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Original Research Article

Java plum (*Syzygium Cumini*) leaves as a potential inhibitor of SARS-CoV-2 3-Chymotrypsine-Like Protease (3CLpro)

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ABSTRACT

The 3-Chymotrypsine-Like Protease (3CLpro) is an enzyme that has a major function in the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) life cycle. It can be utilized as a protein target in search for new medications. Herbal plants are expected to have major contributions to the prevention and treatment of the 2019 Corona Virus Disease (COVID-19), as many herbal plants might have a strong affinity for 3CLpro in the treatment of COVID-19. The purpose of this study is to examine the inhibitory activity, pytochemical components, and antioxidant of extracts from nine herbal plants extracted by the Ultrasound Assisted Extraction (UAE) method that have the potential to inhibit recombinant SARS-CoV-2 3CLpro using an *in vitro* method as a result, it can be used to find novel medication candidates for COVID-19 therapy. Determining antioxidant activity using DPPH and ABTS method, total polyphenol and total flavonoid content using Folin Ciocalteu and quercetin method. From nine herbal plants tested, Java Plum leaves (*Syzygium cumini)* had the most active inhibitory activity with an inhibitory value of 3CLpro (IC_{50} = 226 µg/ml) with a total polyphenol 413±1.83 mg GAE/g extract and total flavonoid as 12.091±0.037 mg QE/g extract. Measurement of antioxidant activity with the DPPH obtained an IC_{50} value of 3.751 ± 0.0149 µg/ml, and with an ABTS gives IC_{50} value 4.43±0.06 µg/ml. This suggests that Java Plum leaves could be a potential source of anti-COVID.

***Keywords*:** SARS-CoV-2, 3CLpro, Indonesia herbal, *in vitro*, Inhibition assay

Introduction

The SARS-CoV-2 epidemic has had a significant negative impact on world health, particularly Indonesia. Corona Virus Disease (COVID-19) cases are steadily declining, which means that the disease will eventually spread globally as an endemic phase.[1] However, COVID-19 infection remains a risk for some of those who are vulnerable. Therefore, anti-COVID-19 alternative drugs are still needed even when this disease becomes endemic. Thus, research to identify new antiviral drugs, especially from natural biodiversity sources, remains important and relevant. To find antiviral drugs, usually researchers use viral enzymes which are used as target proteins. In SARS-CoV-2, there are 3-Chymotrypsine-Like Protease (3CL protease) and Papain like protease (PL protease) which play important roles in the formation of new virus particles. Therefore, preventing these viral proteases from doing their job will directly stop the virus from replicating.[2] The key protein target in drug screening is 3CL protease, also known as the primary protease in SARS-CoV-2, which cleaves proteins at 11 different locations to create a range of non-structural proteins.[2] 3CLpro is also necessary for proteolytic processing of polyproteins which causes maturation of viruses, and crucial for effective viral replication[3,4].This makes 3CLpro a potential target for inhibitor screening or as a drug target for SARS-CoV-2.[5]

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Even though drugs and vaccinations have been developed, research and development of alternative treatments continues, bearing in mind that Indonesia is rich in herbal plants that have potential to serve as antivirals with the same target for Paxlovid that has been suggested by the FDA[6]. Phytochemicals found in herbs can help in the treatment and prevention of SARS-CoV-2 by inhibiting the enzyme 3CLpro enzyme. The screening of active compounds as potential Covid-19 candidates has been the subject of numerous studies recently[4,7–9]. This study was carried out computationally by pairing target proteins such as the SARS-CoV-2 protease with ligands derived from active compounds from herbs and looking at the inhibitory power of the ligands on their targets. However, the above research[4,6,7,9–11] is only *in silico* research. Although it is highly useful for preliminary screening, in vitro bioassay proof is still required. Several *in silico* studies have been conducted on herbal plants in Indonesia which show a high affinity for 3CLpro[11] but no further *in vitro* tests have been carried out. The aim of this study is to search for effective medicinal plants that can reduce the activity of 3CLpro, because inhibition of 3CLpro can halt infection at the early stage of virus replication. This study investigates several natural compound constituents as potential compounds that can target 3CLpro, include flavonoids, phenolics, polyphenols, triterpenes, alkaloids, and glucosinates.[12,13]

The present study focused on nine types of herbal plants that expected to inhibit SARS-CoV-3CLpro enzyme activity including Meniran (*Phyllanthus niruri L*), Tempuyung (*Sonchus arvensis L*), Telang Flower (*Clitoria terntea*), Secang Wood (*Caesalpinia sappan*), Bay leaves (*Syzygium polyanthum*), Guava leaves (*Psidium guajava*), Star Fruit leaves (*Averrhoa carambola L.*), Green Chiretta (*Andrographis paniculata*) and Java Plum leaves (*Syzygium cumini*). Figure 1 shows the hypothesis mechanism pathways of herbal plants that may affect the 3CLpro enzyme. Obtaining the most potential plant results from *in vitro* testing is the first approach in searching for natural compounds as antivirals and as a basis for research to develop plant extracts as prophylaxis for COVID-19 therapy and provide a trusted source of information for the public about effective herbal plants for the treatment

of SARS-CoV-2.[14]The chosen extraction method is Ultrasound Assisted Extraction (UAE), which has the advantages of being safe, economical, requiring little solvent and energy.

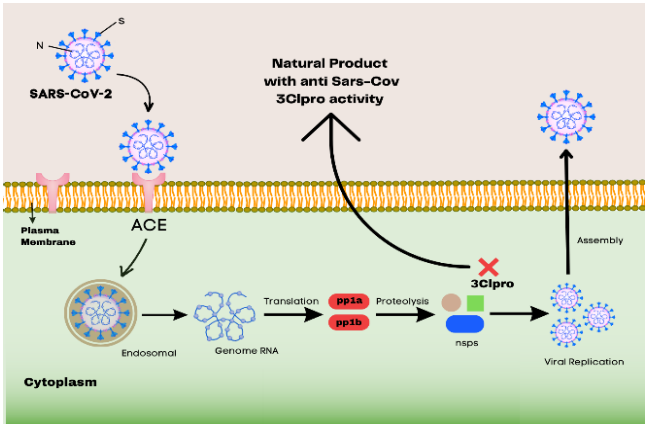

Figure 1: Herbal with anti SARS-CoV-2 3CLpro Activity

Material and Methods

Plants material

The plant material used in this study consisted of nine types of plants was collected on May 2022, namely Secang Wood simplicia (*Caesalpinia sappan*) and Green chiretta (*Andrographis paniculata*) collected from karanganyar-Surakarta, Central Java, Indonesia, Meniran herb (*Phyllanthus niruri* L), Tempuyung herb (*Sonchus arvensis L*), Telang Flower (*Clitoria terntea*), Bay leaf simplicia (*Syzygium polyanthum*), Guava leaf simplicia (*Psidium guajava*), Starfruit leaf simplicia (*Averrhoa carambola L.*) collected from Lampung, Sumatra, Indonesia and Java Plum leaf (*Syzygium cumini*) were obtained from LIPI , West Java, Indonesia. Sample *Caesalpinia sappan, Andrographis paniculate* and *Syzygium cumini* which has been used in previous research was collected on aunthenticated by the Indonesian Institue of Sciences (voucher number: B-809/IPH.3/KS/VII/2020).[15]

Chemicals

The chemicals used in the present study included 3CLpro of SARS-CoV-2 was produced and purified by a research group in Research Center of Applied Microbiology, the National Agency of Research and Innovation (BRIN) Indonesia[16] fluorogenic peptide substrate, LGSAVLQ-Rhodamine 110 substrate (bio techne R&D systems, USA), aluminium chloride ($AlCl_3$), dimethyl solfoxide, folin-ciocalteu, methanol p.a, sodium acetate, etyl acetate, n-Hexan, sodium chloride, sodium hydroxide (Merck, Germany), EDTA (Ethylen Diamine Tetra Acetic Acid) (Biomatics), MiliQ water, HCl, 1,1-Diphenyl-2-picrylhydrazyl (DPPH) (TCI, Japan), ethanol, aquadest (Brataco Chemika, Indonesia), ABTS [2,2'-Azinobis(3 Ethylbenzothiazoline- 6-Sulphonic Acid)], kalium persulfate ($K_2S_2O_8$) ,Tris (Thermo Scientific, USA). The reaction buffer is composed of 20 mM Tris-HCl (pH 7.3),1 mM EDTA, 100nM NaCl, 0.01% BSA (bovine serum albumin), and 1 nM 1, 4-dithiol-D, L-threitol (DTT) (Thermo scientific, Canada)[17] and GC376.[17,18]

Recombinant 3CLpro has functioned well as an active protease by demonstrating its activity against LGSAVLQ Rhodamine 110 substrate.[16,20]

Extraction

Dried plant powder was prepared by weighing 20 g of each plant material followed by the addition of 200 ml of ethanol 70% (1:10 w/v) ratio. Extraction was perfomed using UAE methode at a frequency of 40kHz and a temperature of 40°C for 30 min. The extraction was done three times with a (1:5w/v) solvent ratio. The filtrate from the nine plants was separated using filter paper to obtain the supernatant, which was then evaporated using a rotary evaporator and a water bath to produce a thick extract. The resulting extract was collected and placed in a glass bottle for further testing.

SARS-CoV-2 3CLpro enzyme inhibition assay

Buffer solution was prepared using 100 mM NaCl, 1mM EDTA, 1 mM DTT, and 0.01% BSA[17]and then diluted with MiliQ water up to 50 mL with the addition of TRIS-HCl 20 mM (2.42 g/L) and measured to a pH of 7.3. To obtain 3CLpro 50 nM, the enzyme was diluted with the buffer. Initial stock LGSAVLQ-rhodamine 110-dp substrate concentration of 4mM, which was diluted with assay buffer to a concentration of 20 µM. In order to prepare the extract samples for testing, the extract was dissolved in 50 µl of 5% DMSO and then set to 1 ml in microtubes at a concentration of 1000 g/ml. (final concentration in wells was 250 µg/ml in 348-well plates).

Each 10 µL assay buffer (pH 7.3), 10 µL extract solution (250 µg/ml in well), 10 µL substrate solution, LGSAVLQ-rhodamine 110-dp substrate (4mM), and 10µL 3CLpro enzyme solution 50nM put into the 384 holes well incubated at 37°C. The positive control used GC376 which is a peptidomimetic compound that was reported to be active in inhibiting SARSCoV-2 3CLpro.[19] Each test was carried out 4 times. The Varioskan LUX Multimode Microplate was used to measure the relative fluorescence units (RFU) value at 37°C for 2 hours with an excitation wavelength of 485 nM and an emission wavelength of 535 nM.

Sample blanks were prepared by pipetting 10 µL assay buffer (pH 7.3), substrate LGSAVLQ-rhodamine 110-dp and extract in the absence of enzymes. Sample was prepared by pipetting 30 µl Assay buffer (pH 7.3) and 10 µl LGSAVLQ-rhodamine 110-dp (4mM) Substrate solution, put in 384 well plate. The blank sample was prepared without the addition of 3CLpro enzyme. Blanks controls were prepared by pipetting 20 µl Assay Buffer (pH 7.3), 10 µl 3CLpro enzyme and 10 µl LGSAVLQ-rhodamine 110-dp substrate. This test uses a quenched fluorogenic peptide substrate because this method has been successfully used to measure the activity of the SARS-CoV 3CLpro protease.[12,14,17]

Fluorescence can be used to detect 3Clpro activity based on energy transfer in the substrate. Where the protease cleaves the bond between the fluorophore and the peptide group. A high fluorescence value indicates high protease activity in cleaving the substrate (Rhodamine 110). As a result, the presence of an inhibitor reduces the activity of the protease (enzyme) in cleaving the substrate (Figure 2). Therefore, the intensity of fluorescence signal is proportional to the protease activity.

Total phenolic content

The Folin-Ciocalteu reagent was used to test for total polyphenols, as recommended by the Indonesian Herbal Pharmacopoeia.[21] In short, a vial containing 10 mg ethanol extract was mixed with 10 ml of methanol p.a and stirred until homogeneous with sonication. Then, 1 ml of each series of gallic acid was added to the vials, followed by the addition of 5 ml of folin cicalteu lp solution (7.5% in water). The mixture was incubated for 8 min and then 4 ml of 1% NaOH is added. The mixture was further incubated for 1 hour at 27°C. Various concentrations of gallic acid were prepared as a standard. Absorbance measurements of each solution were taken at the absorption wavelength of gallic acid = 671 nM using a UV-Vis spectrophotometer.

$$\text{Total phenolic content} = \frac{GAE\ X\ V\ X\ D}{W}$$

Gallic Acid Equivalent (mg/L) (GAE) can be calculated by the slope and constant of the calibration curve; Volume of solvent used for extraction (mL) (V); Dilution Factor (D); extract weight (g) (W).

Total flavonoid content

Total flavonoids were measured in accordance with the Indonesian Herbal Pharmacopoeia's guidelines.[21] The ethanol extract accurately weigh ± 10 mg, placed in a vial, added 10 ml of ethanol P, stirred until homogeneous using a sonication. Then, 1 ml of the test solution and each quercetin as a standard solution will be pipetted into the vials, each added 3 mL of ethanol P, 0.1 mL of aluminum chloride ($AlCl_3$) 10%, 0.1 mL of sodium acetate, sufficient with distilled water to 5 mL.

Then, the contents of the vials will be mixed well and incubated for 1 hour at room temperature. The blank solution was measured in the same

manner, but without the addition of aluminum chloride. The test was repeated three times, a calibration curve was made by calculating several concentrations of the test solution so that it could be plotted. The following formula can be used to determine TFC:

$$\text{Total flavonoid content} = \frac{QE\ X\ V\ X\ D}{W}$$

Quercetin equivalent (mg/L) (QE) is obtained using the slope and constants of the calibration curve; Volume of solvent used for extraction (mL) (V); Dilution Factor (D); extract weight (g) (W). The total flavonoid content of the extract was evaluated in terms of catechin equivalents (mg QE/g extract).

Antioxidant activity test

DPPH method

According to modified research,[22] the antioxidant activity can be assessed using the DPPH (1,1-diphenyl-2-picrylhydrazyl) with ascorbic acid used as a reference. A solution was prepared by weighing 10 mg of the extract and dissolving it in 10 mL of methanol p.a in a volumetric flask, the solution was vortexed until all the extracts dissolved. Then a series of solutions were made for several concentrations for extract. To each set of samples in the pipette, 1 mL of DPPH was added, followed by 5 mL of methanol. The solution was vortexed and incubated in the dark at room temperature for 30 minutes. The absorbance was then measured using a UV-Vis spectrophotometer at a wavelength of 515 nM. Positive control using ascorbic acid, the test was carried out with 3 repetitions.

$$\textit{Antioxidant activity} = \frac{(Abs.Blanks - Abs.Sample)}{Abs.Blanks} x100\%$$

The inhibition percentage was calculated for each concentration, and the equation y=a+bx was produced by calculating the linear regression curve, where x is the concentration (g/mL) and y is the inhibition percentage (%). The IC_{50} value was determined by plotting a linear regression graph between sample concentrations and percentage inhibition.

$$IC_{50} = \frac{(50-a)}{b}$$

ABTS method

The ABTS solution [2,2'-Azinobis (3 Ethylbenzothiazoline-6-Sulphonic Acid)] was prepared by mixing ABTS 7.0 mM with $K_2S_2O_8$ 2.45 mM in a ratio (1:1) and incubated in the dark for 12 hours. The extract was weighed as much as 10 mg dissolved in ethanol p.a and a series of concentrations of ethanol extract was made. Each series was added 1 mL of ABTS, sufficient with ethanol p.a up to 5 mL. The mixed solution was vortexed and incubated in a dark room for 30 minutes at room temperature. Then the absorbance was measured using a UV-Vis spectrophotometer with a wavelength of 752nM. The same procedure was carried out for Quercetin as standard. The IC_{50} value was obtained based on the percentage of inhibition of the ABTS radical from the concentration series with the same formula as the DPPH test. The IC_{50} value was obtained by means of a linear regression graph generated from the plot of sample concentration against % inhibition.

Statistical analysis

The experimental values of SARS-CoV-2 3CLpro Enzyme Inhibition Assay, TPC, TFC and antioxidants activity data are expressed as mean values ± SD (n = 3).

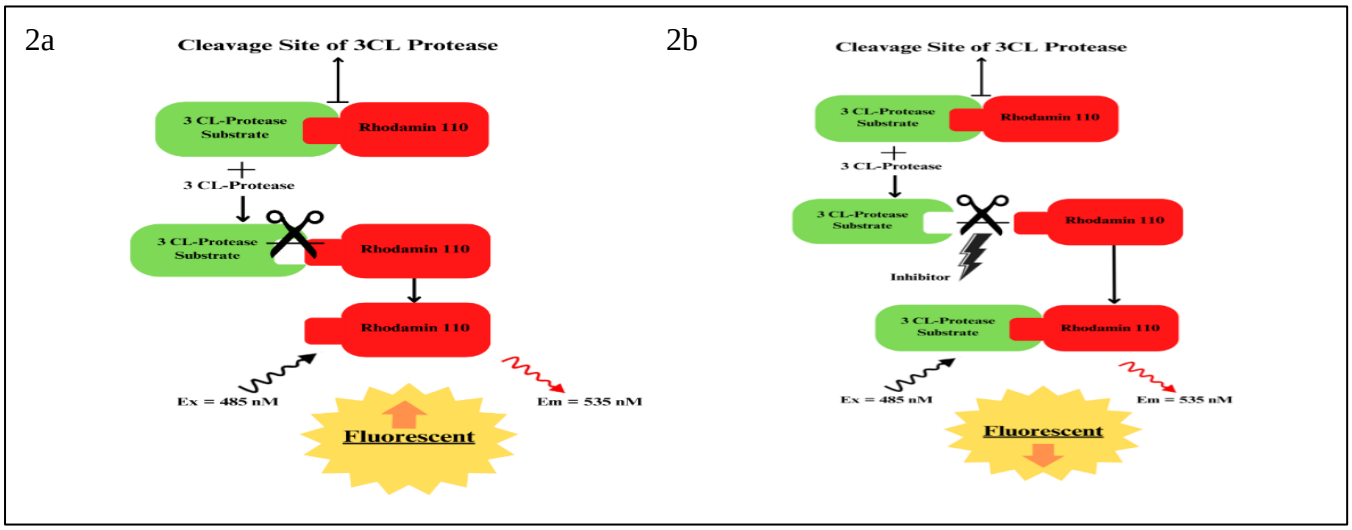

Figure 2. Scematic of Cleavage site of 3CLpro, Rh-110 substrate exhibits high fluorescence in the absence of inhibitors (2a), Rh-110 substrate shows low fluorescence when inhibitor is present (2b).

Results and Discussion

Screening of Plant-Based Extracts for Inhibition of SARS-CoV-2 3CLPro activity

Herbal plants have made significant contributions to the prevention and treatment of Covid-19. *In silico* analyses suggest that various natural compounds present in herbs exhibit a strong affinity for 3CLpro. Specifically, several compounds in *Phyllanthus niruri* and *Sonchus arvensis L* have shown promising results in this regard[11], Telang Flower *(Clitoria terntea)*[23]; Sappan Wood (*Caesalpinia sappan*)[24]; Bay leaf (*Syzygium polyanthum*)[25]; Guava leaves (*Psidium guajava*)[26] ; Starfruit leaves (*Averrhoa carambola L.*)[27];Green Chiretta (*Andrographis paniculata*)[28] and Java Plum leaves (*Syzygium cumini*)[29]. The above-mentioned herbal plants are reported[24–30] to have antiviral, anti-inflammatory, and immunomodulatory activities with potential natural compounds, including a large group of flavonoids, phenolics, polyphenols, triterpenes, alkaloids, and glucosinates. However, the evaluation of the antiviral capacity of these natural compound groups is still limited. Therefore, to assess the inhibition activity of 3CLpro, we conducted *in vitro* testing using nine plants that have the potential to inhibit viral protease function.

The Varioskan LUX Multimode with Fluorescence-based testing was used to assess the inhibitory activity of 3CLpro SARS-CoV-2. The protease action will break the peptide bond between the fluorophore and the peptide group Protease activity in cleaving the substrate results in high fluorescence values. In the presence of inhibitor, it will reduce the activity of the protease resulting in a decrease in the value of fluorescence. At the time of testing, nine extracts created by the extraction procedure known as ultrasound-assisted extraction (UAE) received the same treatment. In a different investigation, GC376 was used as a positive control; this chemical inhibited 3CLpro by 77% at a dose of 100 mM.[27,31]

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The activity of nine ethanol extracts from *Phyllanthus niruri L, Sonchus arvensis L, Clitoria ternatea, Caesalpinia sappan, Syzygium polyanthum, Psidium guajava, Averrhoa carambola L., Andrographis paniculate,* and *Syzygium cumini* against the 3CLpro enzyme using Rhodamine substrate 110 was shown in Figure 3a. The results demonstrated the potential of these nine extracts as 3CLpro inhibitors, as evidenced lower fluorescence value compared to the substrate and enzyme controls. However, four extracts were selected as they showed higher activity compared with GC376 as a positive control.

Four of the nine extracts, namley meniran (*Phylantus Niruri)*, guava leaves (*Psidium guajava*), bay leaves (*Syzygium polyanthum*), and java plum leaves (*Syzygium cumini*) in Figure 3b were found to have high fluorescence values among the nine extracts that demonstrated high inhibitory action against SARS-CoV-2 at the observed times (5 minutes, 1 hour, and 2 hours), while the other five extracts showed moderate to low inhibition. Negative inhibition may be due to the fluorescence interference from the extract which interferes the fluorescence reading.[27] The substrate and enzyme control gave the highest score due to the absence of inhibitors. The GC376 used was at a concentration of 50 µM, resulting good inhibition.

Following plant screening, four extracts that demonstrated inhibitory efficacy were tested again at the same concentration in a lower dose, 100 µg /ml (ppm). Among them, the ethanol extract of Java Plum leaves (*Syzygium cumini*) showed the highest inhibition compared to the other three extracts. Following that, the selected extracts were collected for enzyme testing at various concentrations. Furthermore, the selected extract of Java Plum leaves (*Syzygium cumini*) was prepared with a series of concentrations {0, 50, 100,200, 250, and 1000 µg/ml (ppm)} to test the inhibition of activity of the enzyme 3CLpro. 3CLpro enzyme was prepared with a final concentration of 500 nM. The experiment was conducted with four replications.

Fluorescence can be used to detect 3Clpro activity based on energy transfer in the substrate, where the protease will cut the bond between the fluorophore and the peptide group. High fluorescence value indicates high protease activity in cleaving the substrate (Rhodamine 110), where the lower fluorescene value in the presence of extract showing the inhibition of 3CL protease. The inhibition of protease means the potential of the extract as anti-COVID.

The protease inhibitor 3CLpro suppresses SARS-CoV-2 replication. After entering into the host cell, SARS-CoV-2 releases its genome RNA. The polyproteins pp1a and pp1ab are produced throughout the translation process, which are cleaved into the major protease 3CLpro and nonstructural proteins (nsps). 3CLpro is involved in the production of nsps. Nsps are essential for assembling the viral replication transcription complex (RTC) to engage in RNA synthesis. After an inhibitor from herbal plant enters the cell, it binds to 3CLpro and inhibit the activity of the enzyme. This leads to a failure to assemble virions, and ultimately the host cell fails to release new, intact virions.[30]

Targeting essential enzymes in the virus replication and life cycle is a promising strategy. *In vitro* screening of inhibitors for the 3CLpro protease was conducted on several drugs, including remdesivir, which was previously proven to inhibit SARS-CoV and MERS-CoV, thus increasing understanding of FDA-approved drugs.[32] The Java Plum leaves (*Syzygium cumini*) which belongs to the Myrtaceae family and originates from India, are also known as *Syzygium jambolanum DC* or *Eugenia cumini*. Its reaches the continent of Asia, Australia, and even Indonesia, where it is known as Juwet/Jamblang/Java Plum. Java Plum is reported to contain high levels of phytochemical constituents such as flavonoids, glycosides, myricetin, ellagic acid, anthocyanins, kaempferol, alkaloids, and phenolates.[33] The ethanol leaf extract of Java Plum has been shown to contain high levels of quercetin[34] and to possess antidiabetic, antimicrobial, antioxidant, anticancer, hepatoprotective, gastroprotective, antiparasitic, anti-inflammatory, and immunomodulatory activities.[35,36] Additionally, it has been shown to have antiviral activity against the avian influenza virus (H5N1).[37]

The results of *in silico* research indicate that ellagic acid from Java Plum (*Syzygium cumini*) has the potential as an anti-SARS-CoV-2 agent, as it exhibits the lowest binding affinity towards Spike RB, Helicase, 3CLPro/Mpro, and RdRD from SARS-CoV-2.[29] The toxicity testing results demonstrate that Java Plum is relatively non-toxic, carcinogenicity (non-carcinogenic) and for acute toxicity in rats (3.020 LD_{50}, mol/kg).[38] Histological examination of the kidney, liver, lungs, hearts and pancreas suggested no morphological disturbances, leading to the conclusión that Java Plum (*Syzygium cumini*) does not exert acute or chronic toxic effect.[37]

The results of this study showed that the IC_{50} of the ethanol extract of Java Plum leaves (*Syzygium cumini*) was 226 µg/ml (Figure 4). The flavonoid content of Java Plum leaves and their derivatives may contribute to their ability to inhibit SARS-CoV-2 3CLpro. This study represents the first approach to investigate natural compounds for development aimed at inhibiting the activity of SARS-CoV-2 3CLpro. The ethanol extract of Java Plum leaves (*Syzygium cumini*) has been identified to inhibit the activity of SARS-CoV-2 3CLpro, which ultimately can disrupt virus replication.

These results provide information that Java Plum leaves aid in the treatment of COVID-19 and serve as a basis for further *in vivo* testing and clinical trials. Therefore, further studies may focus on chemically characterizing Java Plum extract and evaluating its fractionation, which would enable the identification of biomolecules and synergistic effects responsible for the inhibitory activity of 3CLpro.

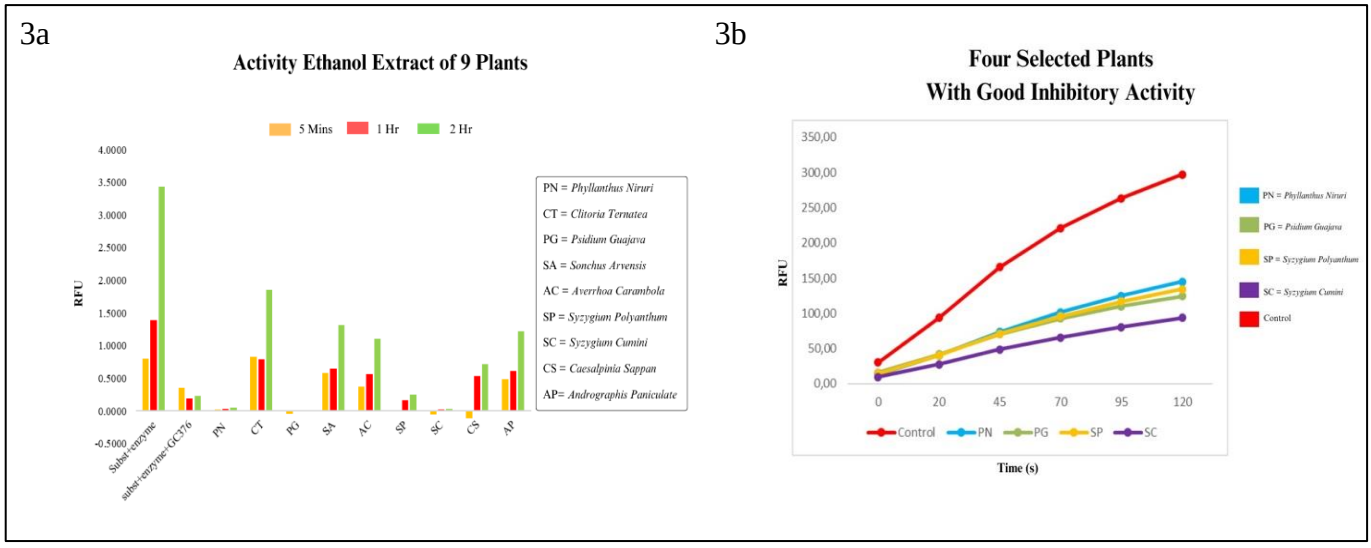

Figure 3: The fluorescence activity of 9 plants was observed at 5 minutes, 60 minutes, and 120 minutes (3a). The activity of 4 selected plants that showed good inhibitory activity (3b).

Table 1: Flavonoid, Phenolic levels and Antioxidant Activity of Java Plum leaves (*Syzygium cumini*) Ethanol Extract

Flavonoid Level (mg QE/g Extract) ± SD*	Total Polyphenols (mg GAE/g Extract)	DPPH *(IC50 (µg/ml) ± SD**)	ABTS (IC50 (µg/ml) ± SD*
12.091 ± 0.037	413 ± 1.83	3.751 ± 0.0149	4.44 ± 0.06

Note: * Data are expressed as mean values ± SD (n = 3).

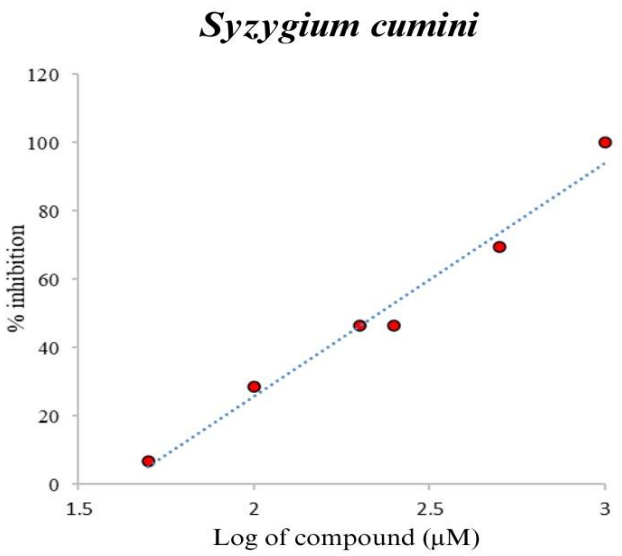

Figure 4: The IC_{50} value of Java Plum leaves (*Syzygium cumini*) was obtained through a linear regression graph plotted between the concentrations of the samples and the percentage of inhibition.

Total Polyphenol and Total Flavonoid

Total level of polyphenols found in Java Plum leaves (*Syzygium cumini*) was 413 ± 1.83 mg GAE/g with a sample concentration extract of 6-50 µg/ml (ppm), these results indicate that the high levels of polyphenols found in java plum leaves. While the total Flavonoid yield was 12.091 ± 0.037 mg QE/g Extract with an extract concentration of 10-50 µg/ml as shown in Table 1.

The antioxidant test of Java Plum leaves (*Syzygium cumini*) ethanol extract using the DPPH method yielded 3.751 ± 0.0149 µg/ml, indicating that the ethanol extract of Java Plum leaves (*Syzygium cumini*) had a very strong inhibition. This value is similar to ascorbic acid, which has a very strong positive control of 2.66 ± 0.001 µg/mL.

Besides using the DPPH method, further test also carried out using the ABTS [2,2'-Azinobis (3 Ethylbenzothiazoline-6-Sulphonic Acid)] method. The principle of the ABTS method is inhibition, the sample is included in the free radical generator and the effect of inhibition on the effects of free radicals will be measured to determine the total antioxidant capacity of the sample.[39] Measurements carried out at a wavelength of 752nM gave very strong results with an IC_{50} value of 4.44 ± 0.06 µg/ml.

Testing with these two methods gave the result that Java Plum leaves ethanol extract (*Syzygium cumini*) has very strong antioxidant activity. Based on the research that has been done, it is known that the antioxidant activity of the Java Plum plant against DPPH and ABTS, shows strong activity (IC_{50}: 3.88 ± 1.09 and 5.98 ± 1.19 µg/mL, resp.).[39] SARS-CoV-2 can trigger Oxidative Stress (OS) where an imbalance occurs between pro-oxidants and antioxidants resulting from the production of more species such as oxidation reactions (ROS). SARS-CoV-2 infection will result in reduced antioxidant reserves, resulting in the possibility of a cytokine storm with inflammation[40] and increased production of oxidants will encourage the formation of ROS which can increase viral replication and exacerbate the severity of COVID-19.[41] The ability of phenolic compounds to scavenge free radicals, will donate atoms, hydrogen atoms, chelate metal cations is connected to their antioxidant activity of an antioxidant compound to gain an electron pair. With a high content of phenolic compounds and very strong antioxidant activity, Java Plum leaves (*Syzygium cumini*) can lower oxidative stress and have a beneficial effect on COVID-19 patients.

Conclusion

The UAE method was used to extract nine herbal plants, including *Phyllanthus niruri L, Sonchus arvensis L, Clitoria ternatea, Caesalpinia sappan, Syzygium polyanthum, Psidium guajava, Averrhoa carambola L., Andrographis paniculate*, and *Syzygium cumini*. The nine herbal plant extracts showed inhibitory action against 3CLpro. The extract of *Syzygium cumini* was shown to be the most active in inhibiting the 3CLpro enzyme, with an IC_{50} value of 226 µg/ml with a Phenolic content of 413 ± 1.83 mg GAE/g Extract, total Flavonoids of 12.091 ± 0.037 mg QE/g Extract and DPPH IC_{50} antioxidant activity. 3.751 ± 0.0149 µg/ml, ABTS IC_{50} 4.44± 0.06 µg/ml.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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