rpm, at 4°C for 45 minutes. The supernatant was collected, then fractionated 20% by ammonium sulfate, stirred overnight at 4°C, and then centrifuged at 10,000 rpm at 4°C for 60 minutes to obtain the deposition of protein. This protein fraction was stored in the refrigerator for enzymatic hydrolysis (Uluko *et al*., 2015).

Determination of protein content

The determination of protein content by the Lowry method was measured by a UV–Vis spectrophotometer with BSA as standard (Ahmad *et al*., 2019).

Preparation of protein hydrolysates

Protein fractions were dissolved 3% (*v/v*) in 20 mM Tris–HCl buffer at pH 7, and protein suspensions were hydrolyzed by pepsin enzyme (EC 3.4.23.1) using a 6% *w/w* enzyme to substrate ratio, at the temperature of 37°C, pH 2 for 2 hours, and the enzymatic hydrolysis ceased by heating at 95°C for 10 minutes (Sugrani *et al*., 2020). The protein hydrolysate samples were placed at −20°C prior to use. The degree of hydrolysis (DH) was calculated using equation (1). Pepsin hydrolysates were separated using membrane molecular weight cut-off (MWCO) membranes (3, 5, and 10 kDa). The fraction was frozen at −20°C for further analysis (Uluko *et al*., 2015).

$$DH\ (\%) = [(h-h0)/Htot] \times 100 \qquad (1)$$

where "h" is the number of peptide bonds cleaved during hydrolysis, "h0" is the content of free amino groups of substrates, and "Htot" is the total number of peptide bonds in the protein substrate (8.6 mEq/g protein) (Cian *et al*., 2012).

Ultrafiltration

The separation of MWCO, which used a Vivaspin 20 Fisher Scientific, was the ultrafiltrates lyophilized hydrolysate process. The fractions were collected from the molecular weight (MW) range of >10 kDa, 5–10 kDa, 3–5 kDa, and <3 kDa and stored at −20°C (Guo *et al*., 2008). These fractions were Peptides Enterobacter from Algae (PEA): PEA1a, PEA1b, PEA1c, and PEA1d.

SDS-PAGE electrophoresis

The purified level and molecular weight of the peptide were determined using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 7% and 10% SDS, using a Mini-PROTEAN 3 cells apparatus (Laboratories of Bio-Rad, Irvine, CA). About 3 µg/µl of peptide and marker was dissolved in the sample buffer and transferred to the vertical slab space electrophoresis system (height 10 cm, width 10 cm, and thickness 0.6 mm). The electrophoresis process was running at 60 mA for 3–4 hours at room temperature (Celebioglu *et al*., 2017) and a protein marker (Fermentas, Lithuania) was used as a molecular weight standard. Finally, the pattern of protein bands was visualized with Coomassie Brilliant Blue R250 described by Fluka USA.

Bioactivity test

Toxicity and antidengue test

Toxicity and the antidengue tests were conducted using Brine Shrimp Lethality (BSLT) and 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) method, respectively. BSLT was used to test the cytotoxic according to Sugrani *et al*. (2019b), and Ahmad *et al*. (2019). Observation data were tabulated, and probit analysis was carried out to get the value of the Lethal Concentration 50 (LC_{50}). Antidengue test of protein fraction and its hydrolysates was carried out against Vero cells infected with the DENV-2 virus. Cytotoxic tests were carried out using Viral ToxGlo™ Assay kit (Promega) based on Karimi *et al*. (2016). The measurements of cells on the viability percentage (%) and the 50% cytotoxic concentration (CC_{50}) value (µg/ml) were calculated from the percentage of inhibition at 20 µg/ml samples administration, based on the probit analysis (Ahmad *et al*., 2019).

Cytotoxicity test

Cytotoxicity of peptide fraction was carried out quantitatively using the MTT method (Boomi *et al*., 2019). HeLa were preserved in DMEM with 10% fetal bovine serum (FBS) and antibiotic-antimycotic solution. The cell was allowed to grow at a temperature of 37°C with a continuous supply of 5% CO_2. HeLa cells (2×10^3 cells/100 µl) inserted into 96-well plates contained 200 µl DMEM media and 10% FBS. The plates were incubated under conditions similar to 5% CO_2. After 24 hours of culture, the used media were removed. Fresh DMEM media with determined concentrations (200; 100; 50; 25; 12.5; 6.25; 3.13; and 1.56 µg/ml) and hydrolysate and peptide samples were added and incubated again under conditions similar to 5% CO_2. The used media were

replaced with 10 µl MTT solutions for each well (5 µg/ml) and incubated for 4 hours at 37°C in a humid incubator with 5% CO_2. After 4 hours, the MTT was removed, and a 100 µl DMSO was added to all the wells. Spectral absorbance was observed at 570 nm. Cells themselves except the addition of peptides/hydrolysates were considered as controls.

Identification of bioactive protein hydrolysates fraction

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis

The pepsin peptide fraction <3 kDa was dissolved in acetonitrile and then filtered by a Millipore filter unit with a size of 0.2 µm. A total of 10 µl of sample filtrate was injected into the LC-ESI-QTOF instrument system. LC-MS analysis was carried out by the UPLC-MS [ACQUITY UPLC®H-Class System (waters, Milford, MA)] equipped with a binary pump. LC was connected to a QTOF mass spectrometer equipped with an ESI ionization source. MS used Xevo G2-X2 QT with positive ionization mode. The ESI parameters used in full scan mode from *m/z* 50–3,000 were carried out with a source temperature of 50°C (column) and 25°C (room). The UPLC column used CORTECS C8 Column (2.7 µm, 2.1 mm × 100 mm). The effluents used were H_2O plus 5 mM ammonium formate and acetonitrile plus 0.05% formic acid, flow velocity: 0.2 ml/minute (gradient) (Wang and Zhang, 2016).

Database searching and in silico activity prediction

The analysis resource and immune epitope database (IEDBe) were used for antidengue prediction (Guevarra *et al*., 2020). The AntiCP was used for anticancer activity prediction (Tyagi *et al*., 2013). Prot Param was used to calculate various chemical and physical parameters of the isolated peptide fraction (Gasteiger *et al*., 2005).

RESULT AND DISCUSSION

Protein isolation and protein hydrolysate production

In this study, protein isolation from the exophytic bacteria *E. agglomerans* SB 5 (1) derived from *Sargassum* sp. (Ahmad *et al*., 2019) was conducted by using a combined method (mechanical and nonmechanical) with Tris–HCl buffer media pH 8.3. The results showed that the extraction yield of 20% protein (PEA) was 7.50%, with a protein content of 27.78 mg/ml (Table 1). The fraction of protein content (22.78 mg/ml) was higher than the level of crude extract (4.45 mg/ml) of the exophytic bacteria *E. agglomerans* SB 5(1) derived from *Sargassum* sp. Compared with our previous study, the combined method obtained higher yield protein (7.50%) than those previously reported in the same resources, which only obtained 2.30%–3.50% of the protein fraction (Ahmad *et al*., 2019; Karim *et al*., 2020).

Furthermore, the isolated protein was hydrolyzed using the commercial enzyme by pepsin (EC 3.4.23.1) to evaluate its biological waste. Enzymatic hydrolysis was carried out to release bioactive bacterial peptides. Protein compounds from algae symbiont bacterial were valuable sources of bioactive peptides, trapped in sequences of primary amino acids, which are released through enzymatic hydrolysis during food digestion in the digestive tract (Beaulieu *et al*., 2015). The DH of PEA1 was more than 10%. According to Morris *et al*. (2008), such a DH has the potential to be used to produce pharmaceutical products.

Bioactivity test

Toxicity and antidengue potential activity

The toxicity test results of the protein fraction (PEA) and pepsin hydrolysate (PEA1) can be seen in Table 2. The smaller the toxicity value, the more the significant toxicity

Table 1. The DH, the protein content fraction, hydrolysate, and peptides of exophytic bacteria *E. agglomerans* SB 5 (1) from *Sargassum* sp.

Samples	Abbreviations	Protein content (mg/ml)	Yield (%)	DH (%)
Crude extract	PE	4.45	–	–
Protein Fraction	PEA	27.78	7.50	–
Pepsin hydrolysate	PEA1	7.86	60.82	23.71
Pepsin peptide fraction >10 kDa	PEA1a	5.75	25.52	–
Pepsin peptide fraction 5–10 kDa	PEA1b	5.40	7.03	–
Pepsin peptide fraction 3–5 kDa	PEA1c	2.58	0.83	–
Pepsin peptide fraction <3kDa	PEA1d	5.06	1.05	–

Table 2. Summary of protein and its hydrolysate for toxicity and antidengue potential after the administration of protein and its hydrolysates fraction.

Samples	Abbreviations	LC_{50} Value (µg/ml)	Inhibition at 20 µg/ml (%)	CC_{50} (µg/ml) in vero Cell
Protein fraction	PEA	27.33	10	>1,000
Pepsin hydrolysate	PEA1	22.20	25	>1,000
Pepsin peptide fraction >10kDa	PEA1a	16.55	50	>500
Pepsin peptide fraction 5–10kDa	PEA1b	10.29	55	255
Pepsin peptide fraction 3–5kDa	PEA1c	4.09	86	154
Pepsin peptide fraction <3kDa	PEA1d	1.77	91	129

activity. Based on this result, PEA1 was continued in the next stage. Overall, the toxicity value, as shown in Table 2, showed that protein and its hydrolysate fractions have different values. Toxicity value of PEA (protein fraction), PEA1 fraction (pepsin hydrolysate), PEA1a fraction (pepsin peptide fraction >10 kDa), and PEA1b fraction (pepsin peptide fraction 5–10 kDa) showed high toxic levels. Meanwhile, PEA1c fraction (pepsin peptide fraction 3–5 kDa) and PEA1d fraction (pepsin peptide fraction <3 kDa) showed remarkably high toxic levels. The various toxicity values of the protein fraction and its hydrolysate are due to the biological activity. Peptides with small molecular weights often exhibit substantial biological activities (Vandanjon *et al*., 2007). Pepsin peptide fraction <3 kDa (PA1d) was the potential peptide with the lowest LC_{50} (1.77 µg/ml) compared to other fractions.

The antidengue properties of the protein and its hydrolysate fractions were confirmed by cytotoxicity test using Vero cells infected with the DENV-2 virus. The antidengue activity of protein fraction and its hydrolysate against Vero cells indicated the potential antidengue. The highest inhibition percentage and CC_{50} were PEA1d (91 %, 129 µg/ml, respectively), followed by PEA1c (86%, 154 µg/ml, respectively). Some peptide sequences exhibited an antiviral effect on dengue in cell culture. For instance, peptide sequence FWFTLIKTQAKQPARYRRFC has been reported as antidengue and displayed an IC_{50} value of 7 µM in a focus-forming unit assay (Costin *et al*., 2010).

Anticancer potential activity

Based on toxicity and antidengue results, it was found that PEA1d is a potential isolated protein. The PEA1d was then tested further in its cytotoxic activity by using the MTT method against HeLa cells as a model of cervical cancer cells. The cytotoxic activity can be observed from the absorbance value (Table 3), which was used to calculate the viability percentage of HeLa cells (Fig. 1). The results showed that PA1d could inhibit HeLa cell growth with an IC_{50} value of 0.75 µg/ml, considered to be a very toxic level. According to E-Kobon *et al*. (2016), anticancer peptide activity is influenced by several things, including amino acid composition and physicochemical properties such as charge, aliphatic index, and amphipathic and structural or confirmation form.

SDS-PAGE electrophoresis

The profile of the extracted protein, protein hydrolysate, and peptide from the exophytic bacteria *E. agglomerans* SB 5 (1) derived from *Sargassum sp.* was determined by running SDS-PAGE (Fig. 2). Estimated MW for each protein was carried out by comparing the distance between sample mobility with protein markers in the SDS-PAGE chromatogram.

Electrophoresis results showed that crude extracts (PE) had scattered and thick bands at various molecular weights. The thickest crude extract tapes were at MW 173.33 kDa, and after fractionation with 20% ammonium sulfate, 20% protein fraction (PEA) was seen having an MW of 27.67 kDa. These results indicated that ammonium sulfate successfully fractionated crude protein samples. This 20% protein fraction was then hydrolyzed using the pepsin enzyme to become protein hydrolysate (PEA1), which appears to have a band at MW of 20.02 kDa, but with a thinner or faded band color, indicating that the hydrolysis successfully occurred. Ultrafiltration of protein hydrolysate showed PEA1c, and PEA1d had low molecular weight at MW of 4.25 kDa and 2.98 kDa, respectively.

Identification of bioactive peptides

LC-MS/MS was chosen for further characterization of peptides. The results of peptide patterns contained in the PEA1d fraction are shown in Figures 3 and 4. Peptide identification was carried out using MASCOT mass fingerprint peptide software (de novo sequencing). De novo sequence peptide was determined from MS/MS spectra data (Fig. 4), fragments PEA1d-1 (344.2310; 345.2341; 346.2357), PEA1d-2 (388.2568; 389.2601; 390.2616), PA1d-3 (432.2827; 436.2856; 434.23; 476.3097; 477.3118; 478.3138), PEAd1-4 (476.3097; 477.3118; 478.3138; 497.2363; 504.3391; 519.3161), PEAd1-5 (520.3341; 521.3376; 522.3398), PEAd1-6 (564.3606; 565.3635; 566.3657; 567.3682), and PEA1d-7 (608.3867; 609.3898; 6109.389). A total of seven sequences of pepsin peptide fraction <3kDa were identified by the MASCOT program (score >60%) with NCBIprot as a protein sequence database (Table 4). All identified peptide fragments had come from bacteria-derived proteins. All peptide fragments had low molecular masses of less than 3 kDa. Of seven peptides, three peptide fragments were negatively charged, three peptide fragments were positively charged, and one was a neutral fragment.

In our study, seven peptide fragment sequences were obtained from the enzymatic hydrolysis process of a protein fraction, namely, MGFIVLGRLEAFVDLCHL (PEA1d-1), MQLDYLECFKGVLGEALRQLGRR (PEA1d-2), NSLKAT-LCLSLTLALAPSL (PEA1d-3), LADGLDSYLELPLFTAVAIL (PEA1d-4), MEELSRNFYIVFIIIGLYSMF (PEA1d-5), SGACT-FGMVFLTGCGLF (PEA1d-6), and VRSIFVRVQLEALYL-CRAIFHDVVF (PEA1d-7). All of the discovered peptide sequences seemed to be rich in leucine amino acid residue, especially in PEA1d3 and PEA1d4 fractions, which contained the leucine-rich

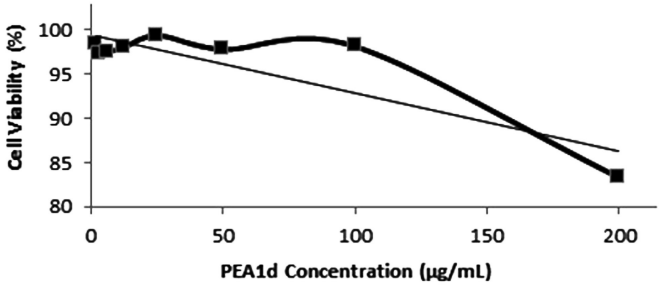

Figure 1. The cytotoxic effect of PEA1d peptides on the viability of HeLa cells.

Table 3. The data of cytotoxic activity against HeLa cell cancer from PEA1d peptides.

Compound	Absorbance at various concentrations								IC_{50} (µg/ml)
Conc. (µg/ml)	1.56	3.13	6.25	12.5	25	50	100	200	0.749
PEA1d	0.688	0.681	0.683	0.686	0.693	0.684	0.686	0.601	

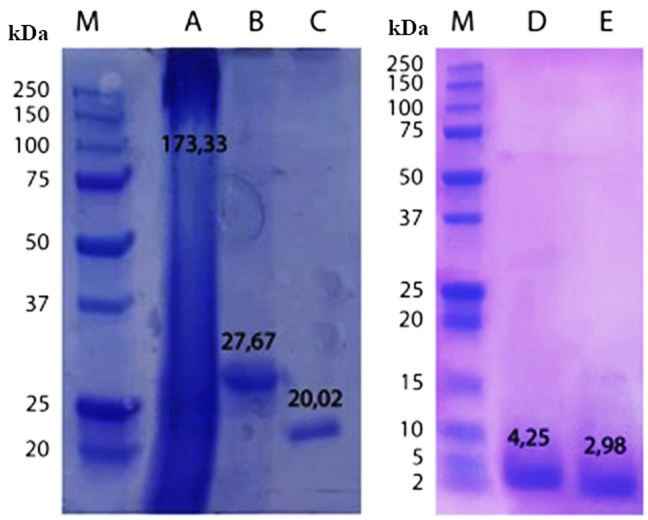

Figure 2. SDS-PAGE chromatogram of protein and its hydrolysate fractions. Marker (M), crude extract (A), protein fraction 20% (B), protein hydrolysate (C), PEA1c peptides 3–5kDa (D), and PEA1d peptides <3kDa (E).

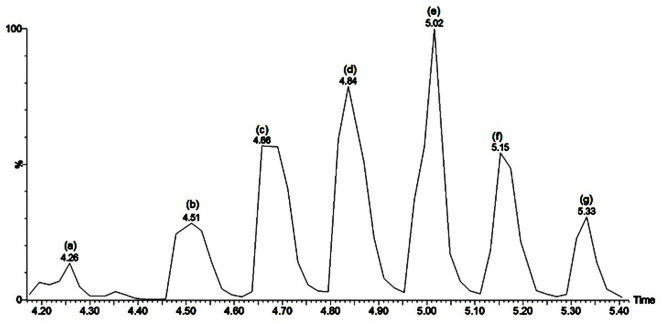

Figure 3. Liquid chromatography (LC) chromatogram of PEA1d-1 to PEA1d-7 peptide fraction: (a) PEA1d-1, (b) PEA1d-2, (c) PEA1d-3, (d) PEA1d-4, (e) PEA1d-5, (f) PEA1d-6, and (g) PEA1d-7.

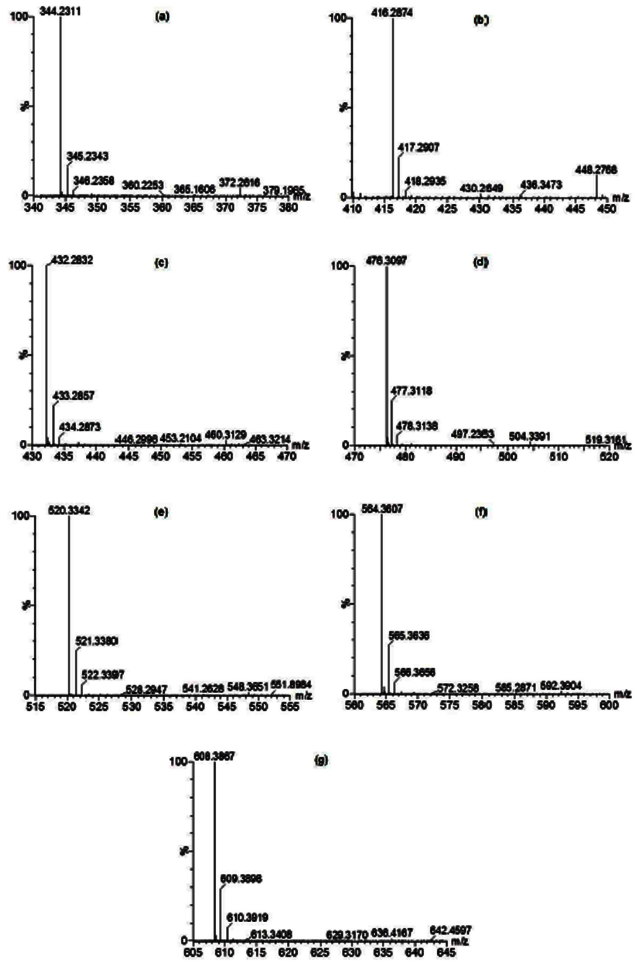

Figure 4. MS/MS spectra of PEA1d-1 to PEA1d-7 peptide fraction: (a) PEA1d-1, (b) PEA1d-2, (c) PEA1d-3, (d) PEA1d-4, (e) PEA1d-5, (f) PEA1d-6, and (g) PEA1d-7.

repeat motifs, and these motifs were not observed in the other five peptide sequences. Thus, these leucine-rich repeat motifs in PEA1d3 and PEA1d4 may contribute to antidengue and anticancer properties (Tables 2, 3, and 5). According to Brogden (2005), leucine-rich repeat motifs had an effect on the unique bioactivity of proteins and peptides. Search results on the NCBIprot and UniProt/SWISS-PROT protein and peptide databases of all peptide sequences (PEA1d-1–PEA1d-7), especially four leucine-rich repeat motifs in PEA1d3 and PEA1d4, did not show any report of these peptides; therefore, these were the first reported peptide sequences.

On the other hand, out of the seven peptide fragments, PEA1d-3 had the highest cationic charge among the others (+2). Cationic charge (positive) determined the antidengue (Guevarra *et al*., 2020; Schmidt *et al*., 2010) and anticancer activity (E-Kobon *et al*., 2016; Hajisharifi and Mohabathar, 2013) of a peptide because the positive charge of the peptide becomes the initial step of electrostatic interaction with cell membranes (cancer, bacteria, or virus) which are negatively charged (E-Kobon *et al*., 2016; Hajisharifi and Mohabathar, 2013). However, the positive charge of active peptides is known to have limits. The seven peptides were further analyzed using *in silico* antidengue prediction of the analysis resource and IEDB (http://www.iedb.org/assay/15063), also anticancer prediction using AntiCP (https://webs.iiitd.edu.in/raghava/anticp) (Table 5). Although *in vitro* and *in vivo* assays have been used, these are both financially and time costly and require more sampling. Conversely, *in silico* analysis can reduce costs, speed up quantification time, and minimize the use of test samples (Sucher, 2014).

There were two peptides based on the *in silico* analysis, which is potentially responsible for the antidengue and anticancer activities from peptides of PEA1d-3 (NS**L**KAT**L**CLS**L**TLA**L**APSL) and PEA1d-4 (**L**ADG**L**DSY**L**ELP**L**FTAVAIL). Interestingly, both of these peptide sequences consist of four leucine-rich repeat motifs (L in a bold and underlined letter), and this specific motif may contribute to the physiological function of peptides, including antidengue and anticancer properties. The exact mechanism of antidengue and anticancer peptides was not identified in this study and would require further work. According to Wang (2020), frequently active peptides were cationic peptides with +3 to +8 charges. Research by Hajisharifi and Mohabatkar (2013) on P38 and P22 peptides from *E. coli* protein, P38 peptides which have a cationic charge +7 active as anticancer and P22 which has a charge +10, showed no activity.

Table 4. Results of PEA1d peptide identification with MASCOT program.

Code	Peptide sequence	ID protein (NCBIprot)	Original protein name	Score (%)	Organisms of origin protein	MW (Da)	Charge
PEA1d-1	MGFIVLGRLEAFVDLCHL	WP_037230068.1	Hypothetical protein	73	*Roseobacter* sp. *GAI101*	2033.48	-0.50
PEA1d-2	MQLDYLECFKGVLGEALRQLGRR	WP_111493865.1	Rrf2 family transcriptional regulator	75	*Marinobacter bohaiensis*	2696.18	1.00
PEA1d-3	NSLKATLCLSLTLALAPSL	WP_086177022.1	Hypothetical protein	81	*Acinetobacter sp. ANC 5054*	1944.36	2.00
PEA1d-4	LADGLDSYLELPLFTAVAIL	WP_055638249.1	Putative baseplate assembly protein	86	*Streptomyces griseoruber*	2150.45	-4.00
PEA1d-5	MEELSRNFYIVFIIIGLYSMF	WP_144701186.1	hypothetical protein	86	*Cohnella* sp. *G13*	2586.10	-1.00
PEA1d-6	SGACTFGMVFLTGCGLF	WP_023928051.1	DUF4156 domain-containing protein	70	*Helicobacter macacae*	1711.04	0.00
PEA1d-7	VRSIFVRVQLEALYLCRAIFHDVVF	SDN75218.1	Hypothetical protein SAMN05443253_11856	77	*Bacillus* sp. *OK048*	2994.59	1.50

Table 5. The amino acid sequences and antidengue and anticancer activity prediction of PEA1d-1 to PEA1d-7 peptides fragment.

Code	Peptide sequence	Antidengue prediction	Anticancer prediction
PEA1d-1	MGFIVLGRLEAFVDLCHL	Non-Antidp	Non-Anticp
PEA1d 2	MQLDYLECFKGVLGEALRQLGRR	Non-Antidp	Non-Anticp
PEA1d-3	NS**L**KAT**L**CLS**L**TLA**L**APSL	Antidp	Anticp
PEA1d-4	**L**ADG**L**DSY**L**ELPLFTAVAIL	Antidp	Anticp
PEA1d-5	MEELSRNFYIVFIIIGLYSMF	Non-Antidp	Non-Anticp
PEA1d-6	SGACTFGMVFLTGCGLF	Non-Antidp	Non-Anticp
PEA1d-7	VRSIFVRVQLEALYLCRAIFHDVVF	Non-Antidp	Non-Anticp

Antidp = antidengue peptide; Anticp = anticancer peptide; LXXX = leucine-rich repeat motif.

Based on the aforementioned facts, it would be very early to state the sequence of PA1d-3 peptides that most contribute to the activity of the pepsin peptide fraction <3kDa (PA1d). Further research is required whether peptides from PA1d play an individual role or synergize with other peptides as Beaulieu *et al.* (2015) stated that the existence of bioactivity of peptides probably can be caused by synergism between several peptides.

CONCLUSION

The PEA1d fraction (pepsin peptide fraction <3kDa) which showed potential antidengue activity and cytotoxic activity with the CC_{50} value of 129 µg/ml against Vero cells and IC_{50} value of 0.749 µg/ml against HeLa cells is considered to be highly toxic. Seven peptide sequences were obtained (PEA1d-1–PEA1d-7) in the PEA1d fraction, but just two novel peptide sequences were identified and predicted to be the source of antidengue and cytotoxic activity, namely, PEA1d-3 (NSLKATLCLSLTLALAPSL) and PEA1d-4 (LADGLDSYLELPLFTAVAIL). It can be concluded that these peptides can be potent candidates for new drug production, especially for antidengue and anticancer from natural products, and developed as synthetic peptides in the future.

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AUTHOR'S CONTRIBUTION

Ahmad A and Massi MN gathered data and were conceptor of this study. Ahmad A, Sugrani A, and Karim H contributed to the study design. Asmi N, Wahid I, Ahmad A, and Karim H contributed to data collection. Ahmad A, Sugrani A contributed to manuscript writing. Ahmad A, Sugrani A, Asmi N, and Wahid I edited the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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