Bioactive Compound Profile and Biological Modeling Reveals the Potential Role of Purified Methanolic Extract of Sweet Flag (*Acorus calamus* L.) in Inhibiting the Dengue Virus (DENV) NS3 Protease-Helicase

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Abstract: *Dengue fever is an infectious disease caused by the dengue virus, and there is no yet effective drug to treat this disease successfully. Our study aimed to identify the bioactive compounds of* Acorus calamus *L. and its potential role in inhibiting dengue virus NS3 protease-helicase. Liquid Chromatography-Mass Spectrometry analyzed phytochemical constituents. Drug-likeness of the predominant compound methanol extract of* Acorus calamus *L. was investigated through the SWISS ADME server. Complex molecular interactions were investigated by Hex 8.0 docking program and Discovery studio 2016. Our study revealed that the five largest phytochemicals in the extract were acoric acid, acorone, acoradin, acoronene, and calamendiol. All predominant compounds are potent to be developed as drug candidates. Molecular docking results showed that the five compounds bind to the Arg599, Pro291, Lys388, Pro431, and His487 of the dengue virus NS3 protease-helicase, the ligand-binding site that plays an essential role in viral replication. The ligand-protein binding pattern exhibited hydrogen and hydrophobic interactions. The interaction of the acoradin-NS3 protease-helicase complex had the lowest binding energy of -299.7 kcal/mol. In summary, we conclude that* Acorus calamus *L. extract may have prospects as a drug for dengue virus infection.*

Keywords: *bioactivity; dengue viral infection; herb medicine; LC-MS;* in silico

■ INTRODUCTION

Dengue is a global burden that infects about 96 million people each year with varying severity of symptoms worldwide. Indonesia is a tropical country with a high prevalence of dengue infection [1]. The agent of dengue is the dengue virus (DENV), transmitted through the mosquito vectors of *Aedes aegypti* and *Aedes albopictus* [2]. The infection of DENV is characterized by several clinical symptoms, including fever, headache, aches, nausea, and subsequent serious consequences such as dengue shock syndrome and morbidity. The high and rapid spread of DENV poses a threat to public health.

Moreover, no antiviral agent successfully cured or prevented this disease nowadays [3-4]. The only dengue vaccine commercially available was CYD-TDV. This vaccine is known as a live-attenuated dengue vaccine to prevent serious infection in priorly infected people. However, the risk of terrible dengue may increase since CYD-TDV was given to individuals who had not previously been infected [5].

The infection of DENV belongs to the family Flaviviridae, consists of four serotypes, namely DENV-

1, DENV-2, DENV-3, and DENV-4. DENV contains a single-stranded RNA and positive polarity genome with 11 kb. The genome encodes both structural proteins and non-structural proteins. Non-structural protein 3 (NS3) is a non-structural protein that plays an essential role in the viral replication and capping RNA [4,6]. Several studies investigated the NS3 of the dengue virus as a molecular target of antiviral drugs against DENV infection [7-8].

Safe and effective vaccine development makes a prominent contribution to avoiding and treating DENV infection [8]. Several plants with antiviral activity might have prospects to be developed as drugs for DENV therapy, such as *Acorus calamus*, *Cymbopogon citratus*, and *Myristica fatua* [3]. Sweet flag (*Acorus calamus* L.) is one of the medicinal plants containing phenolic compounds, alkaloids, flavonoids, tannins, saponins, steroids, triterpenoids, and glycosides [9]. Traditionally, the rhizome of *A. calamus* L. has been beneficial to treat cough, fever, asthma, and digestive disorders [10]. In a previous study, methanol extract of *A. calamus* L. showed anti-dengue activity in vitro and in silico through inhibition of dengue virus NS5 [3]. Non-structural domain 5 of Methyltransferase (NS5) is an enzyme with multiple enzymatic functions enabling synthesis of the RNA synthesis, 5′ RNA cap, and methylation [5]. However, the potential role of *A. calamus* L. methanol extract in inhibiting the NS3 protease-helicase protein is unknown.

Non-structural protein 3 (NS3) is a 69 kDa highly conserved protein among flaviviruses responsible for the viral replication cycle. NS3 consists of two domains, namely the N-terminal protease domain and the C-terminal RNA helicase domain. The N-terminal protease region is involved in cleaving viral polyprotein precursors into individual proteins. The C-terminal RNA helicase domain controls viral RNA synthesis and DENV genome replication [5]. This study aimed to characterize the phytochemical compounds of methanol extract of *A. calamus* L. rhizome using LC-MS analysis. Considering the previous elucidated biological functions, we identified drug-likeness and anti-dengue activity of methanol rhizome extract of *A. calamus* L. through in silico approach.

■ EXPERIMENTAL SECTION

Materials

The rhizome of *A. calamus* L. (local name: jeringau putih) was collected from 2nd Rasau Jaya village, Rasau Raya ditrics, Kubu Raya regency, Kalimantan Island, Indonesia. The chemicals used were methanol (95% chemical purity), Whatman paper grade 1, 0.01% hydrochloric acid in distilled water, and cellulose acetate 0.45 µm. The 3D structure of NS3 helicase-protease, acoric acid, acorone, acoradin, acoronene, and calamendiol was used in silico analysis.

Instrumentation

The instrumentations of a rotary evaporator and a centrifuge were used to extract *A. calamus* L. Extract was purified using a Sep-Pak C18 Vac cartridges, vacuum pump, or aspirator with 40 °C temperature, and stative clam. Shimadzu LCMS was used for characterized the phytochemical compounds of *A. calamus* extract.

Procedure

Extraction of A. calamus *L. rhizome*

The prepared sample was a fresh rhizome of *A. calamus* L. Before extraction, the refined rhizome is needed. Extraction was carried out using 95% methanol solvent [11]. After 24 h of maceration at room temperature, the residue was extracted with 95% methanol (1:5). The combined supernatant was filtered with Whatman paper grade 1, then concentrated using a rotary evaporator (40 °C) [12]. The supernatant was obtained from centrifugation at 5000 rpm for 20 min for purification [13].

Purification of A. calamus *L. rhizome extract*

Briefly, 12 mL of methanol 95% were poured into Sep-Pak C18 Vac cartridges, and then methanol was removed by pouring 18 mL of acidified distilled water. Liquid extract of 5–10 mL was running into the cartridge. Then, the solution was eluted by using methanol 95%. Methanol was evaporated with a rotary evaporator at 40 °C under vacuum conditions. The eluate was re-dissolved with acidified distilled water. Purified extract was stored at 4 °C for further analysis [14].

LC-MS profiling of methanolic extract of A. calamus *L.*

LC-MS analysis was performed using Shimadzu LCMS with the Shim Pack FC-ODS column (2 mm × 150 mm, 3 μm). The solvent consists of 0.01% HCl in water distillation and 95% methanol. We used a flow rate of 0.8 mL/min, maximum pump pressure (P) of 15 kgf/cm^2, the wavelength of 254 nm and 190 nm, the response time of 1 sec, AUX range of 2 AU/V, Lamp of D2 with positive polarity, LC stop time of 100 min, and probe temperature of 250 °C. Mass spectrophotometry (MS) was set as follows: full scan with a positive mode in the m/z 50–500 range. Maintained probe and a curved desolvation line (CDL) at 250 °C. Nitrogen as a nebulizer gas with a 1.5 mL/min flow rate, 1.5 kV gain detector, CDL voltage 25 V, and high voltage probe: +4.5 kV. Compounds are characterized based on the MS spectrum through the LC-MS Post-run Computer Program [15].

Data mining for molecular docking analysis

The target protein in this study is a DENV NS3 protease-helicase. Acoric acid, acorone, acoradin, acoronene, and calamendiol from the *Acorus calamus* L. determined as a ligand. The 3D structure of NS3 protease-helicase (PDB ID: 2WHX) was obtained from RSCB PDB (https://www.rcsb.org/). NCBI Pubchem Database (https://www.ncbi.nlm.nih.gov/) generates chemical structures of acoric Acid (CID: 15558302), acorone (CID: 5316254), acoradin (CID: 126324), acoronene (CID: 15558294), and calamendiol (CID: 12302239) with the SDF format. NS3 protease-helicase preparation includes removing water molecules or ligands using Discovery Studio Software. Then, energy minimization was performed using PyRx software. Furthermore, the docking results with the .pdb format were obtained using Open Babel [16].

Drug-likeness prediction

The biological function of acoric acid, acorone, acoradin, acoronene, and calamendiol of *A. calamus* L. as a drug candidate to treat dengue was investigated through the SWISS ADME server (http://www.swissadme.ch). Physicochemical, pharmacokinetics, and drug-likeness properties are determined to identify potential drug candidates. The Physico-chemical parameters studied are the number of H-bond acceptors, H-bond donors, rotatable bonds, and iLOGP. Furthermore, pharmacokinetic constituents investigated were gastrointestinal absorption, Blood-Brain Barrier Permeability (BBB), and LogKp. Lipinski drug-likeness were analyzed using the SWISS ADME Server [17].

Molecular docking and visualization

Before conducting docking simulation, we validate compounds that really bind to the protein by biological activity prediction tool (http://www.pharmaexpert.ru/passonline/). Hex 8.0 program with Shape+Electro+DARS mode for molecular docking simulations was established. Docking results should be in the .pdb file. Briefly, visualization of the complex interaction of ligand-proteins was performed using the 2016 Discovery Studio program [18]. Furthermore, the binding side, type of chemical bond, and binding energy were determined [16].

■ RESULTS AND DISCUSSION

LC-MS Analysis of Methanol Extract of *A. calamus* L.

The health-beneficial properties of the rhizome *A. calamus* L. are related to the phytochemical component. The LC-MS approach for investigating chemical constituents in methanol rhizome extract of *A. calamus* L. was established. Our study showed 98 phytochemical compounds in the extract of *A. calamus* L. Chromatograms represent the compound component shown in Fig. 1. The predominant compound in the extract of *A. calamus* L. is acoric acid, acorone, acoradin, acoronene, and calamendiol (Table 1). In previous studies, *A. calamus* L. extract positively contain taxifolin-3-glucopopanoside, velutin, methyl digallate, γ-asarone, α-asarone, and β-asarone, which show antioxidant and

Table 1. The bioactive compound profiles of *A. calamus* L. extract with identities of compounds

Peak	Predominant compounds	RT (min)	Product ion (m/z)	Composition (%)
66	Acoric acid	9.086	268	4.0107
63	Acorone	7.291	236	3.6989
83	Acoradin	14.434	417	3.6989
62	Acoronene	7.279	234	3.4221
64	Calamendiol	7.326	238	3.0533

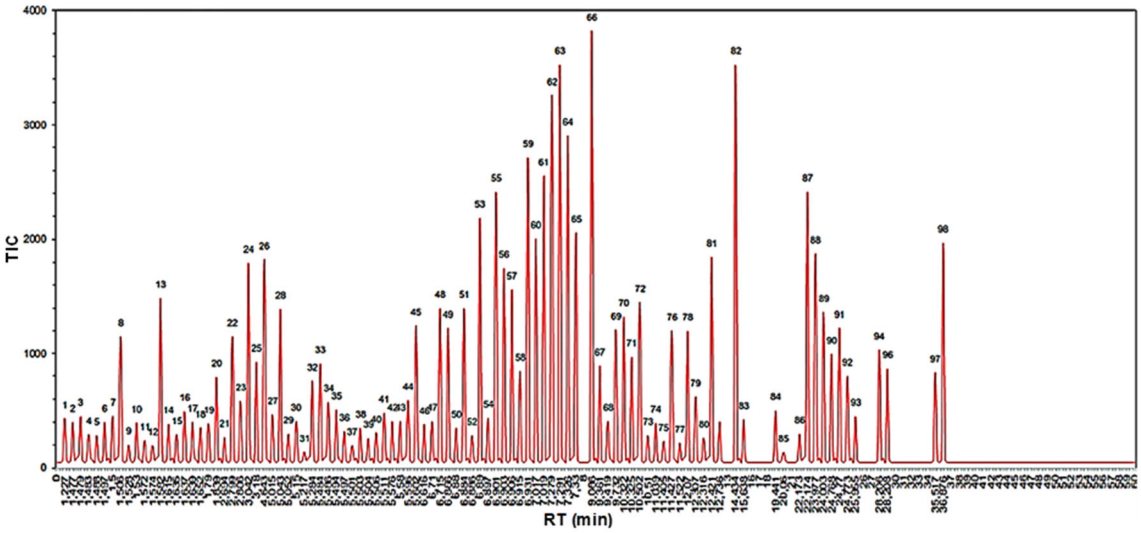

Fig 1. Chromatogram profile of *Acorus Calamus* L. extracts by LC-MS. Peaks show the bioactive compound profiles of *Acorus Calamus* L.

antifungal activity [10,19-20]. Phytochemical components variation is affected by several factors, including geographical, harvest time, solvent, and temperature of extraction [21].

The predominant compound was determined by chemical structure, retention time, composition, molecular weight, and mass spectrum. Acoric acid ($C_{15}H_{24}O_4$) has a molecular weight of 268.3530, indicated by m/z 268; acorone ($C_{15}H_{24}O_2$) with a molecular weight of 236.3550, established by m/z 236. Acoradin ($C_{24}H_{32}O_6$) has a molecular weight of 416.5140 appeared at m/z 417. Acoronene ($C_{15}H_{22}O_2$) and calamendiol ($C_{15}H_{26}O_2$) have a molecular weight of 234.3390 and 238.3710, with m/z of 234 and m/z 238, respectively (Fig. 2). Single-crystal x-ray diffraction studies show that the acoric acid is sesquiterpene from the acarone skeleton. A previous study reported extract ethanol rhizome of *A. calamus* L. (5 μm and 10 μm) have cell proliferation activities on SK-N-BE cell line [22]. In addition, methanol extract of *A. calamus* L. at a dose of 20 g/mL shows DENV-2 to 96.5% inhibitory activity in vitro. Acorone and calamendiol are sesquiterpenoids that may have pharmacological activities as an antifungal. A previous study confirmed that rhizome extract *A. calamus* L. has health benefits for treating disease so that the extract can be employed as a natural treatment [3].

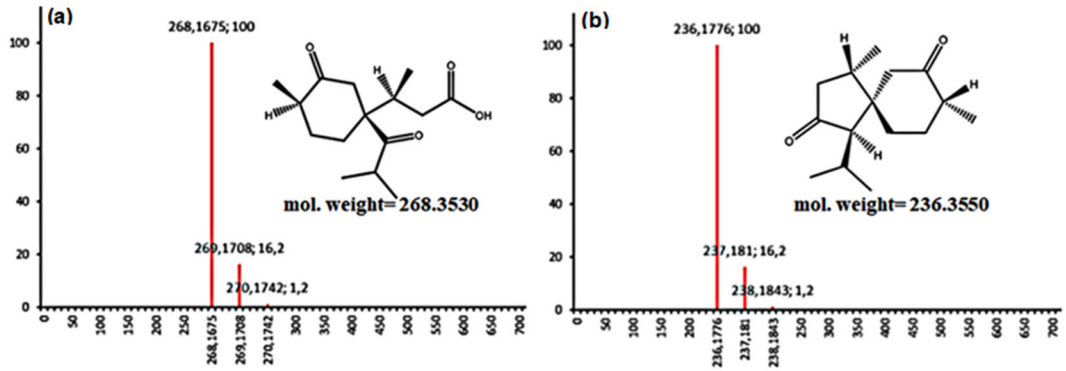

Fig 2. The mass spectra of the predominant compound of methanol rhizome extract of *A. calamus* L. acoric acid (a), acorone (b), acoradin (c), acoronene (d), and calamendiol (e). X-axis shows the m/z, and Y-axis indicates the absorbance (%)

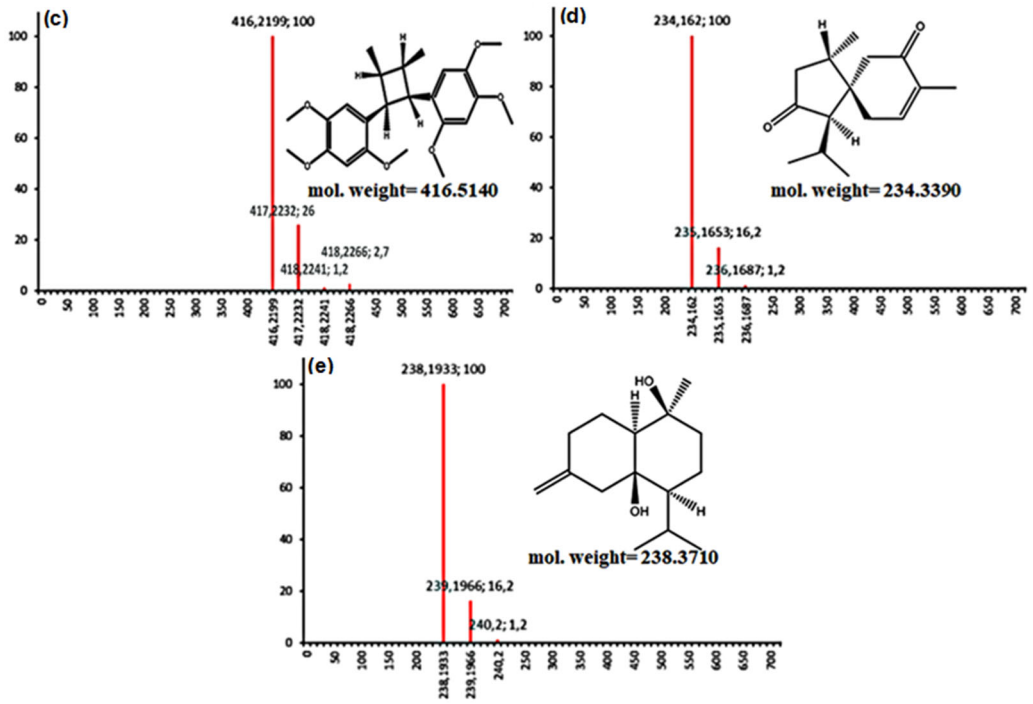

Fig 2. The mass spectra of the predominant compound of methanol rhizome extract of *A. calamus* L. acoric acid (a), acorone (b), acoradin (c), acoronene (d), and calamendiol (e). X-axis shows the m/z, and Y-axis indicates the absorbance (%) (*Continued*)

Physico-chemical Profile, Pharmacokinetics, and Druglikeness of Methanol Extract of *A. calamus* L.

The physicochemical properties of the predominant compounds (acoric acid, acorone, acoradin, acoronene, and calamendiol) of *A. calamus* L. extract are shown in Table 2. Functional groups, lipophilic properties, and the solubility of a natural compound determine the pattern of absorption, distribution, metabolism, and excretion in humans [17]. Based on ADME analysis, all compounds have good gastrointestinal (GI) and Blood-Brain Barrier (BBB) absorption. In addition, the value of skin permeation (KP) was -5.35 cm/sec to -6.65 cm/sec, indicating that the compound could pass through the skin barrier in humans.

Physicochemical is an important parameter related to drug activity. Molecules with a higher number of hydrogen bonds donors and acceptors in their structure may better prevent oxidative stress [16]. In this study, acoradin had the highest total number of hydrogen bonds. Compounds with a molecular weight of 130–725, H–bond donor: 0–6, H–bond acceptor: 2–20, log Kp: -2 to 6.5, and the number of rotatable bonds: 0–15 is considered to the drug clinically (Table 2). A compound with a GI value of more than 80% indicates high absorption capability. The drug's potential to be absorbed by the blood-brain barrier is an essential character related to side effects and drug efficacy. As long as the BBB value is more than 0.3, it exhibits that a compound can permeate the blood-brain barrier and be adequately distributed [23-24].

The drug-likeness analysis performs to investigate a molecule's ability to be a promising drug candidate [25]. One of the parameters for determining potential drug candidates is Lipinski. The Lipinski rule explains the criteria for drug candidates. There are molecular weights (500), high lipophilicity (≤ 5), hydrogen bond donors (≤ 5), hydrogen bond acceptor (≤ 10), and molar refractivity (40–130). At least, drug candidates should adhere to two of their criteria [26]. Based on drug-likeness, acoric acid, acorone, acoradin, acoronene, and calamendiol can be categorized as potent drug candidates (Table 2). After obtaining Lipinski results, we

Table 2. Physicochemical profile, pharmacokinetics, and drug-likeness predominant compounds from the extract of *A. calamus* L. by ADME analysis

Physicochemical properties	Acoric acid	Acorone	Acoradin	Acoronene	Calamendiol
H-bond acceptor	4	2	6	2	2
H-bond donor	1	0	0	0	2
Rotatable bond	5	1	8	1	1
Log $P_{o/w}$ (iLOGP)	1.88	2.48	4.47	2.43	2.88
Pharmacokinetic components					
GI absorption	High	High	High	High	High
BBB permeability	Yes	Yes	Yes	Yes	Yes
CYP1A2 inhibitor	No	No	No	No	No
CYP2C19 inhibitor	No	No	No	No	No
CYP2C9 inhibitor	No	No	No	Yes	No
CYP2D6 inhibitor	No	No	Yes	No	No
CYP3A4 inhibitor	No	No	No	No	No
Log *K*p (cm/s)	-6.65	-5.70	-5.35	-5.86	-6.01
Lipinski	Yes	Yes	Yes	Yes	Yes

investigated the potential role of the predominant compound of *A. calamus* L. as an anti-dengue agent through an *in silico* approach.

In silico Molecular Docking Analysis

Our study demonstrated molecular interactions between acoric acid, acorone, acoradin, acoronene, calamendiol, and non-structural protein NS3 protease-helicase. NS3 protease-helicase was determined as the protein target. The selected ligands include acoric acid, acorone, acoradin, acoronene, and calamendiol from extracts of *A. calamus* L. It was predicted to have a potential function as an anti-dengue drug agent through DENV NS3 protease-helicase binding interactions. Based on biological activity prediction, acoric acid, acorone, acoradin, acoronene, and calamendiol showed pharmacological effects as an antiviral with a Pa value of > 0.4.

The docking results showed five amino acid residues involved in the acoric acid-NS3 protease-helicase complex, namely Arg599, Leu429, Pro431, Pro291, His487 (Fig. 3(a)). Hydrogen bonds and hydrophobic stabilize these interactions. Acorone binds to Arg387, Pro431, Lys388, Arg599, Ile365, His487 residues in the RNA helicase domain of the NS3 protease-helicase. Hydrophobic and hydrogen bonds maintain Arg387, Pro431, Lys388, Arg599, Ile365, His487 residue (Fig. 3(b)). Acoradin and NS3 protease-helicase complex involve Val544, His487, Glu490, Asp409, Ala606, Arg599, Lys388, Arg387, and Pro291. Hydrophobic and hydrogen bonds stabilize the acoradin and NS3 protease-helicase interactions (Fig. 3(c)). The occurrence of molecular complexes is due to non-covalent interactions such as hydrogen bonds, hydrophobicity, and Van der Waals. This binding is related to specific biological activity on the active site of the protein target [27].

The infection of DENV NS3 protease-helicase contains an N-terminal serine protease region with amino acid residues 1–168 and RNA helicase at residues 180–618 linked by 11 amino acids (residues 169–179). The enzyme consists of a trypsin-like serine protease domain with a catalytic triad of His51, Asp75, and Ser135 [4,6]. In this study, nine amino acid residues Ser364, Arg599, Ala602, Arg387, Pro431, Leu443, Arg599, Ile365, and His487, bind to acoronene. The acoronene and NS3 protease-helicase complex was established by hydrogen bond and hydrophobic interactions (Fig. 3(d)). The calamendiol and NS3 protease-helicase complexes had fewer interactions than the acoronene-NS3 protease-helicase complex (Fig. 3(e)). The docking results show that Arg599 and Lys388 are bonding with calamendiol through hydrophobic interaction by alkyl donor. Our study showed that the

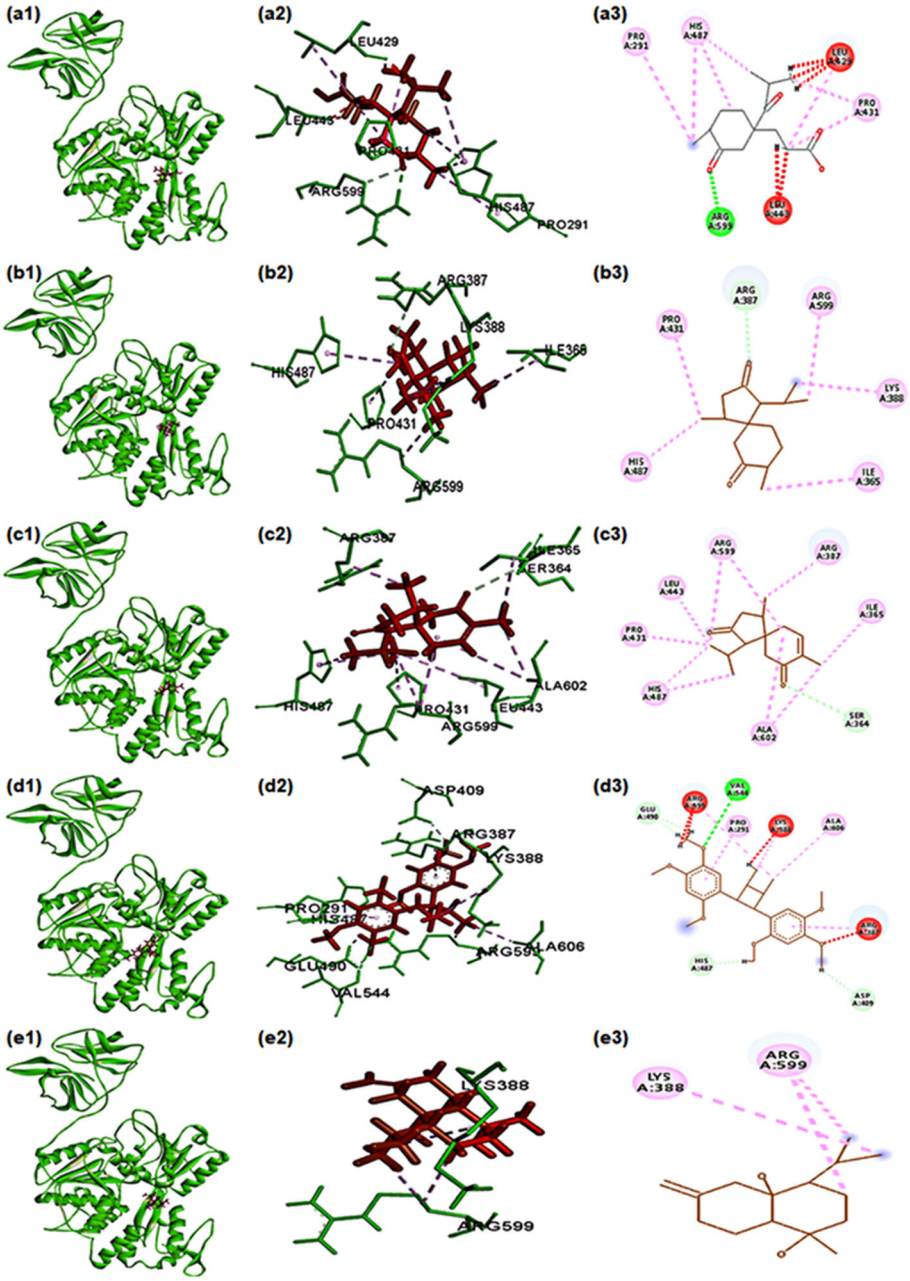

Fig 3. Molecular docking results from the interaction between acoric acid (a), acorone (b), acoradin (c), acoronene (d), and calamendiol (e) complexes with NS3 protease-helicase. Number 1 shows a visualization of the ligand-protein molecular complex. The 3D structure in number 2 and number 3 shows the 2D structure

five predominant compounds of methanol extract of *A. calamus* L. bind with the active site residues Arg599, Pro291, Lys388, Pro431, and His487 of NS3 protease-helicase. A previous study reported that Arg387, Lys388, and Arg599 residues are involved in the molecular interaction of NS3 protease-helicase inhibited by natural compounds [4]. This result suggested that acoric acid, acorone, acoradin, acoronene, and calamendiol might disrupt the enzymatic activity of NS3 helicase-protease, so their function is responsible for the replication cycle could block. Ligand interaction, hydrogen, and hydrophobic effect may cause the protein fold to shift

into a nonfunctional protein [16]. In this study, NS3 helicase-protease signaling might be prevented by five predominant compounds of *A. calamus* L.

Besides chemical bonds, binding energy contributes to the stable molecular complex. Binding energy is formed when intermolecular interactions occur. Therefore, if the binding energy were lower, it would be easier for the molecules to interact. The amino acid residues and the chemical bond between the protein-ligand complex determine the binding energy [16]. Our study found that the acoradin and NS3 protease-helicase complexes required the lowest binding energy of -299.7 kcal/mol, so they could easily bind (Table 3). A hydrogen bond is the main contributor to the structure and ligand-receptor interaction. Furthermore, the efficacy and specificity of the protein target are mainly assessed through hydrogen bonds [28]. In this case, the acoradin and NS3 protease-helicase interaction had the highest number of hydrogen bonds. Consequently, the acoradin and NS3 protease-helicase interaction had the lowest binding energy.

Previous research related to the exploration of anti-DENV investigated the potential activity of 12 compounds from the ethanol root extract of *A. calamus* L. Tatanan A compound was reported to have the highest anti-DENV capacity. In addition, cytotoxicity and cytopathogenic effects induced by DENV2 have successfully reduced (EC50 value of 3.9 M) [29]. Corresponding to the biological function of δ-Selinene and α-Caryophyllene of *Ipomoea batatas* L., exhibited inhibitory of the NS2B/NS3 protease catalytic domains

Table 3. Interaction between acoric acid, acorone, acoradin, acoronene, calamendiol, and NS3 protease-helicase

Interactions	Point interaction	Chemistry bond	Type	Energy binding (kcal/mol)
Acoric acid-NS3 protease-helicase	**A:ARG599:HH12** - :LIG1:O	Hydrogen Bond	Conventional Hydrogen Bond	-247.1
	A:ARG599:CD - :LIG1:O	Hydrogen Bond	Carbon Hydrogen Bond	
	:LIG1:C - A:LEU429	Hydrophobic	Alkyl	
	:LIG1:C - A:PRO431	Hydrophobic	Alkyl	
	:LIG1:C - A:PRO291	Hydrophobic	Alkyl	
	:LIG1:C - A:PRO431	Hydrophobic	Alkyl	
	A:HIS487 - :LIG1	Hydrophobic	Pi-Alkyl	
	A:HIS487 - :LIG1:C	Hydrophobic	Pi-Alkyl	
	A:HIS487 - :LIG1:C	Hydrophobic	Pi-Alkyl	
Acorone-NS3 protease-helicase	**A:ARG387:CD** - :LIG1:O	Hydrogen Bond	Carbon Hydrogen Bond	-226.3
	:LIG1:C - A:PRO431	Hydrophobic	Alkyl	
	:LIG1:C - A:LYS388	Hydrophobic	Alkyl	
	:LIG1:C - A:ARG599	Hydrophobic	Alkyl	
	:LIG1:C - A:ILE365	Hydrophobic	Alkyl	
	A:HIS487 - :LIG1:C	Hydrophobic	Pi-Alkyl	
Acoradin-NS3 protease-helicase	**A:VAL544:HN** - :LIG1:O	Hydrogen Bond	Conventional Hydrogen Bond	-299.7
	:LIG1:H - A:HIS487:NE2	Hydrogen Bond	Carbon Hydrogen Bond	
	:LIG1:H - A:GLU490:OE1	Hydrogen Bond	Carbon Hydrogen Bond	
	:LIG1:H - A:GLU490:OE2	Hydrogen Bond	Carbon Hydrogen Bond	
	:LIG1:H - A:ASP409:OD1	Hydrogen Bond	Carbon Hydrogen Bond	
	A:ALA606 - :LIG1:C	Hydrophobic	Alkyl	
	:LIG1:C - A:ARG599	Hydrophobic	Alkyl	
	:LIG1:C - A:LYS388	Hydrophobic	Alkyl	
	:LIG1 - A:ARG387	Hydrophobic	Pi-Alkyl	
	:LIG1 - A:PRO291	Hydrophobic	Pi-Alkyl	

Table 3. Interaction between acoric acid, acorone, acoradin, acoronene, calamendiol, and NS3 protease-helicase (*Continued*)

Interactions	Point interaction	Chemistry bond	Type	Energy binding (kcal/mol)
Acoronene-NS3 protease-helicase	**A:SER364:CA** - :LIG1:O	Hydrogen Bond	Carbon Hydrogen Bond	-240.3
	A:ARG599 - :LIG1	Hydrophobic	Alkyl	
	A:ALA602 - :LIG1	Hydrophobic	Alkyl	
	A:ALA602 - :LIG1:C	Hydrophobic	Alkyl	
	:LIG1:C - A:ARG387	Hydrophobic	Alkyl	
	:LIG1:C - A:PRO431	Hydrophobic	Alkyl	
	:LIG1:C - A:LEU443	Hydrophobic	Alkyl	
	:LIG1:C - A:ARG599	Hydrophobic	Alkyl	
	:LIG1:C - A:ILE365	Hydrophobic	Alkyl	
	A:HIS487 - :LIG1:C	Hydrophobic	Pi-Alkyl	
	A:HIS487 - :LIG1:C	Hydrophobic	Pi-Alkyl	
Calamendiol-NS3 protease-helicase	**:LIG1:H** - :LIG1:O	Hydrogen Bond	Conventional Hydrogen Bond	-241.3
	:LIG1:C - A:ARG599	Hydrophobic	Alkyl	
	:LIG1:C - A:ARG599	Hydrophobic	Alkyl	
	:LIG1:C - A:LYS388	Hydrophobic	Alkyl	

*bold letters indicate donor atoms

(Ser135 and His51) through hydrophobic interactions [30]. Residue ~170–618 of dengue virus NS3 protease-helicase act encodes NTPase/RNA helicase and 5-RNA triphosphatase. Both enzymes are required for the replication and capping steps of viral RNA [31]. Our study revealed that acoric acid, acorone, acoradin, acoronene, and calamendiol from extracts of *A. calamus* L. have a potential role as anti-dengue through inhibition of the NS3 protease-helicase. This inhibition leads to disruption of RNA replication and translation activities to prevent and treat DENV infection.

■ CONCLUSION

This study confirmed 98 phytochemical compounds in methanol extract of *Acorus calamus* L. Acoric acid, acorone, acoradin, acoronene, and calamendiol were the five major compounds in the extract. These compounds show appropriate physicochemical, pharmacokinetics, and Lipinski rule as drug candidates. Furthermore, molecular docking results showed that acoric acid, acorone, acoradin, acoronene, and calamendiol might interfere with NS3 protease-helicase activity. Thus, the replication cycle of the dengue virus could be blocked.

■ AUTHOR CONTRIBUTIONS

Yuli Arif Tribudi contributed to conception and design, acquisition of data, analysis, and interpretation of data, drafting the article, critically revising the article for important intellectual content, final approval of the version to be published. Ayu Tri Agustin contributed to conception and design, data acquisition, analysis and interpretation of data, drafting the article. Dian Eka Setyaningtyas contributed to the acquisition of data, drafting the article. Dwi Gusmalawati contributed to the acquisition of data, drafting the article. All authors agreed to the final version of this manuscript.

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