Antiviral Potential of Ethanol Extracts of Andalas Endophytic Bacterial Isolate B. J.T.A 2.1 Fermentation Products

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Abstract. The most prevalent infectious disease in tropical and subtropical regions is dengue, which is brought on by the dengue virus (DENV) and endemic illnesses. There is currently no antiviral medication specifically for dengue illness. It is understood that viral load and illness severity are correlated. Andalas Endophytic Bacterial Isolate B.J.T.A 2.1 Fermentation products An important active ingredient responsible for the antiviral activity has been found. This research will examine the antiviral effects of Andalas Endophytic Bacterial Isolate B.J.T.A 2.1 Fermentation products ethanol extract on DENV-2 in vitro with its toxicity in cell line. By using a focus assay and an MTT assay, respectively, vero cells were used to test the antiviral activity (IC50) and toxicity (CC50) in vitro. In this investigation, the IC50 acquired value was 17,91 g/mL, while the CC50 acquired value was 85,4 g/mL. The SI value of Andalas Endophytic Bacterial Isolate B.J.T.A 2.1 Fermentation products was 4.8.

Keywords: Anti Dengue · Endophytic Bacteria · Andalas

1 Introduction

It has been evident in recent years that the most prevalent systemic viral disease affecting people is dengue, which is primarily found in tropical and subtropical regions (Bhatt *et al.*, 2013) Around 390 million individuals worldwide have DENV infections, and there are Each year, there are more than 22,000 fatalities due to dengue, according to the WHO (Anne, 2013) Indonesia comes second after Brazil in terms of how many dengue cases were reported to the WHO between 2004 and and 2010. (WHO, 2009). Bali and DKI Jakarta in Indonesia had the most DHF cases in 2013, totaling 168.5 per 100,000 people (Karyanti *et al.*, 2014) Additionally, Forever dengue has been a significant public Indonesian health problem and has overtaken all other causes of child hospitalization and mortality (Karyanti *et al.*, 2014).

A member of the Flaviviridae family, DENV has four serotypes (DENV-1, DENV-2, DENV-3, and DENV-4) that are characterized by their genetic and antigenic characteristics. One serotype's infection won't provide you immunity to other serotypes

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(CDC, 2009). Asymptomatic infection with DENV is followed by dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS), among other clinical symptoms (WHO, 2009). A member of the Flaviviridae family, DENV has four serotypes (DENV-1, DENV-2, DENV-3, and DENV-4) that are characterized by their genetic and antigenic characteristics. One serotype's infection won't provide you immunity to other serotypes (CDC, 2009). Asymptomatic infection with DENV is followed by dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS), among other clinical symptoms (WHO, 2009).

According to Arajo and Leon (2001), andalas has long been utilized as a remedy in addition to a spice. Researchers have found that the main active ingredients in andalas extract, saphonin and phenolic, have a variety of positive health effects. Additionally, recent experimental research suggest that andalas extract contains antioxidant, anti-inflammatory, antiviral, antibacterial, etc. characteristics (Benzie and Wachtel, 2011). Andalas extract is anticipated to have an antiviral effect on the reproduction of DENV due to its component (Arajo and Leon, 2001; Benzie and Wachtel, 2011). The goal of this study was to assess the potency of an ethanol extract of the Andalas Endophytic Bacterial Isolate B.J.T.A 2.1 as an antiviral against dengue virus infection.

2 Methods

2.1 Andalas Endophytic Bacterial Isolate B.J.T.A 2.1 Fermentation Products Extract Preparation

Andalas Endophytic Bacterial Isolate B.J.T.A 2.1 Fermentation products extract was obtained from Biologi laboratorium Extraction was performed with an alcohol solvent of 90%. The extract is then thickened before methanol-based further fractionation. For in vitro and in vivo tests, the extract was dissolved in either 0.5% carboxymethyl cellulose (CMC) or dimethyl sulfoxide (DMSO) (Sigma, Singapore).

2.2 Vero Cells and DENV-2 Preparation

MEM Medium Fetal Bovine Serum (FBS) at a concentration of 10% was employed to maintain of Vero cells used in this research. NaOH and sodium bicarbonate were added to MEM. The Cells were then incubated at 37 °C with 5% CO^2. Cell was incubated for 4–5 days until confluent. DENV-2 NGC was introduced into a monolayer of vero cells in T-75 flasks for 0.5 FFU/cell MOI. For seven days, cells were incubated at 37 °C with 5% CO2. After collecting the supernatant, it was centrifuged for 5 min at 1000 g. After that, a 0.22 mm syringe-driven filter was used to filter it (Millipore, Co. Bedford MA USA). As previously described by Igarashi *et al.*, 1999, the supernatant was put in a freezer set at -80 °C and tested for the presence of DENV using the Focus assay (Guerrant *et al.*, 2011).

2.3 In Vitro Cytotoxicity (CC50)

Based upon the a's viability vero after cells treatment Combined with the extract, vitro cytotoxicity (CC50) was calculated using the MTT test. 2 x 104 cells/well were put to flat-bottom plates with 96 wells (Corning, USA) and incubated at 37°C with 5 percent CO2. Following a 24-h incubation period, the cells were exposed to extract at concentrations ranging 37°C and incubated with 5% from 2.5 to 80 g/mL CO2. Following the manufacturer's instructions, each well received 20 L of a 3- (4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Promega) salt solution before being incubated for 4 h. Using a micro-plate reader, the absorbance reading of each well was calculated at 490 nm. First, The theoretical percent of toxicity of the samples was calculated by dividing the mean blanked sample ODs by the mean blanked control ODs for each sample. The "Data In" for the curve fit was the computed toxicity percent, with 50 designated as the preferred interpolation. The concentrations of the samples were determined from the curve, and a 50 percent interpolation value was given. At 620 nm, the absorbance was measured. The cytotoxic effect was represented by the viability data. The following equation was used to assess the cells' viability: We were able to calculate CC50 based on the percentage of viable cells. The CC50 represented the means standard deviation of the studies and was determined using graph analysis of concentration-effect curves using nonlinear regression.

2.4 Antiviral Activity Assessment (IC50)

A 96-well plate was seeded with 2x104 cells per well and incubated at 37 °C with 5 percent CO2. The cells were infected with DENV-2 at a MOI of 1 FFU/cell after 24 h. The natural extract concentrations used were 80 g/mL, 40 g/mL, 20 g/mL, 10 g/mL, 5 g/mL, and 2.5 g/mL. 100 L of DMEM + 2% FBS containing different concentrations of natural extract was added after 2 h of infection. Plates were then incubated for a further three days at 37 °C. Focus test was used to measure the titer of the virus after it was extracted (Guerrant *et al.*, 2011) Briefly, a A 10-fold serial dilution of supernatant was injected into a Huh-7 it-1 cell monolayer in duplicate wells. Absorption was performed for two hours at 37 °C in 5% CO2 with agitation. The cell was given 0.5% methylcellulose overlay media, and it was cultured for 3 days at 35 °C with 5% CO2. The labeled infected cells followed a prior publication by Payne *et al.* (2006) with a few minor modifications. First, 10% formaldehyde in PBS was used to fix the infected cells, and they were then let to sit at room temperature for an hour. Cells were three times washed with PBS. 100 L of Nonidet P40 at 1% were added to each well and incubated for 30 min at room temperature to permeabilize the cells. After adding The mixture was then allowed to stand at room temperature for an additional hour in a blocking solution (5% skim milk in PBS). Following washing, the cell was exposed for one hour at room temperature to 1/1000 of human IgG anti-dengue. In 1/1000, anti-human IgG label HRP was added. The substrate was placed after washing, and the infected cell's brown color was visible. The following calculation was used to calculate the IC50 using the Focus assay results: We were able to calculate the IC50 using the percentage of inhibition. The graph reflected the means and standard deviation of the experiments and the IC50 was determined by nonlinear regression analysis of the concentration-effect curves.

2.5 Analytical Statistics

On GraphPad Prism 6, an unpaired t-test was used to analyze the results of the in vitro assay. ANOVA was used to analyze the homogenous and normally distributed data, and the Tukey test was used as a post hoc analysis. Wilcoxon test will be used to analyze data with homogeneous distribution and no sense of normality. Utilizing the statistical analysis program SPSS 21.

3 Result and Discussion

In the vast majority of tropical and subtropical nations, dengue has been a serious issue. Dengue does not currently have a specific treatment. Since maintaining the patient's bodily fluid is so important in treating the severe form of dengue, treatment is only supportive in nature. There are currently no licensed antiviral treatments for dengue. Although studies regarding this have becoming more popular most research, either directly or indirectly natural product as sources for their antiviral study since the active substances for most therapies derived conventionally from natural sources (Muhamad *et al.*, 2010). Additionally, plants were used as the source of almost 50% of medicines that have been approved. However, many plants have cytotoxic properties, which means they could harm cells.

3.1 DENV Inhibition by Andalas Endophytic Bacterial Isolate B.J.T.A 2.1 Fermentation Products Extract

DENV was treated with various extract concentrations, before the vero cells were infected. 80 g/mL, 40 g/mL, 20 g/mL, 10 g/mL, 5 g/mL, and 2.5 g/mL were the concentrations. The positive control was CyclosporinA 2 g/mL. DMSO 0.1% was employed as the negative control. The Each concentration's virus titer data distribution was normal, with a p-value greater than 0.05. The standard deviation of the virus's average titer was shown in Table 1 along with it.

Table 1. DENV-2 Titer after Treatment with varied extract concentration

Treatment extract ug/mL	Average titer (FFU/mL)	SD
80	0	0
40	$0,06 \times 10^2$	3,8
20	$0,75 \times 10^2$	16,5
10	$1,21 \times 10^2$	7,9
5	$1,80 \times 10^2$	11,5
2,5	$1,87 \times 10^2$	18,7
CyA 2 μg/mL (+ control)	$1,73 \times 10^2$	3,6
DMSO 0.1% (- control)	$2,04 \times 10^3$	14,3

To determine the proportion of the virus that was inhibited by therapy, the average virus titer was further computed. To calculate the half, an Inhibitory concentration was established using an equation from the linear regression curve of the % inhibition (IC50).

3.2 Cytotoxic Effect of Andalas Endophytic Bacterial Isolate B.J.T.A 2.1 Fermentation Products Extract

The 50% cytotoxic concentration (CC50) was determined to make sure the extract was not hazardous to the cell. Such was obtained from the MTT assay's results. After being exposed to extract at a concentration of up to 40 g/mL, the cell, viability still displayed a high level (Table 2.). Table 2 shows the vitality of the cells after treatment with varied extract concentrations. (g/mL) Concentration Viability (%) SD 80 51.3 2.39 40 86.5 11.94 20 99.1 3.14 10 97.4 4.01 5 101.8 2.63 2.5 111.7 4.53 The CC50 was analyzed from the linear regression formula for the viability percentage. The CC50 of turmeric was found to be 85.4 g/mL.

The CC50 was examined using the percent viability's linear regression equation. Findings revealed that the CC50 of was examined using the percent viability's linear regression equation. Findings revealed that the CC50 ofturmeric was 85.4 µg/mL.

(Selectivity Index (CC50/IC50) After the CC50 When split by IC50, it was discovered that the extract of Curcuma longa in this investigation had a selectivity index of 4.8. Figure 1 shows a focus picture of a DENV-2 NGC after exposure to different C. longa extract concentrations. (a). 80 µg/mL; (b). 40 g/mL, 20 g/mL, 10 g/mL, 5 g/mL, and 1.5 g/mL and 2.5 g/mL are the concentrations (g). DMSO).

If a substance is cytotoxic but using it might not be safe if it isn't designed to kill cells like an anticancer is. An effective and secure medication is therefore required for therapeutic use. The selectivity index (CC50/IC50) measures a substance's inhibitory and cytotoxic potencies capacities. It might be useful to determine whether a drug is suitable for further research or to gauge a product's efficacy and safety (Muhamad *et al.*, 2010; Fuzo C A and Degrève, 2013). The goal of this research is to identify DENV antivirals that can combat human infection. Vero cells, In this investigation, a human cell line derived from the liver was used. (Fuzo and Degrève, 2013). This study discovered that the CC50 was 85.4 g/mL and that greater concentrations resulted in a lower proportion of cell viability. This means that although not considerably, the extract did kill the cells.

Table 2. The vitality of the cells after exposure to various concentration of

Concentration (µg/mL)	Viability (%)	SD
80	51.3	2.39
40	86.5	11.94
20	99.1	3.14
10	97.4	4.01
5	101.8	2.63
2.5	111.7	4.53

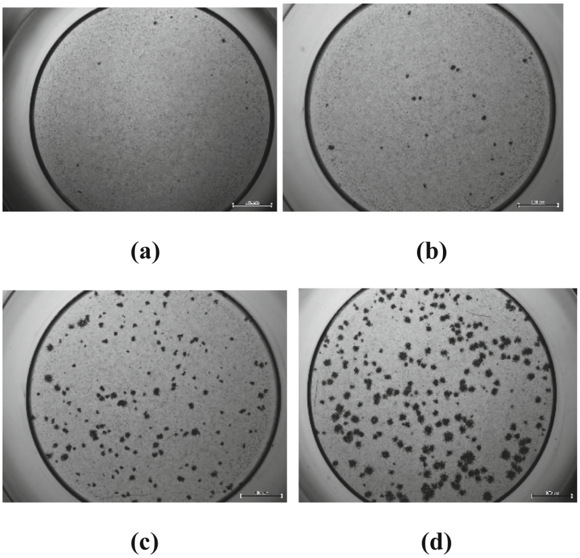

Fig. 1. DENV-2 NGC focus picture following treatment with various concentrations of extract.

A chemical is at a half cytotoxic concentration when it has the potential to impair a cell's viability by 50%. So, in theory, a good chemical ought to have a high CC50. A study of studies on prospective in a study of plants that can be used as anti-dengue remedies, Alternanthera philoxeroides, often known as alligator weed, was discovered to have the least amount of cytotoxicity on cells (CC50 = 535.91) (Kadir *et al.*, 2013).

4 Conclusion

CC50 of Andalas Endophytic Bacterial Isolate B.J.T.A 2.1 Fermentation products extract had an IC50 of 17.91 g/mL and was 85.4 g/mL. An in vivo investigation demonstrated that the extract has an antiviral effect against DENV-2 at dosages of 0.147 mg/mL and reduces the duration of viremia..

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References

1. Karyanti M R, Uiterwaal C S, Kusriastuti R, Hadinegoro S R, Rovers M M, Heesterbeek H et al 2014 The changing incidence of dengue haemorrhagic fever in Indonesia: a 45-year registrybased analysis BMC Infect. Dis. 14 1 412-20.
2. Anne N E 2013 Epidemiology of dengue: past, present and future prospects Clinical Epidemiology 5 299–309.

3. Araújo C C and Leon L L 2001 Biological activities of *Curcuma longa* L. Mem. Inst. Oswaldo Cruz. 96 5 723–8.
4. Badan Pengawas Obat dan Makanan Republik Indonesia 2014 Pedoman Uji Toksisitas Nonklinik Secara In vivo (Jakarta: Pusat Riset BPOM RI) pp 15–27.
5. Benzie I F F and Wachtel-Galor S 2011 Herbal medicine: Biomolecular and clinical aspects 2nd ed (New York: CRC Press) pp 262–82.
6. Bhatt S, Gething P W, Brady O J, Messina J P, Farlow A W, Moyes C L et al 2013 The global distribution and burden of dengue Nature 496 (7446) 504 –7.
7. Bhavani S T N, Shantha N V, Ramesh H P, Indira M A S and Sreenivasa M 1980 Toxicity studies on turmeric (*Curcuma longa*): acute toxicity studies in rats, guineapigs and monkeys. Indian J. Exp. Biol. 18 1 73–5.
8. CDC 2009 Dengue and dengue hemorrhagic fever: information for health care practitioners Dengue Hemorrhagic Fever pp 1–4.
9. Chainani-Wu N 2003 Safety and anti-inflammatory activity of curcumin: a component of tumeric (*Curcuma longa*) Journal of Alternative and Complementary Medicine 9 161–8.
10. Fuzo C A and Degrève L 2013 New pockets in dengue virus 2 surface identified by molecular dynamics simulation J. Mol. Model 19 3 1369–77.
11. Guerrant R L, Walker D H and Weller P F 2011 Tropical infectious diseases: principles, pathogens, and practice 3rd ed (London: Elsevier) pp 504–10.
12. Idrees S and Ashfaq U A 2013 RNAi: antiviral therapy against dengue virus Asian Pac. J. Trop. Biomed. 3 3 232–6.
13. Joshi J, Ghaisas S and Vaidya A 2003 Early human safety study of turmeric oil (*Curcuma longa* oil) administered orally in healthy volunteers. Journal of Association of Physicians of India 51 1055-60.
14. Ammon H P and Wahl M A. 1991 Pharmacology of Curcuma longa.Planta Med. 57 1 1–7.
15. Kadir S L A, Yaakob H and Zulkifli R M 2013 Potential anti-dengue medicinal plants: a review J. Nat. Med. 67 4 677–89.
16. Liju V B, Jeena K and Kuttan R 2012 Acute and subchronic toxicity as well as mutagenic evaluation of essential oil from turmeric (*Curcuma long*a L) Food and Chemical Toxicology 53 52–61
17. Balaji S and Chempakam B 2010 Toxicity prediction of compounds from turmeric (*Curcuma longa* L.) Food and Chemical Toxicology 48 1 2951–9.
18. Moghadamtousi S Z, Kadir H A, Hassandarvish P, Tajik H, Abubakar S and Zandi K 2014 A review on antibacterial, antiviral, and antifungal activity of curcumin Biomed. Res. Int. 1–12.
19. Muhamad M, Kee L Y, Rahman N A, Yusof R 2010 Antiviral actions of flavanoid-derived compounds on dengue virus type-2 Int. J. Biol. Sci. 6 3 294-302.
20. Padilla S L, Rodríguez A, Gonzales M M, Gallego-G J C and Castaño J C 2014 Inhibitory effects of curcumin on dengue virus type 2 infected cells in vitro. Arch. Virol. 159 573–9.
21. Payne A F, Gajewska I B, Kauffman E B and Kramer L D 2006 Quantitation of flaviviruses by fluorescent focus assay J. Virol Methods 134 2 183-9.
22. Schul W, Liu W, Xu H Y, Flamand M, Vasudevan S G and Vasudevan S G 2007 A Dengue fever viremia model in mice shows reduction in viral replication and suppression of the inflammatory response after treatment with antiviral drugs Keystone Symp. Cell Biol. Virus Entry J. Infect. Dis. 195 665–74.
23. Soemadji and Lenny S 2003 Uji aktivitas anti diabetes dari ekstrak kulit jamblang pada mencit diabetes aloksan Indonesia Research Report.
24. Tan S K, Pippen R, Yusof R, Rahman N A, Ibrahim H and Khalid D 2006 Screening of selected zingiberaceae extracts for dengue-2 virus protease inhibitory activities. Sunw. Acad. J. 3 1-7.
25. WHO 2009 Dengue: guidelines for diagnosis, treatment, prevention, and control Spec Program Res. Train. Trop. Dis. 47.

26. WHO 2011 Prevention and Control of Dengue and Dengue Haemorrhagic Fever Resived and expanded pp 17–28.
27. Wilder-Smith A, Ooi E E, Vasudevan S G, Gubler D J 2010 Update on dengue: epidemiology, virus evolution, antiviral drugs, and vaccine development Curr. Infect. Dis. Rep. 12 157–64.
28. Yamanaka A and Konishi E 2009 A simple method for evaluating dengue vaccine effectiveness in mice based on levels of viremia caused by intraperitoneal injection of infected culture cells Vaccine 27 28 3735–43.