

Productive Growing System of *Andrographis Paniculata* and Inhibitions of Reverse Transcriptase and 3clpro Enzymes by the Isolated Andrographolide

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ABSTRACT

Molecular interactions between andrographolide (ADP) and proteins/enzymes of different pathogenic viruses were computationally predicted by using docking method. Binding energy corresponded to a protein-ADP pairing was theoretically accounted in comparison with other current antiviral drugs as positive ligands. *In silico*, interactions between ADP and PLpro (PDB, 6WX4), ADP and 3CLpro (PDB, 7LMD), ADP and ACE-2 receptor-spike protein complex (PDB, 6M0J), as well as ADP and M2 protein (PDB, 6BKK) were stronger than those of between rimantadine, remdesivir, lopinavir, or ritonavir and the described proteins. The inhibitions of ADP against reverse transcriptase and 3CLpro activities were greater than those of lopinavir, ritonavir, and oseltamivir, according to *in vitro* assays. ADP was isolated as a major active compound from *Andrographis paniculata* grown outdoor in newly established, cost-effective, hydroponic system. An average fresh mass per plant was as high as 170 g from a 35x35 cm² spacing hole. Simple and productive procedures were recently suggested for ADP extraction. These acquired data would be applied for pharmaceutical manufacturing.

Keywords *Andrographis paniculata*, Andrographolide, Molecular docking, Reverse transcription, 3C rhinoviral protease, Outdoor hydroponic system

INTRODUCTION

Viruses are biological entities capable of replication in living organisms only. Distinct mechanisms have been used by a virus to affect an organism. This is why a virus that causes illness in cats may not infect humans. There are serious and persistent threats from viruses to humankind at present, due to the emergence, re-emergence, or evolution of drug-resistant strains. Consequently, continuous search of new and potent antiviral drugs is important. Viral life cycle is termed for a group of stages used by a virus to infect cells. These include (i), attachment to host cell membrane; (ii), entry into host cells; (iii), genome uncoating, replication, transcription, and translation; (iv), assembly; and (v), budding and release. Each stage is completed by particular enzymes, which indicated to be dependent on the genome of the infected virus. For instance, replication of most DNA viruses arises in the host cell nucleus and is carried out by the host enzymes. Another large DNA virus, called Vaccinia, is an exception in that replication and transcription occur in the host cell cytoplasm using enzymes encoded by the infected virus. Genetic information of RNA viruses is stored in the form of RNA molecules. Their replication can be achieved by means of RNA-dependent RNA polymerase (RdRp) or RNA-dependent DNA polymerase (so-called reverse transcriptase) for RNA or cDNA synthesis, respectively. Indeed, the viruses must encode reverse transcriptase on their own because of not available from cells of humans. Then, integration between the cDNA and the host genome can be happened, leaving the viral genome persistent in the infected host cells. Consequently, it is helpful to hinder viral infection, replication, and spreading by inactivation of these associating enzymes [1], which have been drug targets for searching new lead compounds by computational methodology.

Andrographolide (ADP) is a major secondary metabolite of *Andrographis paniculata* with diterpenoids structure. The herb has been used in traditional Eastern medicine for treatments such as common cold, sore throat, liver disease, jaundice, diabetes, dysentery, chronic malaria, and snake bite [2,3], and be included in Thai Herbal Pharmacopoeia since 2017. Few years ago, the potentials of ADP and the *A. paniculata* extracts against SARS-CoV-2 were reported [4]. Hard capsules containing dried powder or extracts of the herb were dispensed to COVID-19 patients with mild symptoms in Thailand of which the treatment outcomes were desirable. Later, different interactions between ADP and structural or non-structural proteins of SARS-CoV-2 such as papain-like protease, coronavirus main proteinase, and the spike protein, were demonstrated by many *in silico* researches [5]. By network pharmacological analysis, immune modulatory effects of ADP on p53 signaling and chemokines/natural killer cell mediating cytotoxicity have been apparent, including its inhibitions towards infectious viral strains like herpes simplex virus 1, human immunodeficiency virus, influenza A virus, hepatitis C virus, as well as dengue virus. So far, broad inhibitory spectra of ADP have been indicated [6]. However, molecular mechanisms that underline the described therapeutic actions of ADP are scant at present, leading to the discoveries important.

In this research, molecular docking method was carried out to predict what proteins/enzymes of human infectious strains of viruses are potential as ADP targets. By comparing with other commonly used antiviral drugs, possible/attractive interactions based on binding energy (kcal/mole) could be suggested. Two enzymatic assays [7] were performed *in vitro* for insisting the computationally simulated results. The recently used ADP was purified from *A. paniculata*

grown outdoor in our modified hydroponic garden. Purity and content of the ADP were demonstrated by using HPLC technique [8]. Using our developed procedures for plating *A. paniculata* and extracting ADP, it seemed to increase yield and quality of the extracted ADP (closures on planting of *A. paniculata* and purification of ADP by two petty patents). The obtained data might be applicable in producing a pharmaceutical grade of ADP for manufacturing scale.

METHODS

Chemicals and Reagents

Chemicals and solvents of analytical/chromatographic grades were used. Methanol and absolute ethanol were bought from Merck (Darmstadt, Germany). ADP working standard (98.15% purity) was obtained from Department of Medical Sciences Reference Standards, Ministry of Public Health, Thailand. Abacavir sulfate, lopinavir, ritonavir, and oseltamivir phosphate were purchased from Sigma-Aldrich. Mouse muscle cell line, C₂C₁₂, was acquired from ATCC. Chemicals/reagents for cell culture technique such as Dulbecco's Modified Eagle medium (DMEM), fetal bovine serum (FBS), penicillin/streptomycin, fungizone, RIPA buffer, etc., were purchased from Gibco (Life Technologies, NY, USA). Oligonucleotide primers were ordered from Macrogen HQ (Seoul, Republic of Korea). The HRV 3C protease activity assay kit (Colorimetric) was bought from BioVision (MA, USA).

Molecular Docking Study

Three-dimensional structures of ADP (Pubchem ID: 5318517) and oseltamivir (Pubchem ID: 65028) were downloaded from PubChem database, while those of lopinavir (ChemSpider ID: 83706) and ritonavir (ChemSpider ID: 347980) were from ChemSpider server. Translation to Protein Data Bank (PDB) format files by using Online SMILES Translator and Structure File Generator (<https://cactus.nci.nih.gov/translate/>) was then performed. Crystal structures of enzymes were acquired from RCSB protein database (<http://www.rcsb.org>). These included influenza A neuraminidases: H1N1 (PDB ID: 6HP0), H3N2 (PDB ID: 4GZT), H3N8 (PDB ID: 4WA4), N4 (PDB ID: 2HTW); influenza A M2 protein (PDB ID: 6BKK); hepatitis C virus nonstructural protein 5B (NS5B) isolate 1 (PDB ID: 4TLR) and subtype 1b (PDB ID: 5CZB); hepatitis B virus mutant capsid proteins: (PDB ID: 5T2P) and (PDB ID: 6J10); SARS CoV-2 proteases: 3-chymotrypsin-like protease (PDB ID: 7LMD) and papain-like protease (PDB ID: 6WX4); and ACE-2 receptor-spike protein complex (PDB ID: 6M0J). Any attached molecules including water were dissected from the crystal structures. But polar hydrogen atoms were added. These were operated by using AutoDock4. Files of target proteins and test ligands were saved as PDBQT format for molecular docking. Again, AutoDock4 was used for selection of active site residues and molecular docking Grid, and docking protocols of the active site predictions. Grid site was set spacing at 0.375 Å. The x-y-z dimensions were set at 120-120-120 Å³. The rigid entity of protein structures was used. But the ligand was set as a flexible molecule. Docking study was rendered by using the Lamarckian genetic algorithm (GA) and implemented by AutoDock4. The number of GA runs was 50 with a popular size of 200. Binding energy (ΔG_{bind}) was analyzed by using ADT. Interactions were visualized by using BIOVIA Discovery Studio (BIOVIA, 2020). Protein-ligand structure at the binding site was visualized by using Visual Molecular Dynamics (VMD) package.

Inhibition of Reverse Transcriptase Activity

Total RNA Extraction:

Extraction of total RNA was proceeded according to the manufacture's protocol (Invitrogen). C₂C₁₂ cells were routinely cultured in a T25 flask in DMEM supplemented with 10% FBS and 1% antibiotic/antimycotic at 37°C in a 5% CO₂ incubator. RNA extraction was carried out using 95% confluent cells as follows. The old culture was removed. A 0.5-ml TRIzol™ reagent was added, incubated at room temperature for 5 min, and transferred to a 1.5-ml tube. Next, 0.1 ml of chloroform was added, rigorously mixed for 2 min, and centrifuged at 12,000×g, 6°C, 15 min. The clear supernatant was carefully transferred to a new 1.5-ml tube. Then, 0.25 ml of isopropanol was added, thoroughly mixed, incubated for 10 min, and centrifuged at 12,000×g, 6°C, 10 min. RNA was precipitated as white gel-like pellet at the tube's bottom. The supernatant was removed. The pellet was resuspended using 0.5 ml of 75% ethanol and re-centrifuged at 7500×g, 6°C, 5 min. The supernatant was discarded. The extracted RNA was air-dried for 10 min and dissolved in 200 µl RNase-free water.

Purity and Concentration:

The absorbance at 260 and 280 nm of the extracted RNA was measured. The purity was estimated from the A₂₆₀/A₂₈₀ ratio. The ratio of ~2 is considered pure. RNA concentration was calculated by using the formula: A₂₆₀ x dilution x 40 = µg RNA/ml.

Reactions Set Up for Reverse Transcriptase Inhibitory Assay:

Abacavir (AV) is a reverse transcriptase inhibitor and was used as positive control in this study. Stock solutions of abacavir (AV-SS) and andrographolide (ADP-SS) at a concentration of 6.6 mM in absolute ethanol were separately prepared. Using the guidelines of SuperScript® VILO™ cDNA Synthesis Kit (Invitrogen), a reaction was set up by sequentially adding a component as shown in Table 2. The reaction was operated on G-STROM Thermal Cycler (Open CELL Labs, United Kingdom) as 25°C, 10 min; 42°C, 60 min, and 85°C, 5 min. The resulting cDNA was next used in qPCR experiments. Glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) is a housekeeping gene in mammalian cells, thereby constantly expressed. The magnitude of its transcription has been used for comparing gene expression data [7]. Recently, qPCR reactions using Brilliant II SYBR Green QPCR kit (Agilent Technologies) were prepared. The oligonucleotides: 5′-catcactgccacccagaagactg-3′ and 5′-atgccagtgcgcttcccggttcag-3′ were of forward and reverse primers of *gapdh* gene, respectively. In a 10-µl qPCR reaction, it contained 5 µl of 2x Brilliant II SYBR Green QPCR Master Mix, 2 µl of each primer (1µM), and 1 µl of the cDNA template. The reaction was operated on MIC Real Time PCR Cycler (Labgene scientific SA, Switzerland) by a two-step cycling program: 1 cycle at 95°C, 10 min, followed by 40 cycles at 95°C, 30 sec and 60°C, 60 sec. The corresponding C_q was recorded. The percentage of reverse transcriptase inhibition was calculated by the following formula:

$$\% \text{ Inhibition} = \frac{C_q(\text{test}) - C_q(\text{blank})}{C_q(\text{test})} \times 100$$

Inhibition of 3CLpro Human Rhinovirus

HRV 3C protease inhibitory assay kit was used for screening test samples that specifically inhibit the protease activity. In brief, cleavage of chromogenic peptide substrates by the

protease results in release of pNA that is spectroscopically quantified at 405 nm. However, in the presence of an inhibitor, the cleaving ability is reduced or abolished resulting in decrease or loss of the absorbance. Changes of the absorption were recorded by using a kinetic mode on Varioskan LUX™ Multimode Microplate Reader (Thermo Fisher Scientific).

Table 2: Set-up reaction mixtures for reverse transcriptase inhibitory assay

Sample's name	Volume added in a sequential order (μl)					
	5x VILO Reaction Mix	10x SuperScript Enzyme Mix	Total RNA	AV-SS (Final conc, mM)	ADP-SS (Final conc, mM)	UltraPure water
Blank	4 (All tubes)	2 (All tubes)	10 (All tubes)	-	-	4.0
AV-1				0.5 (0.165)	-	3.5
AV-2				1.0 (0.330)	-	3.0
AV-3				1.5 (0.495)	-	2.5
AV-4				2.0 (0.660)	-	2.0
AV-5				2.5 (0.825)	-	1.5
AV-6				3.0 (0.990)	-	1.0
AV-7				4.0 (1.320)	-	0.0
ADP-1				-	0.5 (0.165)	3.5
ADP-2				-	1.0 (0.330)	3.0
ADP-3				-	1.5 (0.495)	2.5
ADP-4				-	2.0 (0.660)	2.0
ADP-5				-	2.5 (0.825)	1.5
ADP-6				-	3.0 (0.990)	1.0
ADP-7				-	4.0 (1.320)	0.0

Determination of ADP Content

HPLC method described by Limsiriwong et al. was used for determination of ADP content in the extract [8]. The chromatographic conditions were as follows: column C₁₈ (15 cm x 3.9 cm), mobile phase: 50% methanol in water, flow rate: 1 mL/min, detector: UV 224 nm, and injection volume: 20 μl. Calculation of the % ADP was carried out by comparing with the corresponding standard curve.

Statistical Analysis

All *in vitro* experiments were done in triplicate. Results were reported as means±S.D. The difference between results was evaluated by using one-way ANOVA of IBM SPSS Statistics version 23, and *p*-values < 0.05 were considered significantly different.

RESULTS AND DISCUSSION

Computational Methodology

Molecular docking has been used for prediction of binding sites for test compounds on particular targets of enzymes or receptors. In this project, bonding/interaction of ADP towards a specified protein was examined in parallel with various commonly used antiviral drugs. Results of the best predicted conformations were summarized in Table 1. The binding affinity between ADP and reverse transcriptase was comparatively low ($\Delta G_{\text{bind}} < -7$ kcal/mole) and

consequently not shown in Table 1. In comparison with rimantadine, remdesivir, lopinavir, and/or ritonavir, ADP was exhibited to preferentially interact with PLpro (the papain-like protease), 3CLpro (the 3-chymotrypsin-like protease), and ACE-2 receptor-spike protein complex of SARS-CoV-2, as well as M2 protein of influenza A. To our knowledge, these proteases have been responsible for cleavage of viral polyproteins during replication, and serious inflammatory responses as observed in some COVID-19 patients have been due to extreme production of cytokines and be presumptive to mediate the shearing effects of the enzymes as well [9]. Thus, antagonizing the enzymes' activities might lead to replication inhibition of the SARS-CoV-2 virus. Meanwhile, M2 protein has been evident to associate the pathogenicity of influenza A virus. Once inducing it to interact with mitochondrial antiviral signaling proteins, aggregation of the proteins occurs. This has been the cause of mitochondrial deformation, resulting in increased production and release of reactive oxygen species (ROS). The released ROS then activates mitochondrial autophagy, leading to compromising host immune responses and facilitating the virus attach [10]. To this regard, using *A. paniculata* as dried powder or extracts for treating common cold has been possible by counteracting M2 protein of the pathogenic virus. Based on the single-stranded RNA as their genome, it was important in this study to access reverse transcriptase inhibitory activity of the extracted ADP *in vitro* before executing it for relevant preclinical trials.

Reverse Transcriptase Inhibitory Activity

To determine reverse transcriptase (RT) inhibitory effect of the ADP, two-steps qPCR method was set-up. cDNA synthesis by the RT action and amplification of *gapdh* gene by *Taq* polymerase were subsequently monitored using SYBR green dye on MIC Thermal Cycler. Abacavir, an approved RT inhibitor for treating human immunodeficiency virus 1 infection, was a drug as positive control. A concentration range of 0.15-1.5 mmol/L was investigated for both substances. Results were graphed in Figure 1. There was dose dependent effect at the concentrations ≤ 1 mmol/L, but the ADP was more potent than abacavir. The RT inhibition reached maximum at 1 mmol/L for these compounds. At concentrations higher than 1 mmol/L, the RT inhibitory power of the ADP was stabilized, but of abacavir it was decreased.

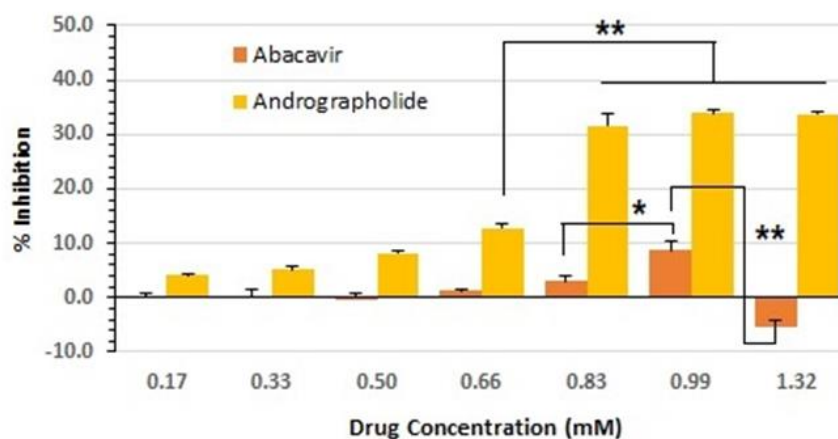


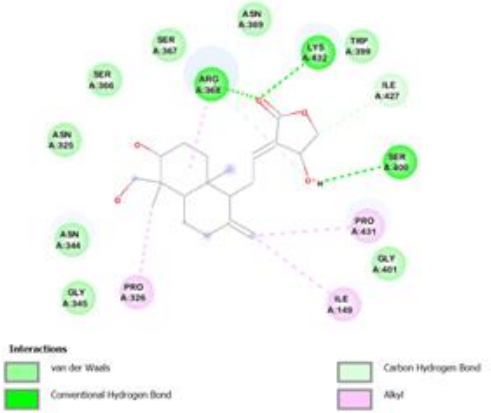
Figure 1: The % inhibition of abacavir and ADP on reverse transcriptase activity as determined by using two-steps qPCR method of *gapdh* gene; * $p < 0.05$, ** $p < 0.01$

Nucleoside- and nonnucleoside-RT inhibitors are two major classes of drugs capable of inhibiting the RT activity. Nucleoside triphosphate is the form to be added into the growing cDNA chain in competition with native nucleotides as functioned by RT. Zidovudine, lamivudine, abacavir, emtricitabine and tenofovir are of the examples and recently used for AIDS treatment. Of the second nonnucleoside-RT inhibitors, their molecules are usually small and hydrophobic such as nevirapine, efavirenz, etravirine and rilpivirine. Bonding between the drug and the enzyme is intrinsically non-specific, resulting in uncertain mechanisms of action. To be clinically successful, combining the drugs from these two different groups has been practiced. For example, tenofovir/emtricitabine/nevirapine combination has been a highly active recipe against retroviruses [11]. Instead of the insignificant ΔG_{bind} for the ADP-RT complexes, ADP was newly classified as a non-nucleoside RT inhibitor accordingly.

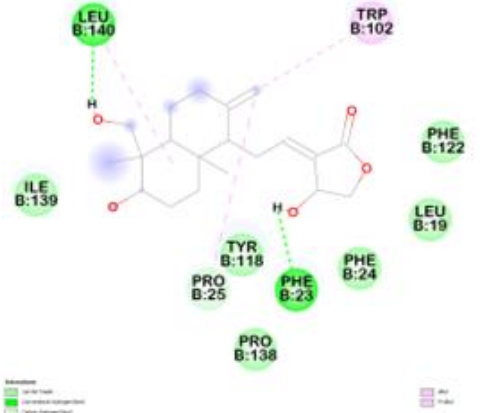
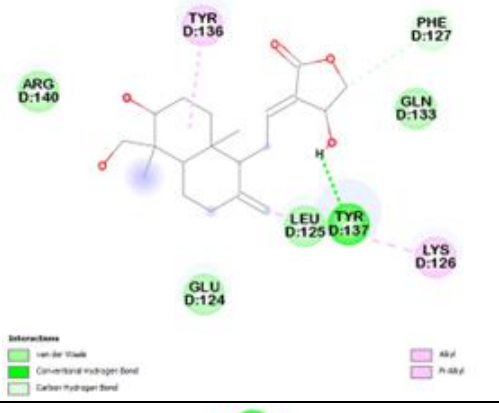
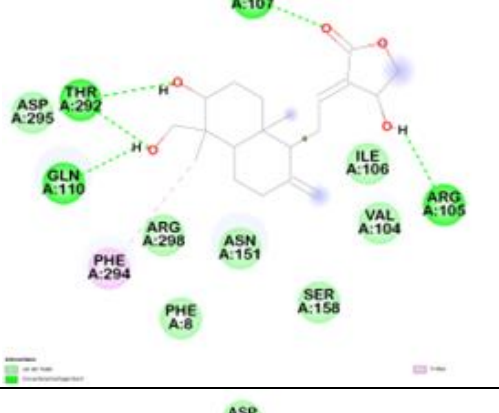
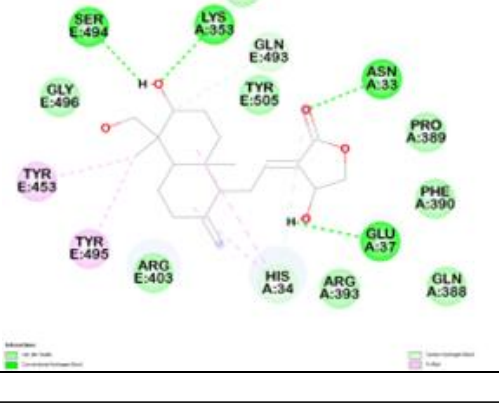
Inhibition of 3CLpro

According to the molecular docking data (Table 1), interactions between ADP and 3CLpro of SARS-CoV-2 were comparatively stronger than those of remdesivir-, lopinavir-, and ritonavir-3CLpro bonding. To date, the 3CLpro is known to antagonize host innate immunity. Meanwhile, spreading of this virus is facilitated by the matured protein [12]. Since *A. paniculata* has been commonly used for cold treatment, this might hint us to find out whether ADP additionally exerts inhibitory effect on human rhinovirus 3CLpro (HRV 3CLpro). To this regard, a kit for screening HRV 3CLpro inhibitory activity was performed *in vitro* by monitoring the changed OD₄₀₅ after colorimetric peptide substrates were cleaved by the enzyme. Identical concentrations at 20, 40, and 80 $\mu\text{mol/L}$ of the ADP, lopinavir, ritonavir, and oseltamivir were investigated. In Figure 2, lopinavir and ritonavir at these three concentrations were not found to inhibit the enzyme activity. But neutralization of the enzyme activity was indicated for oseltamivir and the ADP at 80 $\mu\text{mol/L}$. Inhibition of the 3CLpro by the ADP was dose dependent. Instead, this was dose independent for oseltamivir.

Table 1: Data of binding energy and interactions between ADP/known inhibitors and a viral protein target, evaluated by molecular docking method

	Binding energy (kcal/mol)	Interaction with andrographolide
	Influenza A-neuraminidase H1N1	
Andrographolide	-8.31	
Oseltamivir	-8.12	
Zanamivir	-7.33	
	Influenza A-neuraminidase H3N2	
Andrographolide	-7.61	
Oseltamivir	-6.37	

Zanamivir	-7.02	<p>Interactions</p> <ul style="list-style-type: none"> van der Waals Conventional Hydrogen Bond 25-Allyl
	Influenza A-M2 protein	
Andrographolide	-10.41	<p>Interactions</p> <ul style="list-style-type: none"> van der Waals Conventional Hydrogen Bond 25-Allyl
Rimantadine	-6.87	
	Hepatitis C-nonstructural protein 5B	
Andrographolide	-9.00	<p>Interactions</p> <ul style="list-style-type: none"> van der Waals Conventional Hydrogen Bond 25-Allyl
Nesbuvir	-12.65	
	Hepatitis B-capsid protein	
Andrographolide	-7.29	
Ciclopirox	-6.21	

Sulfamoylbenzamide	-6.06	
Papain-like protease of SARS-CoV-2		
Andrographolide	-7.28	
Remdesivir	-5.88	
Lopinavir	-6.50	
Ritonavir	-5.57	
3-Chymotrypsin-like protease of SARS-CoV-2		
Andrographolide	-8.62	
Remdesivir	-6.09	
Lopinavir	-6.00	
Ritonavir	-5.45	
ACE-2 receptor-spike protein complex of SARS-CoV-2		
Andrographolide	-9.43	
Lisinopril	-6.04	
Lopinavir	-7.20	
Remdesivir	-6.14	
Ritonavir	-6.56	

So far, the results of computational simulation and *in vitro* enzymatic assays concerning RT and 3CLpro inhibitions were consistent. Diverse pharmacological effects of ADP have been reviewed [6]. But perspectives as new antiviral candidate or a lead of ADP were recently discussed. Impediment of viral entry to host cells by ADP might be due to membrane receptors' blockage [12], consisting with the most negative ΔG_{bind} of it on ACE-2 receptor-spike protein complex of SARS-CoV-2 (Table 1). Another essential pharmacological effect of ADP was RT inhibitory activity, because the replication of the entered RNA viruses could be inhibited [13,14]. Moreover, inactivation of protease enzymes responsible for polyprotein processing by ADP might induce the replicated viruses defective or immature, leading to incapability to spread out of the infected cells [15]. Therefore, ADP would be potential as an antiviral drug according to the mechanisms of actions described.

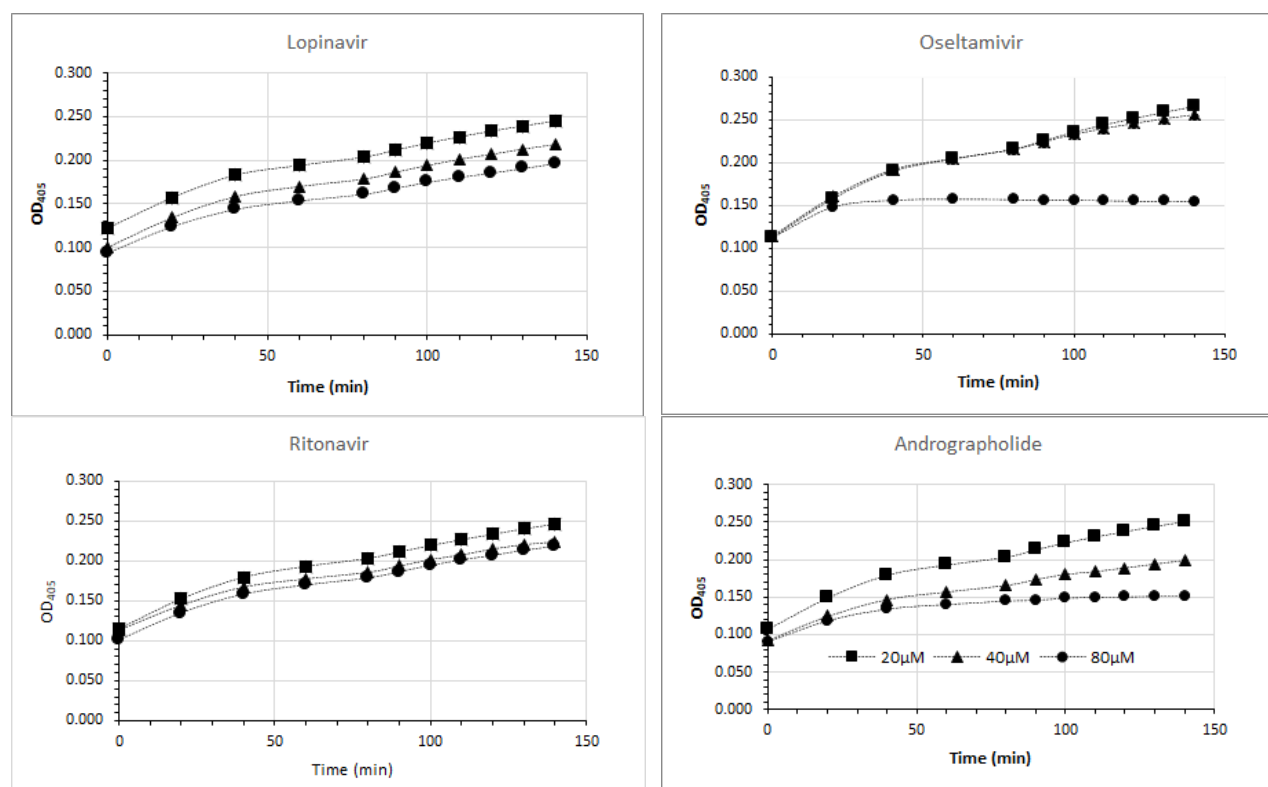


Figure 2: Screening of HRV 3CLpro inhibitory activity of lopinavir, ritonavir, oseltamivir, and the ADP at concentrations of 20, 40 and 80 µmol/L. The cleaved chromogenic peptide substrates were spectrophotometrically monitored at 405 nm for 2 h.

Extraction of ADP from the Planted *A. paniculata*

Recently, *A. paniculata* was cultivated outdoor in newly established hydroponic conditions for 110 days. Photography of the mature plant was shown in Figure 3. A sprout was seeded in a hole of 35x35 cm². The planted plant got maturation in 110 days, obtaining 112±18 g and 55±5 g fresh weight of the airy part and the root, respectively. After drying in the sun for 2 days, the corresponding weights were decreased to 14±3 g and 9±2 g, respectively. The ADP extraction was carried out using 30 g of the finely ground and homogeneously mixed powder and a total 450-mL ethanol with 5 times repeated. The solvent was removed by using a rotary evaporator,

resulting in viscous extract. Amorphous crystals were obtained after cooling the viscous extract at 4-6°C overnight. Thin Layer Chromatography (TLC) was performed for checking the extract's purity in a solvent system containing chloroform, methanol, and ethyl acetate at 8:1.5:1 volume ratio. The TLC result was shown in Figure 4A. HPLC technique was used for determination of ADP content in the extracts. The chromatograms of ADP standard and those from the 1st, the 2nd, or the 4th round extraction were shown in Figure 4B-E. Up to 17±2% yield and 85±2% purity of the ADP was obtained.



Figure 3: The morphology of whole plant (A) and the root (B) of *A. paniculata* cultivated in an outdoor hydroponic system for 110 days

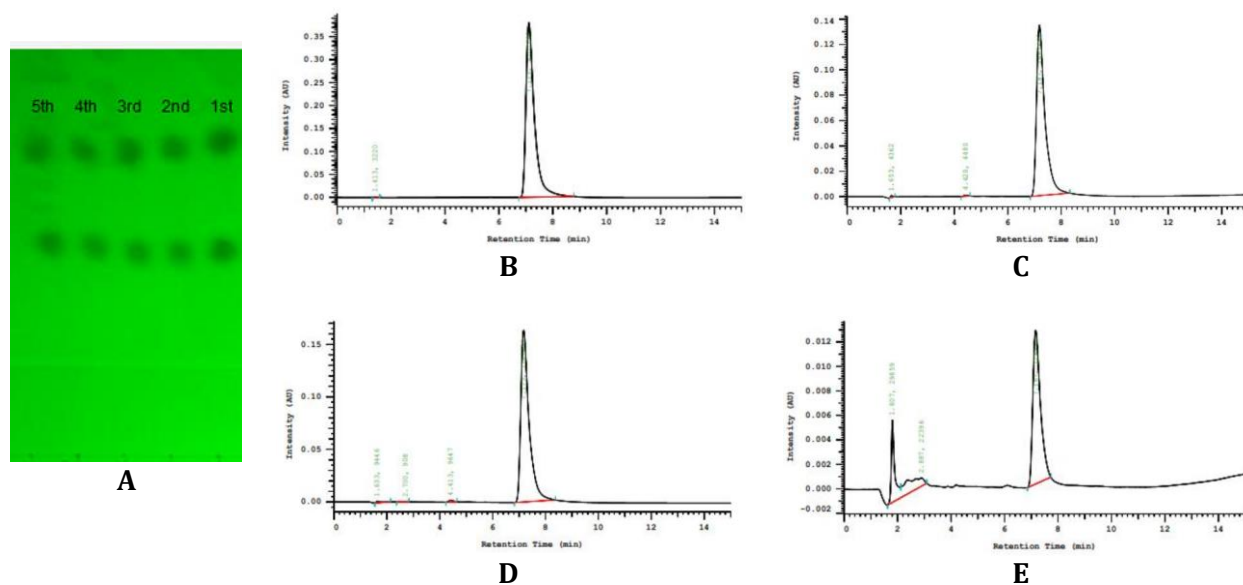


Figure 4: (A), TLC results of the extractant from the 1st, 2nd, 3rd, 4th, or 5th round; HPLC chromatograms of ADP standard (B), the fraction from the 1st (C), the 2nd (D) or the 4th (E) round, respectively

Instead, when a conventional method of seed sowing in a space of 30×15 cm² was applied, an average biomass of 3 Metric Ton per hectare of *A. paniculata* was obtained after 110 days of growth. The number of 222 thousand plants per hectare and of ~13.5 g fresh weight per plant were revealed for this [16]. Thus, greater biomass was comparatively acquired from our

established cropping methodology. The technique of plant tissue culture has been applied for large scale agriculture, indicating that the regenerated plants produce higher amounts of ADP than the mother plants [17]. However, this advanced technique might not be suitable/applicable to poor farmers due to high investment cost. A report has shown high content of ADP in the root, which is secondary to the leaves [18]. The recently developed protocol provided additional advantage because the whole plant could be harvested for ADP extraction, resulting in increased % yield. Methanol, acetone, ether, dichloromethane, and/or chloroform have been organic solvents commonly used for ADP extraction previously [19]. However, to sustain the planet, a pharmaceutical grade ethanol alone was used for ADP extraction recently. The recent consecutive methodology provided the ADP yield of as high as 17% with 85% purity. In contrast, other previous reports have indicated the yield of around 2%. Since 1970, "Health for All" has been a strategy of the World Health Organization (WHO) for promoting health and human dignity, and enhancing the quality of life, the basis of which is primary health care. Afterwards, traditional medicine has been accepted as a game changer on this by its own innovation and sustainability [20]. *A. paniculata* is among various herbs contributed to health promotion. Few years ago, the herb has been of great demand for use in treatment of the pandemic COVID-19 in many countries around the world, and be deposited in the primary health care of Thailand since 2017. Cultivating it in rural areas of the country plays an important role to the poor for their income as well. However, the repeated gardening is a major cause of soil infertility. Thus, growth inferiority as well as reduced/inconsistent metabolisms in production of ADP are assumed. New outdoor hydroponic system for planting *A. paniculata* was recently developed (patented). Solar energy is considerable for the countries located near the equator and is an inexpensive energy source, thereby profitable for opened field agriculture. B-Veggie (our patented bio-control) was additionally supplemented in the circulated water of the hydroponic system in order to promote root sprouting and decrease the water's temperature. The used water could be directly released to environments because it was already treated by the inoculated bio-control. At the meantime, simple, cost-effective, and sustainable protocol for ADP extraction was established for obtaining high % yield and purity of the extracted ADP (patented). These consecutive protocols could be applied for rural farmers. It was hopeful for improving household economy and quality of life.

CONCLUSION

Among medicinal plants, *A. paniculata* is vastly utilized for health promotion by eradicating a range of symptoms as cold/fever and liver/cardiovascular diseases. Such uses are based on knowledges that pass down from generation to generation. An intimate knowledge was obtained from the recent study. Simple and efficient methodologies associating *A. paniculata* growth and ADP isolation were introduced. The engaged techniques might be transferred to poor farmers in rural areas. In using these, large amount and high quality of the plant material was obtained, leading to increasing their incomes. Moreover, high productivity and purity of the extracted ADP was advantageous for pharmaceutical manufacturing. Computational simulation was carried out to evaluate interactions between ADP and a protein/enzyme of particular viruses. Some attractive interactions were further investigated for designing new drug/lead as antiviral. RT and 3CLpro were of two most relevant enzymes bonded by ADP that were re-investigated *in vitro* by using specific commercial kits. The computational results and

those of wet-lab analysis were consistent. However, actual benefits to a range of population of ADP would be acquired from pre-clinical and clinical trials that are awaited.

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Author Contributions

All the authors have accepted responsibility for the entire content of this manuscript and approved submission. Jasadee Kaewsrichan and Paweena Wongwitwichot designed and performed research, Ashara Pengnoo contributed new reagents or analytic tools, Jasadee Kaewsrichan and Varomyalin Tipmanee analyzed data, and Jasadee Kaewsrichan wrote the paper.

Competing Interests

The authors have no conflict of interests to declare.

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