Advances in Biological Sciences Research, volume 22
7th International Conference on Biological Science (ICBS 2021)

Potential of *Dendrobium* spp. Secondary Metabolites as Medicinal Source for SARS-CoV-2

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ABSTRACT
Coronaviruses have long been a severe issue due to their capacity to mutate and infect the respiratory tract. *Dendrobium* orchids are the most diverse orchid species in Indonesia. Polysaccharides, bibenzyl, phenanthrene, coumarin, sesquiterpenoids, alkaloids, and steroids were among the phytochemical substances discovered in *Dendrobium spp.* On a group of alkaloids, flavonoid, and terpenoid, Dendrobium's anti-coronavirus action has been thoroughly researched *in silico*, *in vitro*, and *in vivo*. Secondary metabolites were evaluated on methanolic extracts of *Dendrobium phalaenopsis* and *Dendrobium lineale* leaves in this study. In a computer simulation, antiviral activity against SARS-CoV-2 was also discovered. Thin-layer chromatography (TLC) was used to determine the concentration of alkaloids, flavonoids, and terpenoids in dried leaf extracts of *D. phalaenopsis* and *D. lineale*. The online Covid-19 Docking Server (https://ncov.schanglab.org.cn/index.php) was used as an autodocking molecular docking tool to find potential secondary metabolites of *Dendrobium* spp. as SARS-CoV-2 antiviral. Screening of the leaves of *Dendrobium phalaenopsis* and *Dendrobium lineale* using methanolic extracts revealed positive results for alkaloid, flavonoid, and terpenoid content. According to the results of molecular docking, Medioresinol, (+)-Lirioresinol B, Acanthoside B, Dendrocandin B, Denthyrsinone, and Dendroside D had the lowest affinity energy.

Keywords: *Dendrobium, In silico COVID 19, Secondary metabolite.*

1. INTRODUCTION

The phytochemical content of the *Dendrobium* is known to have various roles as raw materials for Traditional Chinese Medicine (TCM). *Dendrobium* is known as an anticancer, antioxidant, and anti-inflammatory [1]. One of the potentials of secondary metabolites is the activity as antiviral. Various phytochemical compounds were found in *Dendrobium* spp. including polysaccharides, bibenzyl, phenanthrene, coumarin, sesquiterpenoids, alkaloids, and steroids [2]. Alkaloids, flavonoids, and terpenoids have been extracted from *Dendrobium anosmum-gigantea* through thin layer chromatography [3]. Johnson and Janakariman (2013) found alkaloids, flavonoids, and triterpenoids in *Dendrobium panduratum* leaf and stem extracts [4].

Terpenoids can act as antitumors, anti-inflammatory, antiviral, and other biological activities [5]. Alkaloids are a diverse collection of chemicals that have antibacterial properties by disrupting DNA and RNA polymerase enzymes, preventing DNA and RNA production. Alkaloids also inhibit cell wall formation by interfering with the formation of peptidoglycan which causes cells to lyse [6], on animal and human organisms, there are both unpleasant and positive effects. Alkaloids from many plant species have anti-inflammatory, chemotherapeutic, analgesics, local anesthetic and pain relief, neuropharmacologic, antiparasitic, antibacterial, antispasmodic, anticorrosive, antiapoptotic, anti-HIV, and insecticidal effects [7]. Flavonoids are reported to be able to act as antioxidants, antibacterial, anti-inflammatory, antiangiogenic, anti-mutagenic, and anti-cancer [8].

Coronaviruses have long been known to cause severe infections and other health problems. One of the global issues brought on by coronavirus is the Covid-19 pandemic. SARS-CoV-2 was first detected in late 2019 in Wuhan, China, and has since spread throughout the world, resulting in a pandemic with 222.7 million confirmed cases and 4.6 million deaths as of September 8, 2021. There haven't been any medications identified yet that can stop SARS-CoV-2 from growing in host cells.

Along with advances in research technology, many secondary metabolites were found to provide effective antiviral activity. Anti-coronavirus activity in *Dendrobium* has been extensively studied through *in silico*, *in vivo* and *in vitro* analysis. Recent studies also found out that secondary metabolite on *Dendrobium* can be used as antiviral therapeutic against coronavirus.

In this study, secondary metabolites were screened on the methanolic extracts of the leaves of *Dendrobium phalaenopsis* and *Dendrobium lineale. Dendrobium phalaenopsis* and *Dendrobium lineale* were chosen because until now there has been no further study on the types of secondary metabolites in these two orchids. The secondary metabolite *D. anosmum* was used as a ligand for molecular docking to detect the antiviral activity of SARS-CoV-2 *in silico*. The activity of *D. panduratum* secondary metabolites against the SARS-CoV-2 virus RdRp target protein was determined using *in silico* analysis..

RNA-dependent RNA polymerase is an enzyme found in RNA viruses that plays a key role in the life cycle of viruses such as coronaviruses (CoVs) [9]. Antiviral agents that inhibit RdRp are classified as a necessary group of nucleotide and nucleoside analogs. The RdRp site is a conserved region of the SARS-CoV-2 protein that is widely used as a target for molecular docking [10]. It plays an important role in viral replication and is widely used as a target for molecular docking. Ribosomes bound to the endoplasmic reticulum (ER) translate the structural proteins S1, S2, envelope, and membrane, which are used as preparations for viron assembly. The nucleocapsid (N) is made up of genomic RNA and is found in the cytoplasm. After that, it combines with virion precursors, which are subsequently transferred from the ER to the cell surface via tiny vesicles via the Golgi Apparatus. [11]. RdRp enzyme was chosen to be used as a docking target to determine whether the metabolite compound could inhibit enzyme activity in replication so that the virus could not reproduce.

In silico study like molecular docking would predict the interactions between RdRp and candidate of antiviral agents. The *in silico* study on drug discovery makes this research more goal-oriented and save the test resources [12]. In this study, data on the antiviral activity of secondary metabolites will also be collected as further evidence that *Dendrobium* has potential as an anti-coronavirus.

2. METHODS

2.1. Dendrobium spp. Leaf Extraction. and Secondary Metabolite Screening

The dry leaves of *D. phalaenopsis* and *D.lineale* were extracted by maceration method. The leaves were crushed to form simplicia, and dissolved in methanol at the ratio of 1 gram of simplicia to 10 ml of methanol. The extract was shaken for 24 hours, the extract was filtered twice with filter paper, and evaporated for 3 days. Thin-layer chromatography was used to examine secondary metabolites in dried leaf extracts of D. phalaenopsis and D. lineale (TLC). The stationary phase in this technique is a Silica Gel 60 F254 plate, and the mobile phases are n-hexane and ethyl acetate (7:3). In this TLC test, the content of secondary metabolites of alkaloids, flavonoids, and terpenoids was tested. In the alkaloid test, Dragendroff's stain-seeking reagent is used, if there are alkaloids it will be marked with an orange-brown color. In the flavonoid test, the citroborate stain-seeking reagent is used, if there are flavonoids it will be marked with a yellow color. In the terpenoid test, a vanillin sulfate staining reagent is used, if there are terpenoids it will be marked with a purple color.

2.2. Potential Secondary Metabolites of Dendrobium spp. As an Antivirus SARS-CoV-2

Potential secondary metabolites of *Dendrobium* spp., such as antiviral SARS-CoV-2, were identified by *in silico* research. In August 2021, an *in silico* analysis was performed by obtaining the phytochemical compounds of the genus Dendrobium spp. using the KNApSAcK: Metabolite Ecology Distribution website. The 3-dimensional structure of the phytochemical *Dendrobium* spp. was downloaded in .sdf format on the PubChem page. Online Covid-19 Docking Server as an autodock molecular docking tool developed by Kong *et al.* (2020) https://ncov.schanglab.org.cn/index.php. RdRp RNA site was used at coordinates x = 10.85; y =12.58; z = 68.72 (r = 12). Native ligands as positive controls for target proteins reference were sought in relevant journals. RNM native ligand compound was obtained (.smi) at https://pubchem.ncbi.nlm.nih.gov/.

Docking secondary metabolite with the lowest affinity energy will be analyzed with Software Discovery Studio 2020 to observe interactions and determine residual contact occurs. Compound visualization was performed with Discovery Studio 2020 software in the Receptor-ligand interaction option. The profiles of the ligands used in this study were accessed from http://www.swissadme.ch/ index.php

using Lipinski's five rules, which included molecular weight, LogP (lipophilicity), number of hydrogen bond donors, number of hydrogen bond acceptors, and violation, along with the SMILES of each ligand obtained from the PubChem page.

3. RESULT AND DISCUSSION

Based on secondary metabolite screening in the methanolic extracts of the leaves of *Dendrobium phalaenopsis* and *Dendrobium lineale* showed positive results on the Alkaloid, Flavonoid, and Terpenoid test using the TLC method (Table 1 & 2) Dragendroff for the alkaloid group is marked with a brownish orange color, citroborate for the flavonoid group is marked in yellow, and vanillin sulfate for the terpenoid group is marked in purple. The TLC results also obtained different Rf values for each compound. Based on the results of molecular docking binding affinity value, from 128 secondary metabolite on *Dendrobium,* there were only 6 compounds with the lowest affinity energy than NRM. Medioresinol and (+)-Lirioresinol B is a compound from the lignan group. Acanthoside B is a compound from the protoalkaloid group. Dendrocandin B is a class of bibenzyl-dihydrophenanthrene compounds. Denthyr- sinone is a new class of phenanthrene-phenanthraquine compounds. Dendroside D is a sesquiterpene guaiane group compound.

Based on Figure 1, visualization of the ligand-protein interactions of the six secondary metabolites showed that there were only four strongest metabolite modes that interacted with RNA-dependent RNA-polymerase (RdRp). The strongest metabolite mode was viewed from its low binding affinity and its interaction with key amino acids. According to Table 4, the metabolite Medioresinol has the highest chance of binding to RdRp, with a binding affinity of -8 kcal/mol.

3.1. Dendrobium spp. Leaf Extraction and Secondary Metabolite Screening

Extracts of the leaves of *Dendrobium phalaenopsis* and *Dendrobium lineale* showed positive results on the alkaloid, flavonoid, and terpenoid. The Rf values for each compound are shown in the TLC results. The Rf value is obtained by dividing the compound's distance covered by the distance traveled by the solvent from the lower limit of the silica plate. The Rf value is influenced by the adsorbent and eluent composition [13].

Factors that affect the Rf value include the mobile phase, adsorbent properties, plate thickness, the chamber used, temperature, sample mass, and chromatographic technique used. The larger Rf value indicates that the polarity of the compound group is different from the polarity of the stationary phase. Nonpolar compounds are attracted to nonpolar solvents and are not held back by the stationary phase, which is

Table 1. Screening results of secondary metabolites of *Dendrobium phalaenopsis* leaf methanolic extract

Compound Group	Visible Light	UV Light	*Rf*	Stain Detection Reagent	Stain Color	Presence of Compounds
Alkaloids	Light Green	Green	0,03	Dragendroff	Brown	+
Flavonoids	Greenish Yellow	Yellow	0,49	Citroborate	Yellow	+
Terpenoids	Purple	Purple	0,64 0,75	Vanilline Sulfate	Purplish Blue	+

Table 2. Screening results of secondary metabolites of *Dendrobium lineale* leaf methanolic extract

Compound Group	Visible Light	UV Light	*Rf*	Stain Detection Reagent	Stain Color	Presence of Compounds
Alkaloids	Light Green	Green	0,04	Dragendroff	Brown	+
Flavonoids	Greenish Yellow	Yellow	0,26	Citroborate	Yellow	+
Terpenoids	Purple	Purple	0,71 0,94	Vanilline Sulfate	Purplish Blue	+

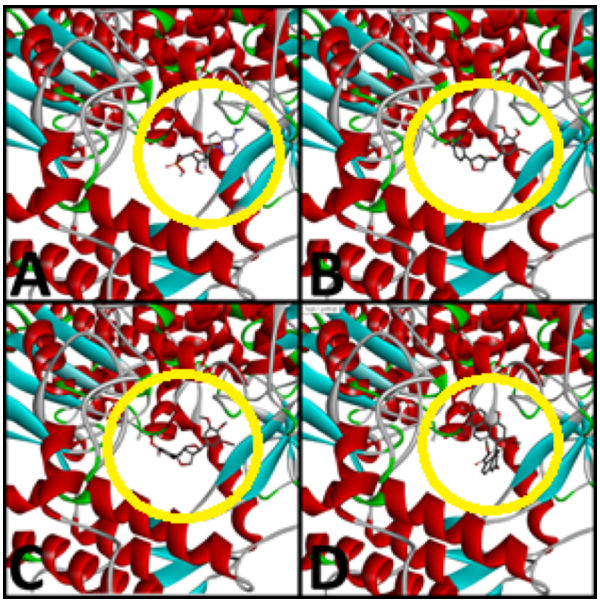

Figure 1 Docking analysis visualization of RdRp binding with the strongest mode of secondary metabolites. (A)RNM (native ligand) (B) Medioresinol (C) Liriosenol (D) Denthyrsinone

polar. Methanol is used for extraction because it is a good solvent for all primary and secondary metabolites because it penetrates well into the cell content, so it is the most commonly used solvent unless you want to extract specific compounds [14].

Potential of alkaloid, flavonoid and terpenoid as antivirus have been already studied. Flavonoid are a phenolic derivative which have aromatic link. Terpenoid is a natural product which structure is composed of several isoprene units. Alkaloid is a natural product which the structure have nitrogen atoms that are generally in a cyclic system combination.

Dendrobine, which is discovered as a group of alkaloids in the orchid *Dendrobium nobile*, has antiviral efficacy against influenza A viruses such as A/FM-1/1/47 (H1N1), A/Puerto Rico/8/34 H274Y (H1N1), and A/Aichi/2/68 (H3N2). Dendrobine's low IC50 value suggests that it could be used as a possible anti-coronavirus agent [15]. According to Fielding et al. (2020), alkaloid can modify host factors to produce antiviral imunomodulators, as well as decrease SARS-CoV-2 replication and have cytophatic effects *in vitro* stages [16].

Chiow *et al*. (2015) tested flavonoids in the form of quercetin, quercitrin and rutin on *Houttuynia cordata* Thunb. plants, these flavonoids have antiviral action against the influenza virus, the herpes simplex virus-1, and human immunodeficiency virus-1 *in vitro* [17]. Alzaabi et al. (2001) also discussed the potential of flavonoids as antiviral drugs against the SARS-CoV-2 virus [18]. Naringenin as a secondary metabolite of flavonoid which is also found in *Dendrobium* has been investigated to have potential against M^{pro} in SARS-CoV-2 through molecular docking [19].

Brezani et al. (2018) extracted 12 pure compounds from Eucalyptus globulus leaves and branches and investigated their anti-herpetic effectiveness against replication of HSV-1 and HSV 2 type antigens. The findings revealed that tereticornate A (triterpenoid) molecules had the highest anti-HSV-1 action, even surpassing acyclovir, the standard antiviral medication [20]. Brezáni *et al.* (2018) also explained about the mechanism of action bioactive terpenes and their derivates against SARS-CoV-2, some of terpenes can be inhibit the isomerization of coronavirus protease and induced apoptosis [20].

3.2. In silico Prediction of Potential Secondary Metabolites from Dendrobium spp. as SARS-CoV-2 Antivirus

In silico prediction is one of the research projects that has been developed to provide accurate data before *in vitro* and *in vivo* examinations. *Dendrobium*'s potential as an antiviral against SARS-CoV-2 was investigated using *in silico* methods in this research.. One mechanism of secondary metabolite activity is by inhibiting the active site of viral enzymes. The RNA-dependent RNA polymerase (RdRp) enzyme is involved in structural protein RNA replication. The RdRp RNA site is a protected area for the SARS-CoV-2 protein, which is commonly exploited as a molecular docking target and plays a important role in viral replication. Ribosomes coupled to the endoplasmic reticulum (ER) translate the structural proteins S1, S2, Envelope, and Membrane, which are employed as virion assembly preparations. The nucleocapsid (N) is made up of genomic RNA and is found in the cytoplasm. After that, it combines with virion precursors, which are then transported from the ER to the cell surface via tiny vesicles via the golgi apparatus. [21].

The selection of ligands as drug candidate molecules must also follow Lipinski's five guidelines, in addition to referring to the database connected to the selection of secondary metabolites/ligands of *Dendrobium* orchids. The results of the evaluation of the ligands showed that from the 6 compounds tested in this study were accepted by Lipinski's five rules except Dendroside D. By knowing that Dendroside D had Lipinski's violation = 3, then Dendroside D was suspected to be unsuitable as raw material for making medicine (undraggable). Meanwhile, all ligands other than Dendroside D have a violation value of 0 so that these compounds can temporarily be used as medicine candidates (druggable) and used as ligands in the docking process.

Table 3. List of Molecular *Docking* Energy Affinity

Group	Metabolite name	Binding Affinity Kcal/mol	*Rf*
Lignin	Medioresinol	-8	5.62
Lignin	(+)-Lirioresinol B	-8	5.70
Alkaloid	Acanthoside B	-8	5.98
Bibenzyl	Dendrocandin B	-8.6	6.50
phenanthrene	Denthyrsinone	-8.2	6.52
Sesquiterpenes (Terpenoid)	Dendroside D	-8	5.51
Native Ligand	RNM	-7	5.46

Table 4. Amino acid binding pocket on the strongest mode of *Dendrobium* secondary metabolite with RdRp

Metabolite	Bind. Aff	Amino acid binding pocket*
NRM	-7	**ALA558 ARG553 ARG624 ASP623 THR556 THR680** TYR456
Medioresinol	-8	**ALA558 ARG553 ARG624 ASP623** MET542 **THR556 THR680** TYR619
Liriosenol	-8	**ALA558 ARG553 ARG624 ASP623** CYS622 LYS621 MET542 TYR619
Denthyrsinone	-8,2	**THR556** ARG555 SER682 **ARG624 ASP623** ASP760 LYS545

*the amino acid in bold is the key of interaction of RdRp

Medioresinol has a molecular weight of 388.41 Da, a log P of 3.29, and H-bond donors 2 and acceptors 2 and 3. 7. The molecular weight of lirioresinol is 418.44 Da, with a LogP of 3.52, H-bond donor 2 and H-bond acceptor 8. Denthyrsinone has a molecular weight of 522.50 Da, a Log P of 2.41, and H-bond donors 3 and 8. Because the ligand does not violate more than two of Lipinski's five rules, these compounds can be employed as therapeutic candidates in the short term (druggable).

According to Schrödinger (2010), the affinity energy value <-10 represents a good bond so that it will show the best results during laboratory experiments, while values of -9 to -8 are quite good and show good results during laboratory experiments (Table 3.). The affinity energy value is determined by atoms conformation between molecule of ligand and target. There are various conformation-dependent scoring function in autodock vina which is used to various interaction [22].

The interactions formed by Denthrysinone and RdRp are hydrogen bonds, electrostatic interactions, and hydrophobicity with amino acids ALA558, ARG553, ARG624, ASP623, THR556, THR680 (Table 4.). Hydrogen bonds and hydrophobic interaction have a big impact to scoring of affinity energy because they have a big scoring coefficient [23]. Interaction between ligand and target in key amino acid in target would affect the role of target metabolism [24]. The SARS-CoV-2 RdRp's performance is expected to be inhibited by the interaction between the ligand and the protein receptor.

In summary, using the TLC screening method, it can be proven that the methanol extracts of the leaves of *D. phalaenopsis* and *D. lineale* are positive for alkaloids, flavonoids, and terpenoids. Dendrocandin B, Dendroside D, Medioresinol, and Denthyrsinone had the highest possibility of binding to RdRp in the *in silico* analysis and have a strong potential for usage as a treatment drug for the SARS-CoV-2 virus. Medioresinol, Liriosenol, and Denthyrsinone create the most stable bond, which gives this compound phytochemical action to block viral replication in the RdRp RNA section of the SARS-CoV-2 site. More research is needed to determine the toxicity, bioavailability, and practicality of the chemical.

AUTHORS' CONTRIBUTIONS

MR as the first author conducted screening, writing full paper and editing, TR doing screening, writing and editing full paper, AK doing molecular docking, writing

full paper, reviewing, AEH doing visualization, writing full paper, ES as the Principle Investigator of the KDM Research Grant providing direction in conducting research and finalizing the manuscript.

ACKNOWLEDGMENTS

We would like to express our sincere thanks to Lisna Hidayati, Hiqmah Yusi Yana, Abby Crisma Dentama, and Eria Rosanti for help in TLC, Lipinski rules, and sampling orchid. Lastly, we would also like to thanks to Student-Lecturer Collaboration (KDM) Research Grant 2021 from The Faculty of Biology UGM.

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