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Elimination of Dengue Virus with Antiviral Compound and Appropriate Technology

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ABSTRACT

Infection with the dengue virus by the *Aedes aegypti* mosquito vector is in the form of dengue hemorrhagic fever (DHF), which can cause a decrease in platelets and even death. The parasitic drug niclosamide, which is effective against dengue virus serotype 2 (DENV-2) is used to prevent further dengue virus infection. Many tests were carried out using inhibitors such as doxorubicin (SA-17), glycoside inhibitors in the form of deocynojirimycin (DNJ) and castanospermine (CSP), carbohydrate-binding agents (CBA), and the use of heparan sulfate aimed at inhibition of the adsorption process and replication process, as well as improper protein folding to prevent the conformation of virus merger. The elimination process can also be carried out using antiviral compounds found in the leaves of *Psidium guajava* and *Carica papaya*, which have inhibitory activities of 92.6% and 89.5%, respectively; propyl gallate, with a percent inhibition of dengue virus envelope protein serotype 2 of 53-9.85%; isobutyl gallate, with CC_{50} values of 167.19 g/mL and an inhibitory value (IC_{50}) of 4.45; Cissampelos Pariera Linn methanol extract, with progressive inhibition as the Cipa extract concentration increased with an IC_{50} value of 6.1µg/ml Preventive methods are also carried out in several ways, namely by utilizing hydrophobic liquid in the form of silicone oil (low-viscosity polydimethylsiloxane, or L-PDMS), the use of eave tubes in home tubes inserted with insecticides, and utilizing ultrasound with a frequency of 100 kHz and 90 dB to repel mosquitoes carrying dengue virus vectors.

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1. Introduction

Dengue virus is a member of the family Flaviridae that can be grouped into four different serotypes, namely serotypes 1-4. This virus can cause infection in humans through the bite of the *Aedes aegypti* mosquito, resulting in a disease commonly called dengue hemorrhagic fever. Humans infected with ether serotypes will produce lifelong immunity. The DENV-3 serotype is a serotype in that dominance often occurs. Based on the World Health Organization for 2022 the first symptom caused is a high fever in the patient. The symptom caused is decreased platelets in the body [1]. Cases of dengue hemorrhagic fever will increase during the rainy season because there are more and more puddles in the environment which become a place for *Aedes aegypti* mosquitoes to lay eggs [2]. Based on the Directorate General of P2P of the Ministry of Health of the Republic of Indonesia for 2020, in 2019 there were still 138,127 people who were affected by dengue hemorrhagic fever. Where this is known to have increased from the previous year as many as 65,602 thousand residents [3]. At this time, it is known that there is still no type of drug or vaccination that can specifically fight dengue virus infection in Indonesia [3].

Dengue Hemorrhagic Fever (DHF) will generally be treated with the antipyretic drug Acetaminophen and the anti-hepatic drug Sofosbuvir. It is known that both drugs are able to reduce the symptoms of dengue infection to a certain extent [4]. The most common use of antipyretic drugs in patients is paracetamol. Whereas the use of this drug is almost certainly not to inhibit the activity of the dengue virus, but rather to reduce pain followed by fever, which is the first symptom of dengue virus infection [3]. Another treatment given is an infusion of crystalloid fluid with isotonic properties. It is aimed at replacing the volume of plasma lost from blood vessels. However, the administration of this fluid provides side effects, namely patients experiencing inflammation, lactic acidosis, hemodynamic instability, and hemoconcentration [5]. Several countries have developed vaccines for the dengue virus, namely the Dengvaxia vaccine, and have been licensed in 20 countries. However, it is known that the vaccine is able to cause side effects in the form of complications in seronegative patients. Another vaccine is the mRNA-LNP Dengue Virus Serotype 1 vaccine, which has been tested in vitro and provides a protective immunological response and good results. The mechanism of action of the vaccine is with siRNA and targets DENV-NS4B and -NS5 so that it can inhibit the disease progression of all dengue virus serotypes (serotypes 1-4) [6]. Some studies have also tried to create anti-dengue products from natural compounds. One of them is geraniin, which is an ellagitannin compound obtained from several plant species able to limit the receptor process of viral cells by inhibiting the dengue virus replication process so that it can reduce the activity of viremia caused by the dengue virus [7]. Besides that, many such phenolic compounds, such as Fisetin, Naringin, Catechin, and Delphinidin, that showed inhibitory effects on the DENV in pre- and post-treatments, and their mechanisms of inhibition have yet to be clarified.

This shows that efforts to eliminate the dengue virus (DENV 1-4) continue with the aim of finding compounds or drugs that can specifically inhibit the activity of the dengue virus. Not only are some studies testing the active compound present in plants or the result of chemical synthesis, but some are also trying to develop new drugs that were previously used as antivirals to increase the effectiveness of these drugs. Therefore, a review of previous research is needed

with methods or types of antivirals developed for eliminating the dengue virus in the hope of creating better innovations in the future.

2. Drugs and Inhibitors for Dengue Virus Infection

In some countries, such as Indonesia, the treatment for patients infected with the dengue virus is to provide drugs such as paracetamol and also antipyretic which basically only reduce the pain and fever produced by infection with the dengue virus, and do not inhibit the activities of the dengue virus specifically. Many studies are aimed at finding a specific cure to inhibit the dengue virus. One of them is a study conducted by [8] regarding the use of the parasitic agent or drug niclosamide to inhibit infection with dengue virus serotype 2. In addition to its function as an anthelmintic drug, niclosamide has been widely reported to confer broad antiviral activity. Its previous use was to inhibit the arthropod-borne Zika virus. Zika and DENV infection through an undefined targeting of flavivirus NS2B-NS3 protease. In previous studies, it was stated that niclosamide is a drug that can play a role in carrying protons that are used to inhibit the process of decreasing endosomal pH so that it can inhibit rhinovirus infection and influenza in humans [9].

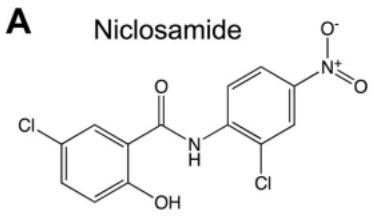

Figure 1. Chemical Structure of Niclosamide [10]

In vitro and in vivo testing of niclosamide-niglosamida to combat infection from a dengue virus serotype is being conducted. In vitro, it was carried out using murine Neuro-2a cells (ATCC, CCL131), human cells A549 (ATCC, CCL185), and *baby hamster kidney* cells (BHK-21), which were then cultured and given dengue virus infection treatment, and their cytotoxicity was assessed. Then the cells already infected by the dengue virus are extracted with buffer fluid, and the proteins are separated using SDS-polyacrylamide gel electrophoresis and transferred onto the polyvinylidene difluoride membrane. The process will produce spots, which will be examined with antibodies developed with chemiluminescence so that the relative density of each identified protein can be calculated. The ELISA kit was used to detect antibodies produced by cells (IFN-) in cell culture media [8]. While in vivo, it was done with means of Genetically and pharmacologically targeting V-ATPase effectively inhibits DENV.

The cytotoxicity of cells A549 and BHK-21 is determined in vitro by the release of LDH protein. The result is that the administration of niclosamide at all doses causes a minor cytotoxic effect on cells. cytotoxic concentrations of 50% (CC_{50}) in BHK-21 cells given the drug niclosamide using the LDH assay showed that its value was less than 10 μM. Niclosamide was significantly able to stem the release of viral RNA with a value of $P<0.05$ [11]. Niclosamide does not alter the translation of the virus in cells already infected with the dengue virus. The anti-dengue activity of niclosamide does not depend on cells infected with the

dengue virus, which contains host and viral factors to replicate, but rather inhibits the virus by targeting the formation of NS2B-NS3 proteases from dengue viruses [12]. Based on this, it can be concluded that niclosamide inhibits the formation of NS2B and NS3 DENV complexes. In human cells, the use of niclosamide has been shown to reduce the replication of the dengue virus in human A549 cells [13]. Currently, through the screening of 2,816 approved and investigational drugs, niclosamide has been identified as a potential viral inhibitor targeting the formation of the NS2B-NS3 protease of the flaviviruses Zika and DENV. Therefore it can be said to be a good drug candidate to reduce the dengue virus [8].

Then some inhibitors that are used to inhibit or eliminate the dengue virus began to appear. Among them is a derivative of the antibiotic doxorubicin (SA-17) with a structure similar to tetracycline, which is able to interact with the hydrophobic pockets of envelope proteins so as to prevent conformation and the incorporation of viruses. Besides that, NITD448, selected in docking experiments, was demonstrated to inhibit DENV-2 fusion by binding to the hydrophobic pocket of the E-protein. Doxorubicin has an antiviral activity to treat dengue virus serovars 1, 2, and 3 that have been tested on Vero cells and C6/36 cells by conducting docking tests and physicochemical algorithms using structural data from enzyme proteins, small molecules, and peptides that target hydrophobic bags in dengue viruses [14].

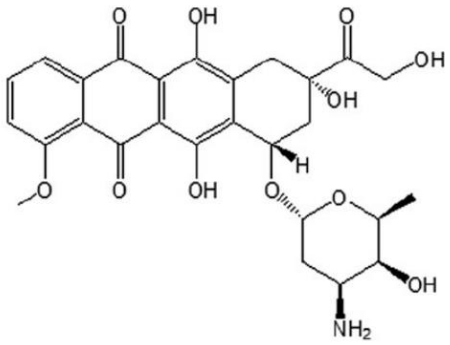

Figure 2. Chemical Structure of Doxorubicin [15]

The second is a glycosidase inhibitor that appears to deal with the risk of an increase in antibody dependence in DHF patients. This inhibitor is used to target host cell processes, one of which is the glycosylation process. One of the inhibitors used is a -glycosidase inhibitor, which affects viral protein N-glycosylation and the endoplasmic reticulum. Compounds that can be used to inhibit protein folding are the imino sugars deoxynojirimycin (DNJ) and castanospermine (CSP), which have a structure similar to glucose [15, 16]. DNJ and CSP are able to inhibit dengue virus serotype 1, 2, 3, and 4 because they are able to reduce the number of particles secreted due to improper folding of glycoproteins and reduce the infectious power of secreted dengue virus particles. This is because CSP is one of the natural alkaloid compounds derived from black beans and is water-soluble with low cytotoxic effects. However, NN-DNJ and a CSP derivate both reduced significantly viremia in a dengue fever mouse model. Further optimization of the chemical structure of the imino sugar DNJ leads to the production of N-pentyl- (1-hydroxy cyclohexyl)-DNJ (OSL-9511), an iminocyclitol with

a DNJ head group, which showed reduced cytotoxicity and retained antiviral activity against DENV [17].

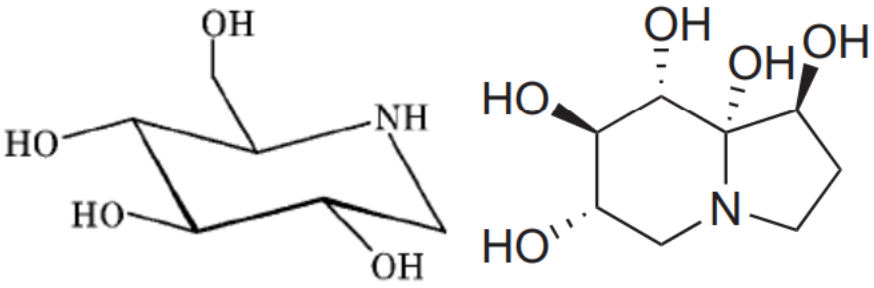

Figure 3. Left (Chemical Structure of Deoxynojirimycin) [18], Right (Chemical Structure of Castanospermine) [19]

The third is the carbohydrate-binding agent (CBA) which is known to have an antiviral activity to fight dengue viruses. Where this research was conducted by Alen et al., 2009 to evaluate the effect of CBA on dengue virus infection using Raji cells transfected with dendritic cells-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN) [14, 20]. DC-SIGN can be expressed in dendritic cells and alveolar macrophages that are one of the c-type transmembrane receptors and consist of four domains, namely the cytoplasm, the transmembrane domain, seven to eight extracellular neck repetitions, and the carbohydrate recognition domain. DC-SIGN is able to form bonds with different pathogens, for example, in HIV (Human Immunodeficiency Virus), HCMV (Human Cytomegalovirus), hepatitis C virus, *Mycobacterium tuberculosis*, and in some parasites and yeast [21]. CBA can prevent infection of cells efficiently by being mediated by DC-SIGN on Ruji cells. The mechanism of action of this CBA is that when newly isolated monocytes are treated with IL-4 to increase the expression of DC-SIGN, they become susceptible to the dengue virus and the CBA exhibits strong antiviral activity. where CBA will inhibit the adsorption of DENV to cells, and it can be assumed that CBA can interact with envelope proteins in the dengue virus because the protein has two potential N-related glycosylation sites [14].

The fourth is the use of heparan sulfate, which can target envelope protein interactions in the dengue virus. Heparan Sulfate is also expressed by Vero cells, CHO cells, and BHK cells which are widely used in the study of dengue virus infection because of the easy cell growth conditions. Heparan Sulfate very often acts as an attachment factor to concentrate the virus on the cell surface to facilitate binding to a second receptor. The use of heparan sulfate will be more effective when combined with soluble GAG, which is a highly charged polyanion. GAG and heparin have a higher sulfate content in proteins compared to heparan sulfate. This causes both types of inhibitors to be able to prevent the entry of the dengue virus into vero cells and BHK cells [14]. In a study conducted in vitro by Lee et al. (2005) to determine the infectivity of dengue virus, it was found that BHK cells infected with dengue virus serotype 2 for 15 minutes without treatment with heparin 20 g/mL showed that 43% of infected BHK cells were based on cell populations that showed specific fluorescence intensity of envelope proteins that

exceeded that of uninfected cells. After being treated with heparin, there was a reduction of approximately 7 times the infectiousness of the virus, which is only 6% of infected cells [22].

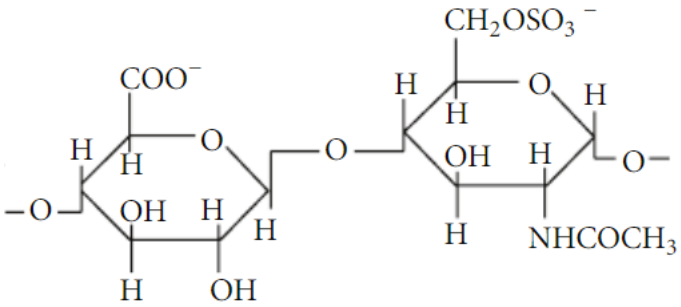

Figure 4. Chemical Structure of Heparan Sulfate [23].

3. Anti Virus (Anti-Dengue) Compounds

Psidium guajava and Carica papaya Leaf Extracts

Guava Leaf Extract (*Psidium guajava*) contains flavonoid compounds, such as quinic acid, caffeoyl glucose, quercetin, gallate acid, and myricetin. The compound flavonoid quercetin in guava can only be found on the leaves and the skin, where the amount contained in the leaves is in the range of 56±0.0058 μg/mg and in the skin of the fruit is 10.09±0.012 μg/mg [24]. This quercetin content has the potential to suppress the intracellular replication process of dengue virus serotype 2 (DENV-2) to reach 75.7% ± 1.57 at a concentration of 50 μg/mL. In addition, the quercetin compound inhibits the release of the ATPase enzyme in dengue virus serotype 4 [25].

While papaya leaf extract (*Carica papaya*) contains active compounds papain, namely chymopapain, cystatin, tocopherol, ascorbic acid, flavonoid cyanogenic-glucosides, and glucosinolates [26]. The presence of papain compounds in *Carica papaya* leaf extract can increase platelet count in dengue fever patients [27]. This is because papain can initiate the presence of IL-6 synthesis so that there is an increase in the process of thrombopoietin expression in the liver. This can lead to the presence of megakaryopoiesis so that platelets can increase [24].

Research conducted by Saptawati et al., 2017 aimed at determining plants that have potential against dengue virus through in vitro testing produced results in accordance with the statement above. The test was carried out using a cell viability test and a cytotoxicity test on normal cells infected with dengue virus serotype 2 and then given treatment using *Psidium guajava* and *Carica papaya* leaf extracts. The results obtained showed that the leaf extracts of *Psidium guajava* and *Carica papaya* had inhibitory activities of 92.6% and 89.5%, respectively, and did not show any toxicity in cells because cell viability was still greater than 50% [24]. This is in accordance with previous studies, which stated that quercetin in *Psidium guajava* leaf extract was able to inhibit the replication process of dengue virus serotype 2 significantly, namely with an IC value of 50:35.7 g/mL, while in *Carica papaya* leaf extract it was able to inhibit NS2B-NS3 protease. This mechanism of inhibition is also similar to the flavonoid content present in the two extracts, namely inhibiting RNA polymerase [25].

Isobutyl Gallate and Propyl Gallate Compounds

Research related to gallate acid which has the potential to be an antiviral against the dengue virus has already been carried out. Whereas gallate acid has the potential to be the primary phenolic element found in some plants and functions as a powerful antioxidant, particularly for patients suffering from dengue fever, asthma, cancer, neurological benefits, antiviral and antifungal properties, and is not toxic to normal cells [28]. Derivatives of gallate acid compounds such as isobutyl gallate and propyl gallate are among the most effective derivatives to help fight DNA and RNA viruses. Isobutyl gallate is commonly used as a nutraceutical because it contains anti-inflammatory and antioxidant content. Polyphenol compounds obtained through the esterification process are one of the ingredients in gallate acid that act as an antiviral. Gallate acid has been shown in previous studies to inhibit the activity of the hepatitis C virus, influenza virus, and poliovirus [29]. In addition, propyl error is also one of the compounds that have the potential to be a strong antiviral compound for the dengue virus. Where in the test conducted by Kusuma et al., 2022, it is stated that the percentage of inhibition of propyl compounds against the envelope protein of dengue virus serotype 2 is 53±9.85% [30].

In the research conducted by Dewi et al., 2018 related to the antiviral properties of gallate acid derivative compounds, namely isobutyl gallate, toxicity test methods, focus tests, and antivirus tests were used. The test was carried out by treating several concentrations of isobutyl gallate with dengue virus serotype 2 (DENV-2) that had been infected in vero cells. Use the focus test to determine the inhibition concentration (IC_{50}) and the toxicity test to determine the cytotoxic concentration (CC_{50}) using the MTT assay. The results showed that the isobutyl compound did not have cytotoxic properties in Huh 7 IT-1 cells with a CC_{50} value of 167.19 g/mL and an inhibitory value (IC_{50}) of 4.45 g/s tests, and antivirus tests were used. The test was carried out by treating several concentrations of isobutyl gallate with dengue virus serotype 2 (DENV-2) that had been infected in Vero cells. Use the focus test to determine the inhibition concentration (IC_{50}) and the toxicity test to determine the cytotoxic concentration (CC_{50}) using the MTT assay. The results showed that the isobutyl compound did not have cytotoxic properties in Huh7 IT-1 cells with a CC_{50} value of 167.19 g/mL and an inhibitory value (IC_{50}) of 4.45 g/mL so that a selectivity index (SI) value of 25.69 was obtained [29]. High SI values indicate that compounds are selective against pathogens and are a significant value [25].

While the effectiveness of propyl gallate compounds against dengue virus serotype 2 was carried out by Kusuma et al., 2022 using toxicity tests with focus tests and MTT tests and followed by in silico tests. The results obtained were the value of propyl resistance to receptors was 9±2.65% and envelope protein in dengue virus serotype 2 was 53±9.85%. In the test, it was proven that propyl compounds are not toxic because the viability of the cells is 125±1%. With the in silico test, it was found that the binding energy possessed by propyl to the envelope protein was -3.21 kcal/mol, indicating spontaneous bonds and good affinity [30]. With these results, it can be stated that propyl compound has inhibitory power against dengue virus serotype 2 and does not provide toxic properties when given at the virus attachment stage. Propyl gallate has lipophilic properties, so when administered to a cell, it is possible for it to

enter the cell. This shows that propyl gallate compounds are able to provide protection against oxidation by hydrogen peroxide and oxygen free radicals by showing the presence of catalytic effects. The existence of this property gives affinity to the cell membrane, which has an impact on its interaction with mitochondria and microsomes so that it can inhibit the process of attachment of the dengue virus to cells [31]. Then in silico testing gives a result of binding energy is -3.21 kcal/mol which means that the bond produced by the envelope protein with propyl gallate is 3.21 kcal/mol. This suggests that propyl has a greater potential to bind to and inhibit envelope proteins possessed by dengue virus serotype 2 [30].

Anti-Dengue and Cytotoxic Activity of Hydrolysates Protein Exophitic Bacteria of Brown Algae (Sargassum sp.)

Sargassum sp. is a type of algae that can symbiosis with bacteria. With this symbiosis, bioactive compounds can be produced that have great potential as basic medicinal and nutraceutical ingredients [32]. *Sargassum sp.* is one of the ingredients used to make carrageenan. However, bacteria capable of symbiosis with these algae have antibacterial, antifungal, anticancer, and anti-dengue properties. The anti-dengue activity of this bacterium is due to the presence of peptide molecules that are able to inhibit the replication and infection processes of the dengue virus [33].

Research related to this is by isolating proteins owned by algae symbiont bacteria, namely *Enterobacteria agglomerans* SB5 using the ammonium sulfate fractionation method by Ahmad et al., 2019. Then a toxicity test was carried out to determine the toxicity properties of the possession using the BSLT (Brine Shrimp Lethality Test) and MTT methods. The results obtained were the discovery of bioactive proteins with high toxicity indicated by an LC_{50} (Median Lethal Concentration) value of 48.67 μg/ml. The next test is by anti-dengue test using Vero cells with a result of percent inhibition (IC_{50}) is 70% and cytotoxic concentration (CC_{50}) is 260.37 μg/ml, this means that the protein still does not have potential as an anti-dengue agent.

Therefore, the research was resumed in 2021 by Ahmad et al., 2021 with an additional method, namely the extraction and hydrolysis of pepsin enzymes to obtain peptides with bioactive properties. In pepsin peptide fraction <3kDa gives a positive result against anti-dengue activity against Vero cells. Where the percent inhibition is 91% with the CC_{50} value being 129 μg/mL. This fraction still has toxic properties in shrimp larvae after a BSLT test with an LC_{50} value of 1.77 μg/mL [33]. In this study, it was found that peptide fragments PEA1d-3 (NSLKATLCLSLTLAPSL) had the highest cationic charge. Where the presence of this charge will determine the anti-dengue properties possessed by the peptide. This is because the positive charge possessed by peptides is one of the stages of initiation of electrostatic interactions possessed by cells with viruses that have negative charges [34].

Cissampelos Pareira Linn methanol extract (Cipa Extract)

Cissampelos pareira Linn belonging to the Menispermanceae family is an upright or climbing herb, known as ambastha or lahupatha in traditional Indian medicine. Cissampelos pareira contains many secondary metabolites such as berberine, biologine, cissampeline, pareirubrine A and B which have been tested for their medicinal value and therefore have great

potential to produce drugs. This plant is conventionally propagated by seeds and root cuttings. From a phytochemical perspective, the roots of *Cissampelos pareira* contain hyatin, hytinin, haytidine, and berberine alkaloids. From the leaves, it is also known that this plant contains a flavone dimer, namely cissampeloflavone [24].

The methanol extract of *Cissampelos pariera* Linn (Cipa extract) was reported to be potent in inhibiting four serotypes of the dengue virus [24]. Initial screening in the study conducted by Sood et al., 2015, it was shown that only the methanol extract showed antiviral activity when tested against DENV-2 or DENV-3. Hydroalcoholic and aqueous extracts of all 19 plants selected for the study did not show any antiviral activity when tested against these two DENV serotypes (IC_{50} >> 100 μg/ml). Consequently, all subsequent studies were performed using methanol extracts.

In a study conducted by Sood et al., 2015 regarding Cipa extract which has antiviral activity in dengue, it was tested using in vitro and in vivo methods. The interaction between paracetamol and Cipa extract was assessed in vitro using the type-1 test format and in effect. *in vivo* Cipa extract in the presence and absence of paracetamol was assessed using a rat Wistar fever model. Wistar rats (weighing 180–220 gr) of both sexes were used. Since the data so far indicate that Cipa extract has strong pan-DENV inhibitory activity, it is considered worthwhile to explore the feasibility of its therapeutic use. Since DF is usually treated with paracetamol, it is important to ascertain the nature of the interaction between Cipa and these drugs.

The results of the study on Cipa extract and paracetamol by type-1 assay were carried out in which DENV-3 was pre-incubated with serial dilutions of Cipa extract. It was observed that the infectivity of DENV-3 was inhibited progressively with increasing Cipa extract concentration, with an IC_{50} value of 6.1 μg/ml. The addition of up to 100 μg/ml paracetamol into the DENV-3/Cipa extract pre-incubation mixture did not significantly affect the inhibition profile of Cipa. The calculated IC_{50} values in the presence of paracetamol at 1.10 and 100 μg/ml were 8.4, 7.4, and 8.5 μg/ml, respectively. Paracetamol alone at all tested concentrations had no effect on DENV infectivity (the number of plaques obtained with DENV-3 alone and DENV-3 plus paracetamol at 100 μg/ml were 43±3 and 45±4, respectively; n = 3). The next experiment examined the effect of Cipa extract on the antipyretic activity of paracetamol using the Wistar rat pyrexia model. Interestingly, this experiment revealed that Cipa extract has an intrinsic antipyretic effect. When mice, whose fever was induced by subcutaneous injection of brewer's yeast, were treated with Cipa extract, the fever was suppressed with efficiency comparable to that of paracetamol. Interestingly, the co-administration of Cipa extract with paracetamol has a synergistic effect, resulting in a more pronounced decrease in body temperature [35].

4. Mosquito Repellent Innovative Technology and Method

Mosquito Repellents DueTo Tarsal Contact with Hydrophobic Fluids

As previously known, the dengue virus requires a vector in the form of a female *Aedes aegypti* mosquito to be able to enter its host [2]. The infection caused by the dengue virus is in the form of dengue hemorrhagic fever (DHF), which will increase during the rainy season. This is because mosquitoes will start laying eggs and larvae can develop rapidly due to many

puddles. While laying eggs in puddles, mosquitoes use their legs, which are hydrophobic because they have a smooth surface so that they can support their bodies above mosquitoes they can help mosquitoes to lay eggs and remove adult mosquitoes from the pupae to be able to fly [36].

The process of infection from the dengue virus begins with the presence of mosquito bites on the skin's surface. Mosquito legs support and stabilize posture when perched on the skin's surface and are followed by blood-sucking behavior [37]. The dengue virus that causes dengue fever is in the mosquito's salivary glands. When the female mosquito sucks blood, she injects saliva into the bite wound. This is where then change places, from mosquito saliva into our bodies. This mosquito gets the virus after it bites a victim who has been infected with the dengue virus. Then he bit another person and contagion ensued. After incubation of the virus for eight to ten days, infected mosquitoes are capable of being carriers of the virus for the rest of their lives. Because of this, researchers are developing new mosquito repellents in order to prevent blood-sucking and dengue virus infection. In general, the method used to repel mosquitoes is to utilize the sense of smell of mosquitoes, which is emitted by volatile active compounds. One of them is using DEET (N,N-diethyl-3-methyl benzamide), which is proven to be the most effective repellent with a duration of 6 hours. However, using excessively high concentrations can cause inflammation and is hazardous to children and infants [38].

Research conducted by Iikura et al., 2020, is in line with existing conditions related to mosquito expulsion methods. It is carried out by observing the behavior of mosquitoes when landing on oil-coated surfaces and how long it takes for mosquitoes to come into contact with surfaces coated with an oily liquid (hydrophobic). The oil used is silicone oil (low-viscosity polydimethylsiloxane, L-PDMS). In the physical properties of the liquids such as surface tension and viscosity by comparing the wetting and mosquito contact time with medium- and high-viscosity polydimethylsiloxane (M-PDMS and H-PDMS, respectively) to that with L-PDMS. These liquids have very different viscosities owing to the different degrees of polymerization, yet they have similar surface tensions because they all comprise the same repeating units. The result is that mosquitoes cannot come into contact with the surface for more than 3 seconds. This is because the liquid will wet the mosquito legs around the contact area [39].

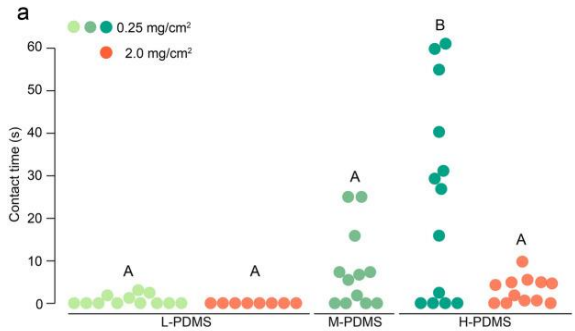

Figure 5. Mosquito Contact Time on PDMS Coated Glass Substrate (All Mosquitoes Leave L-PDMS Coated Surface With Time Less Than 3 Seconds and Up To 61 Seconds With H-PDMS) [39].

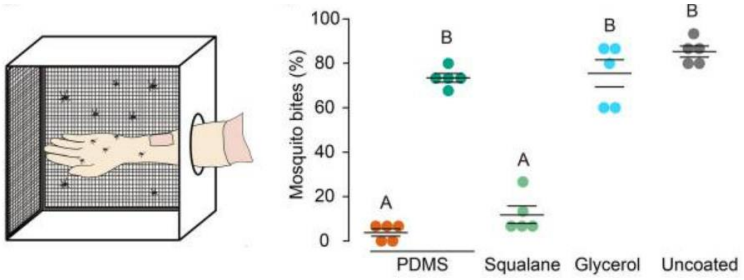

Figure 6. Left (Mosquito Bite Test Method With Forearm Exposure Area 4 cm x 5 cm), Right (L-PDMS Coated hand (Orange) Only 4% of Mosquitoes Land and Bite) [39].

Then the treatment was given by increasing the number of applications from 0.25 to 2 mg/cm2. The result obtained is that the contact time of the tarsal with the surface becomes shorter, going from 3 seconds to 0.18 seconds. It is also directly proportional to the number of mosquito bites on the surface of the skin. Only 4% of mosquito bites occur on skin with a layer of L-PDMS, compared to 85% on the skin without a layer of L-PDMS. The results show that the mosquito legs have hydrophobic properties, and when exposed to a liquid with the same properties, they will show an escape response because the meniscus is formed from the given liquid. Hydrophobic liquids with low viscosity will produce a faster tensile caliper force to produce rejection on the surface [39].

Eave Tubes Control Mosquito

Methods for controlling the ingress of mosquitoes into the house today are limited only to the use of insecticides indoors or with insecticidal mosquito nets. Mosquitoes can get into the room as a result of cracks or open roofs. Where it can be used to prevent mosquitoes from entering the house in search of a host [40]. Research conducted by Snetselaar et al., 2017 used eave tubes as a household protection product by looking at the natural behavior of malarial mosquitoes to find hosts. Where the existence of this method is expected to give rise to further research related to the inhibition of *Aedes aegypti* mosquitoes [41].

This study used a building with a mud wall and a roof made of corrugated iron sheets. The house has a tube that will be inserted through an eave tube with deltamethrin (a type of insecticide) and bendiocarb treatment. A few days later, there were mosquitoes with strains of *Anopheles gambiae* and *Anopheles arabiensis*. The examination process was carried out using a standard exposure bioassay for 3 minutes [41].

Figure 7. a. Experimental House With Mud Wallas and Corrugated Iron Sheets Roof with Nets Inside, c. Eave Tubes Sidelined with Insecticides (Deltamethrin and Bendiocarb) [41].

The result was that the treatment using an open eave tube was able to release 71% of *Anopheles gambiae* mosquitoes and be recaptured. Where the results were obtained, 92% were captured indoors, and 31% were recaptured using the CDC's light beam. And for mosquitoes, *Anopheles arabiensis* can be caught by 46%, with 76% of them caught indoors [41].

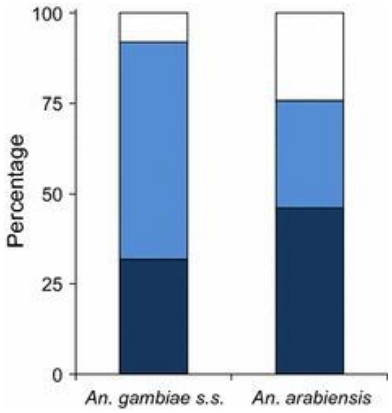

Figure 8. Percentage of Mosquitoes Caught From Indoors, *Anopheles gambiae* 92% and *Anopheles arabiensis* 76% [41].

The result is due to the response of mosquitoes that recognize the host's smell from the tube. *Anopheles gambiae* mosquitos are more common than *Anopheles arabiensis* mosquitos. One of the factors is that the general endophilic and endophagic properties of the mosquito *Anopheles gambiae* are more dominant than those of the mosquito *Anopheles arabiensis*. The use of insecticides in the form of deltamethrin and bendiocarb increases the effectiveness of eave tubes to block mosquitoes. Furthermore, bendiocarb has the ability to kill female mosquitos [41]. Mosquitoes can significantly move away from the house when part of the house is treated with mosquito nets and eave tube inserts on PVC pipes. The mechanism that occurs is when there is an increase in temperature during the day, causing body odor, or the host will go to the roof. This invites mosquitoes to enter the house from the roof. The use of mosquito nets and eave tube inserts in PVC pipes can reduce the number of mosquitoes in a room by more than 90% [42]. This proves that the use of eave tubes can reduce the entry of viruses that require vectors in the form of mosquitoes, such as malaria and dengue hemorrhagic

fever. For the prevention of dengue virus, which requires vectors in the form of Aedes aegypti mosquitoes, it is necessary to carry out further tests to be further confirmed [43].

Ultrasound Technology to Mosquito Repellents

The use of mosquito-repellent chemical drugs with a high concentration can have a bad effect on human health, namely dizziness and itching. At the moment, widely used electronic methods are used to repel mosquitoes [44]. Research conducted by Kim et al., 2021, used ultrasound with different frequencies and sound pressures to determine the behavior of female *Aedes aegypti* mosquitoes in searching for their hosts. Where the use of ultrasound is aimed at determining the presence of changes in mRNA expression [45]. Ultrasound is one of the sound waves with a frequency of more than 20 kHz, but cannot be heard by humans. While mosquitoes have good hearing, especially *Aedes aegypti* mosquitoes are able to hear up to 2 kHz [46].

The study was conducted by ultrasound to determine changes in the behavior of mosquitoes in search of their hosts. It is performed by creating a wind tunnel test and combining ultrasound, CO_2, and airflow similar to real conditions. The CO_2 tube and ultrasound speaker are placed at the endpoint to rattle and repel mosquitoes simultaneously. as well as using a fan that is opposite the endpoint with the aim of directing airflow toward the starting point. released 20 mosquitoes to the starting point, continued to open the sliding wall, and the CO_2 tube was activated along with the fan. Each test was conducted at frequencies of 20, 60, and 100 kHz with pressures of 50, 75, and 90 dB for 5 minutes [45].

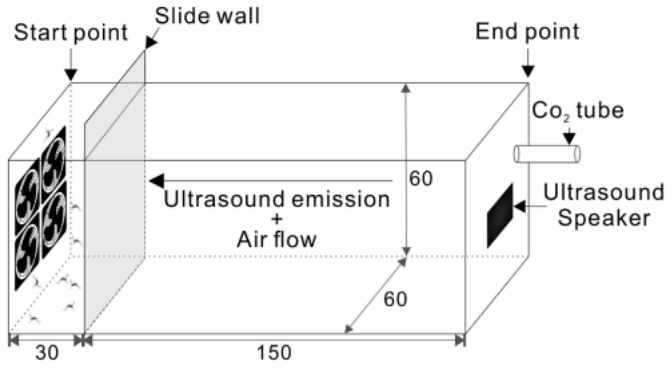

Figure 9. Mosquito Expulsion Test with Wind Tunnel [45]

The results obtained were from tests with an ultrasound frequency of 100 kHz and a pressure of 90 dB to provide the best mosquito rejection results. Treatment by exposing humans to ultrasound for a long period of time, more than 24 hours, at a frequency of 100 kHz (90 dB), can reduce the behavior of *Aedes aegypti* mosquitoes to make contact with or find a host on human skin [45].

Examination of the presence of a host search disorder was proven by the absence of human bites inserted in the sample box that had been given a 90 dB pressurized caterpillar ultrasonic pre-treatment with frequencies of 30 kHz and 100 kHz. This is due to physiological changes related to olfactory expression, CO2 sensing, and the gene that regulates *Aedes aegypti* hearing

caused by 100 kHz/90 dB ultrasound exposure for 24 hours and continued mRNA extraction from the mosquito head that has been exposed. The result obtained was that there were no significant changes in the odor receptor (AaOrco) of Aedes aegypti, but there was a decrease in the CO2 receptor, namely gustatory receptor 3 (AaGr3) [45]. AaGr3 is one of the mutant genes that serve to detect human skin at close range. Based on this, it shows that pre-treatment with ultrasound can reduce the exposure of the AaGr3 gene, which plays a big role in communication with humans, so that the dengue virus infection process can be avoided [47].

5. Conclusions

The methods used for the elimination of the dengue virus have been widely developed, such as the presence of inhibitors, antiviral compounds, and innovations in mosquito expulsion. Inhibitors that can be used are doxorubicin (SA-17), glycosidase inhibitors in the form of deocynojirimycin (DNJ) and castanospermine (CSP), carbohydrate-binding agents (CBA), and the use of heparan sulfate aimed at inhibiting the adsorption process and replication process, as well as improper protein folding to prevent the conformation of virus mergers. The antiviral compounds that have been found are in the leaf extracts of *Psidium guajava* and *Carica papaya,* which have inhibitory activities of 92.6% and 89.5%, respectively; propyl gallate with a percent inhibition of the dengue virus envelope protein serotype 2 is 53±9.85%, isobutyl gallate with a CC_{50} value of 167.19 μg/mL and have an inhibitory value (IC_{50}) of 4.45 μg/mL, and bioactive proteins owned by *Enterobacteria agglomerans* symbiosis with the brown algae *Sargassum sp.* has its percentage inhibition is 91% with a CC_{50} value of 129 μg/mL. Preventive methods for mosquito expulsion are also carried out in several ways, namely by utilizing hydrophobic liquid in the form of silicone oil (low-viscosity polydimethylsiloxane: L-PDMS), the use of eave tubes in home tubes inserted with insecticides, and utilizing ultrasound with a frequency of 100 kHz/90 dB.

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