The study of *Calotropis Gigantea* leaf metabolites from Ie Brouk geothermal area Lamteuba-Aceh Besar using molecular docking

G M Idroes¹, T E Tallei², R Idroes³,⁴, Muslem⁵, M Riza¹ and Suhendrayatna¹,*

¹ Department of Chemical Engineering, Faculty of Engineering, Universitas Syiah Kuala, Kopekma Darussalam, Banda Aceh 23111, Indonesia
² Department of Biology, Universitas Sam Ratulangi, Manado 95115, Indonesia
³ Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Kopekma Darussalam, Banda Aceh 23111, Indonesia
⁴ Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Kopekma Darussalam, Banda Aceh 23111, Indonesia
⁵ Department of Chemistry, Faculty of Science and Technology, Universitas Islam Negeri Ar-Raniry, Banda Aceh 23111, Indonesia

*Corresponding E-mail: suhendrayatna@unsyiah.ac.id

**Abstract.** Analysis of the metabolite contents of *C. gigantea* leaf from the Ie Brouk geothermal area has been done. The metabolites were determined by Gas Chromatography Mass Spectrometry and proteins from Main Protease (6LU7) and Spike Glycoprotein (6VXX) was studied by molecular docking. The leaf sample was prepared by extraction procedure using 96% ethanol solvent. The yield obtained was analyzed by using Gas Chromatography Mass Spectrometry. Molecular docking between metabolite and proteins showed strong interactions from Urs-12-en-24-oic acid 3-oxo methyl ester, Lupenyl Acetate, Beta Amyrenyl Acetate with high binding affinity of -15.1, -14.3, and -14.1 kcal/mole to 6VXX, and -12.3, -11.1, and -11.6 kcal/mole to 6LU7, respectively. The visualization of the interaction between metabolite to an amino acid proteins showed well pocket number. The pocket number in 6LU7 were higher than 6VXX, which was contributed from a compact state structure of 6VXX. The results showed that the ethanol extract of *C. gigantea* from the Ie Brouk geothermal area has an enormous potential to be further developed.

1. **Introduction**

Main protease (Mpro)/3CLpro (Protein Data Bank (PDB) code, PDB: 6LU7), and Spike glycoprotein (S) (PDB code: 6VXX) are the protein parts represented the SARS-CoV-2 [1]. The proteins usually are used as an object in identifying the virus infection or virus drug studies. Even though an effective solution for the deadly virus is a vaccine [2], drug treatment is also needed to treat the patients who had been infected [3]. A compound which has antiviral activity is a good option for drug purpose. The compound is against the virus by inhibiting the virus infection so that the infection level can be reduced during an incubation period [4].

Metabolites are abundant resources in a tropical country like Indonesia, especially from a plant. The metabolites produce itself by the plant [5], [6], or by endophyte [7], [8]. The local society has been even
used directly from natural resources, which they call medicinal plants [9]. Incredible biodiversity and geographical diversity provide various metabolites even by the same plants in different origins [10]. The compound is very potential to be drug material because it reported having various medicinal activities, such as antimicrobial [11], [12], antibacterial [13], [14], antibiofilm [15], and antioxidant [16], [17], and also antiviral [18], [19].

*Calotropis gigantea* (C. gigantea) is one of the medicinal plants reported to have antiviral activities [20]. The metabolite contained in plants is highly variable because it grows in a diverse geographic area [21]. It was reported that plants lived in high mineral soil such as coast [22] and geothermal areas [23]–[26] contained higher metabolite yield [27]. In the said condition, the metabolite kinds were also more diverse [28]. It is interesting to identify each metabolite containing in this plant, including its therapeutic activity.

In modern treatment, medicinal plants' use has been through specific isolation, and identification of metabolites contributed to medical function [29]. Each metabolite was also tested against specific pathogens and disease so that the drug ability will be optimal [30]. Usually, studying the interaction between chemical compounds and protein receptors is done through a spectroscopic approach [31], [32]. However, this in vivo approach in searching for a potential drug, which also involves laboratory procedures [33], takes a lot of time. This is not a reasonable effort given the conditions of this emergency pandemic. So, a virtual screening by molecular docking is the best option. This computational method provides a shortcut, which is possible to minimize several bio laboratory procedures [34], [35].

In this research, the metabolites in *C. gigantea* leaf were extracted through solvent extraction. The yield obtained was analyzed using Gas Chromatography Mass Spectrometry (GCMS). The potential drug activities of dominant metabolites against SARS-CoV-Proteins of 6LU7 and 6VXX were studied by molecular docking.

2. Materials and methods
2.1. Materials
The materials used were *C. gigantea* leaf and analytical grade ethanol 96% (purchased from Merck KgaA, Germany).

2.2. Sample preparation
*C. gigantea* leaf sample was collected from Ie Brouk geothermal area. The sample was cleaned using distillate water and dried at room temperature. It was then grounded until smooth. The powder obtained was extracted using ethanol 96% though the maceration procedure for 48 hours. The extract was filtered and evaporated to obtain extract yield for GCMS analysis.

2.3. GCMS instrumental conditions
The sample was analyzed using the GCMS 7890A Agilent Technologies equipped by EA 01.00 ChemStation, Mass Spectrometer detector, and HP Ultra 2L column. The carrier gas is helium (1.0 mL/min). The oven temperature is programmed start from 60 °C for 0 minutes in increments of 3 °C/minute to 150 °C for 1 minute and 20 °C/minute to 280 °C for 26 minutes. The chromatography process was finalized in 56.833 minutes. The mass detector is operated by an impact electron system at 70 eV.

2.4. Receptors
All receptors were downloaded from the Protein Data Bank site (https://www.rcsb.org/) contained a single repository of 3D protein structures. Receptors used in this research were Main Protease (Mpro) (PDB 6LU7) and Spike (S) glycoprotein (closed state) (PDB 6VXX).
2.5. Ligands
All ligand structures were downloaded from the PubChem site (https://pubchem.ncbi.nlm.nih.gov/) contained chemical information and biological assay activities. Ligands used in this research were metabolites from *C. gigantea* leaf extract determined by GCMS.

2.6. Preparation of receptors and ligands
Downloaded receptors were opened using BIOVIA Discovery Studio Visualizer 2020. Water molecules and native ligands were removed, then the receptors are stored in .pdb format. Next, the hydrogen atoms were added to the receptors using Autodock Tools. Files were stored in the .pdbqt format. Downloaded ligands were opened using Open Babel and converted to .pdb format. Next, the torque is adjusted, and the file was saved in .pdbqt format.

2.7. Molecular docking simulation and visualization
Molecular docking simulation was performed using Autodock Vina. All .pdbqt files from ligands and receptors were copied into the Vina folder. Vina configuration was typed in notepad, and the files were saved as conf.txt. The command prompt was used to execute the Vina program. The molecular docking results were evaluated based on the best pose between ligands and receptors, which showed the most negative free binding energy value. The visualization was performed using the Biovia Discovery Studio 2020.

3. Results and discussion
Medicinal plants have been known and used long ago in traditional medical practices to cure various diseases. Bioactive compounds, which are secondary metabolites contained in these plants, are highly varied depending on the environment, especially from the soil on which they are grown. Plants grown in extreme locations such as geothermal area have unique secondary metabolites which need to be explored for their potential as agents against the causes of infectious diseases. One of these plants is *C. gigantea*, which well grows in high bicarbonate soil. Research conducted by [36] showed that bicarbonate (HCO$_3^-$) presence played a role in metabolomic changes in plant cells, such as in Arabidopsis cells.

Researchers are focusing their works on finding antiviral agents during a pandemic, among others sourced from plants. [20] The molecular docking approach reported that the lignan glycoside compound from *C. gigantea* latex showed an anti-influenza virus activity. Because most viral proteins can be used as drug targets, this study aims to understand the potential of the metabolite from *C. gigantea* leaf extract in inhibiting the two main receptors of SARS-CoV-2, namely Main Protease (6LU7) and spike glycoprotein (6VXX). Research has shown that these two receptors were potential targets for the development of anti-SARS-CoV-2 [1], [19].

There were 16 metabolites in the ethanol extract of *C. gigantea* leaves detected by GCMS. The results of the molecular docking between these compounds with 6LU7 and 6VXX proteins showed that 9 compounds had very good binding affinity (Table 1), namely (A) Urs-12-en-24-oic acid 3-oxo methyl ester; (B) Lupenyl Acetate; (C) Beta Amyrenyl Acetate; (D) Alpha-Amyrin; (E) beta-Amyrin; (F) psi-Taraxasterol acetate; (G) beta-sitosterol; (H) Squalene, and (I) Methyl tetracosanoate. The visualization of Interaction between amino acids of the 6VXX and 6LU7 receptors and metabolite are presented in Figures 1 and 2. Compounds A, B, and C have a binding affinity of -15.1, -14.3, and -14.1 kcal/mole to 6VXX, and -12.3, -11.1, and -11.6 kcal/mole to 6LU7, respectively. This high binding affinity is supported by the number of hydrogen bonds between the ligands and receptors.
The 2nd International Conference on Agriculture and Bio-industry
IOP Conf. Series: Earth and Environmental Science 667 (2021) 012072
doi:10.1088/1755-1315/667/1/012072

Table 1. Binding affinity between the metabolites (ligands) found in *C. gigantea* and SARS-CoV-2 proteins of 6LU7 and 6VXX (receptors).

| Symbol | Ligand                                         | PubChem CID | Receptors |
|--------|------------------------------------------------|-------------|-----------|
|        |                                                |             | 6VXX      | 6LU7      |
| A      | Urs-12-en-24-oic acid 3-oxo methyl ester       | 612822      | -15.1     | -12.3     |
| B      | Lupenyl Acetate                                | 45050622    | -14.3     | -11.1     |
| C      | Beta Amyrenyl Acetate                          | 345510      | -14.1     | -11.6     |
| D      | Alpha-Amyrin                                   | 73170       | -9.7      | -7.9      |
| E      | Beta-Amyrin                                    | 73145       | -9.5      | -7.3      |
| F      | Psi-Taraxasterol acetate                       | 13970054    | -9.2      | -7.7      |
| G      | Beta-Sitosterol                                 | 222284      | -8.0      | -6.7      |
| H      | Squalene                                       | 638072      | -7.1      | -5.7      |
| I      | Methyl tetracosanolate                          | 75546       | -6.5      | -4.7      |
| J      | Phytol                                         | 5280435     | -5.7      | -5.1      |
| K      | Methyl linolenate                               | 5319706     | -5.3      | -4.1      |
| L      | 9-Octadecenoic acid, ethyl ester               | 5364430     | -5.0      | -4.6      |
| M      | Methyl linoleate                                | 5284421     | -4.9      | -5.0      |
| N      | Methyl palmitate                               | 8181        | -4.7      | -4.7      |
| O      | Neophytadiene                                   | 10446       | -3.9      | -4.7      |
| P      | Methyl isocyanate                               | 12228       | -3.0      | -2.5      |

The active sites of the spike glycoprotein (6VXX) and Main protease (6LU7) receptors were determined by CASTp 3.0 [37]. However, the number of ligand-receptor interactions is determined by the pocket numbers which are available on the active sides. Basically, pockets are areas that can be accessed because they have an open structure to allow the ligands to interact optimally [38]. As shown in Tables 2 and 3, there are 18 and 19 active site residues at 6LU7 and 6VXX, respectively. The results of determining the 6LU7 pocket in this study were similar to the results reported by [1]. Although the binding affinity values of the ligands studied were higher at 6VXX, the interaction of ligands to amino acids at the pockets were higher in 6LU7 than 6VXX (Tables 2 and 3). The most binding to amino acids in the 6VXX pocket were ligands F and G. The few ligands bonds to the 6VXX pocket were caused by the receptor structure condition, which is in a closed state. So, it was difficult for ligands to bind to the host membrane. The Spike Ectodomain (6VYB) is an example of a structure in an open state which has a very high probability for ligands to bind to the host membrane [38].

According to [39], the important amino acid interaction residues between remdesivir and main protease were Cys 145, His 164, Glu 166, Pro 168, and Asn 142. [40] reported that the binding site of Mpro is located on the active sides of Cys 145, Lys 137, and His 41. Their results were consistent with the findings in this study which the ligands A, B, E, and F met these criteria. This means that the four ligands bind very tightly to Mpro appropriately at the active site. [41] found that His 41 and Cys 145 residues were included in the Mpro catalytic dyad. Nelfinavir also produces hydrogen bonding interactions with the residue of His 41. In current studies, compounds A, B, C, E, and F showed bonds at this position. Thus, His 41 is a very important area for substrate binding. Nelfinavir also binds to Met 165, where A-F compounds also bind to the same residue.
Figure 1. The residues from the interaction between Main Protease (6LU7) receptors and metabolites (ligands) of (A) Urs-12-en-24-oic acid 3-oxo methyl ester; (B) lupenyl acetate; (C) beta amyrenyl acetate; (D) alpha-amyrin; (E) beta-amyrin; (F) psi-taraxasterol acetate; and (G) beta-sitosterol.
Figure 2. The residues from the interaction between spike glycoprotein (6VXX) receptors and metabolites (ligands) of (A) urs-12-en-24-oic acid 3-oxo methyl ester; (B) lupenyl acetate; (C) beta amyrenyl acetate; (D) alpha-amyrin; (E) beta-amyrin; (F) psi-taraxasterol acetate; (G) beta-sitosterol; (H) squalene, and (I) methyl tetracosanoate.
Table 2. Interactions of ligands to amino acids at the 6LU7 pockets.

| Amino acids in the active sites | Interactions of ligands to amino acids |
|--------------------------------|---------------------------------------|
|                               | A | B | C | D | E | F | G |
| A 24 THR                       | √ |   |   |   |   |   |   |
| A 25 THR                       | √ | √ |   |   |   |   |   |
| A 26 THR                       | √ |   |   |   |   |   |   |
| A 27 LEU                       | √ |   |   |   |   |   |   |
| A 41 HIS                       | √ | √ |   |   |   |   |   |
| A 45 THR                       | √ |   |   |   |   |   |   |
| A 46 SER                       |   |   |   |   |   |   |   |
| A 49 MET                       | √ | √ |   |   |   |   |   |
| A 140 PHE                      |   |   |   |   |   |   |   |
| A 141 LEU                      |   |   |   |   |   |   |   |
| A 142 ASN                      | √ | √ |   |   |   |   |   |
| A 143 GLY                      | √ | √ |   |   |   |   |   |
| A 144 SER                      | √ | √ |   |   |   |   |   |
| A 145 CYS                      | √ | √ |   |   |   |   |   |
| A 163 HIS                      |   |   |   |   |   |   |   |
| A 165 MET                      | √ | √ |   |   |   |   |   |
| A 166 GLU                      | √ | √ |   |   |   |   |   |
| A 172 HIS                      |   |   |   |   |   |   |   |

Table 3. Interactions of ligands to amino acids at the 6VXX pockets.

| Amino acids in the active sites | Interactions of ligands to amino acids |
|--------------------------------|---------------------------------------|
|                               | A | B | C | D | E | F | G | H | I |
| A 128 ILE                      |   |   |   | √ |   |   |   |   |   |
| A 168 PHE                      |   |   |   | √ |   |   |   |   |   |
| A 170 TYR                      |   |   |   | √ |   |   |   |   |   |
| A 203 ILE                      |   |   |   | √ |   |   |   |   |   |
| A 227 VAL                      |   |   |   | √ |   |   |   |   |   |
| A 229 ASP                      |   |   |   | √ |   |   |   |   |   |
| A 780 GLU                      |   |   |   | √ |   |   |   |   |   |
| A 784 GLN                      |   |   |   | √ |   |   |   |   |   |
| A 1016 ALA                     |   |   |   | √ |   |   |   |   |   |
| AC 1019 ARG                    |   |   |   | √ |   |   |   |   |   |
| AC 1020 ALA                    |   |   |   | √ |   |   |   |   |   |
| A 1026 ALA                     |   |   |   | √ |   |   |   |   |   |
| A 1029 ARG                     |   |   |   | √ |   |   |   |   |   |
| A 1030 SER                     |   |   |   | √ |   |   |   |   |   |
| Amino acids in the active sites | Interactions of ligands to amino acids |
|--------------------------------|-------------------------------------|
| A 1024 LEU                     | √                                   |
| A 1040 VAL                     | √                                   |
| A 1041 ASP                     | √                                   |
| A 1042 PHE                     | √                                   |
| A 2016 ALA                     | √                                   |
| A 2017 THR                     | √ √ √                               |
| B GLU 725                      | √                                   |
| B 1023 ASN                     | √                                   |
| B 1024 LEU                     | √                                   |
| B 1027 THR                     | √                                   |
| B 1028 GLY                     | √                                   |
| B 1039 ARG                     | √ √                                 |
| B 1041 ASP                     | √                                   |
| B 1117 THR                     | √                                   |
| B 1118 ASP                     | √                                   |
| C 1021 SER                     | √                                   |
| C 1023 ASN                     | √ √                                 |
| C 1026 ALA                     | √ √ √                               |
| C 1027 THR                     | √ √                                 |
| C 1028 LYS                     | √                                   |
| C 1030 SER                     | √ √                                 |
| C 1031 GLU                     | √                                   |
| C 1034 LEU                     | √                                   |
| C 1039 ARG                     | √                                   |
| C 1040 VAL                     | √ √ √ √                             |
| C 1041 ASP                     | √ √ √                               |
| C 1042 PHE                     | √                                   |
| C 1044 GLY                     | √                                   |
| C 1045 LYS                     | √                                   |
| C 1064 HIS                     | √                                   |
| C 1068 VAL                     | √                                   |

Hydroxychroloquine is one of the therapeutic agents for COVID-19. According to Goyal and Goyal [42], this compound binds to 6VXX with the binding affinity ranging from -5 to -6 kcal/mole. It can be seen that the A-G compounds used in this study have greater binding affinity than Hydroxychroloquine. These compounds also have a lot of interactions, especially the hydrogen bonding interaction with 6VXX.
This research is still the basis for the development of further research, including bioavailability and toxicity. Molecular dynamics is also needed to evaluate the stability of the bond between the ligand and the receptor. We believe that our research can add more significance to the following findings to help and to manage the COVID-19 pandemic.

4. Conclusion
The compounds from the ethanol extract of C. gigantea leaf showed the enormous potential to be further developed as anti-SARS-CoV-2 agents. The interaction between the metabolite and the SARS-CoV-2 protein produced high binding energies and interaction numbers. Molecular docking between metabolites and SARS-CoV-2 proteins showed strong interaction from Urs-12-en-24-oic acid 3-oxo methyl ester, Lupenyl Acetate, Beta Amyrenyl Acetate with a high binding affinity of -15.1, -14.3, and -14.1 kcal/mole to 6VXX, and -12.3, -11.1, and -11.6 kcal/mole to 6LU7, respectively. The visualization of the interaction between metabolite to an amino acid of SARS-CoV-2 proteins showed well pocket numbers. The pocket numbers in 6LU7 were higher than 6VXX, contributing from a close state structure of 6VXX. Further research is recommended to be directed at studies regarding bioavailability, toxicity, and also the stability of the interaction between ligands and receptors through molecular dynamics.

Reference
[1] Rakib A, Paul A, Chy M N U, Sami S A, Baral S K, Majumder M, Tareq A M, Amin M N, Shahriar A, Uddin M Z, Dutta M, Tallei T E, Emran T Bin and Simal-Gandara J 2020 Molecules 25 3936
[2] Thanh Le T, Andreadakis Z, Kumar A, Gómez Román R, Tollefsen S, Saville M and Mayhew S 2020 Nat. Rev. Drug Discov. 19 305–6
[3] Mehra M R, Desai S S, Kuy S, Henry T D and Patel A N 2020 N. Engl. J. Med. 382 e102
[4] Mitjà O and Clotet B 2020 Lancet Glob. Heal. 8 e639–40
[5] Nuraskin C A, Marlina, Idroes R, Soraya C and Djufri 2019 IOP Conf. Ser. Mater. Sci. Eng. 523
[6] Paristiowati M, Moersilah M, Stephanie M M, Zulmanelis Z, Idroes R and Puspita R A 2019 J. Phys. Conf. Ser. 1402 055041
[7] Tallei T E, Linelejan Y T, Umboh S D, Adam A A, Muslem and Idroes R 2020 IOP Conf. Ser. Mater. Sci. Eng. 796 012047
[8] Zulfendi, Idroes R and Khairan 2019 IOP Conf. Ser. Mater. Sci. Eng. 523 12013
[9] Tallei T E, Pelealu J J, Pollo H N, Pollo G A V, Adam A A, Effendi Y, Karuniawan A, Rahimah S and Idroes R 2019 Data Br. 104681
[10] Price E J, Wilkin P, Sarasan V and Fraser P D 2016 Sci. Rep. 6 29136
[11] Estevam E, Griffin S, Nasim J, Zielinski S, Aszyk J, Osowicka M, Dawidowska N, Idroes R, Bartoszek A and Jacob C 2015 Nat. Prod. Commun. 10 1733–8
[12] Ningsih D S, Idroes R, Bachtiar B M and Khairan 2019 IOP Conf. Ser. Mater. Sci. Eng. 523 12009
[13] Nuraskin C A, Marlina, Idroes R, Soraya C and Djufri D 2020 Open Access Maced. J. Med. Sci. 8 181–4
[14] Rahmad R, Earlia N, Nabilah C, Inayati I, Amin M, Prakoeswa C R S, Khairan K and Idroes R 2019 IOP Conf. Ser. Mater. Sci. Eng. 523 012011
[15] Pratiwi S U T, Lagendijk E L, de Weert S, Idroes R and indrawan C 2015 Int. J. Appl. Res. Nat. Prod. 8 1–13
[16] Suhartono E, Sinha M, Santos M B, Idroes R and Indrawan M S 2019 Journal of Physics: Conference Series 1374 12057
[17] Nasution R, Idroes R, Amna U and Al. E 2020 Maced. J. Med. Sci.
[18] Marcello A, Cerva A, Milan Bonotto R, Nascimento Alves L, Rajasekharan S, Giacobone C, Caccia C, Cavalli R, Adami M, Brambilla P, Lembo D, Poli G and Leoni V 2020 Redox Biol. 36 101682
[19] Tallei T E, Tumilaar S G, Niode N J, Fatimawali F, Kepel B J, Idroes R and Effendi Y 2020 Preprints 2020040102
[20] Parhira S, Yang Z-F, Zhu G-Y, Chen Q-L, Zhou B-X, Wang Y-T, Liu L, Bai L-P and Jiang Z-H 2014 PLoS One 9 e104544
[21] Wahid P A, Valiathan M S, Kamalam N V., Eapen J T, Vijayalakshmi S, Prabhu R K and Mahalingam T R 2000 J. Plant Nutr. 23 329–38
[22] Jaleel C A, Gopi R, Manivannan P, Sankar B, Kishorekumar A and Panneerselvam R 2007 Colloids Surfaces B Biointerfaces 60 195–200
[23] Idroes R, Yusuf M, Alatas M, Subhan, Lala A, Saiful, Suhendra R, Idroes G M and Marwan 2018 IOP Conf. Ser. Mater. Sci. Eng. 334 012002
[24] Idroes R, Yusuf M, Alatas M, Subhan, Lala A, Muslem, Suhendra R, Idroes G M, Suhendrayatna, Marwan and Riza M 2019 IOP Conf. Ser. Mater. Sci. Eng. 523 012010
[25] Idroes R, Yusuf M, Alatas M, Subhan, Lala A, Muhammad, Suhendra R, Idroes G M and Marwan 2019 IOP Conf. Ser. Mater. Sci. Eng. 523 012012
[26] Idroes R, Yusuf M, Saiful S, Alatas M, Subhan S, Lala A, Muslem M, Suhendra R, Idroes G M, Marwan M and Mahlia T M I 2019 Energies 12 4442
[27] Nuraskin C, Marlina, Idroes R, Soraya C and Djufri 2020 Rasayan J. Chem. 13 18–23
[28] Nuraskin C A, Marlina, Idroes R, Soraya C and Djufri 2019 Res. J. Pharm. Technol. 12 5247
[29] Wang B Q 2010 J. Med. Plants Res. 4 2813–20
[30] Das K, Tiwari R K S and Shrivastava D K 2010 J. Med. Plants Res. 4 104–11
[31] Suhartono E, Thalib I, Aflanie I, Noor Z and Idroes R 2018 IOP Conf. Ser. Mater. Sci. Eng. 350 12008
[32] Suhartono E, Noor Z, Edyson, Budianto W Y and Idroes R 2019 AIP Conf. Proc. 2108 020025
[33] Witkowski B, Amaratunga C, Khim N, Sreng S, Chim P, Kim S, Lim P, Mao S, Sopha C, Sam B, Anderson J M, Duong S, Chuar C M, Taylor W R J, Suon S, Mercereau-Puijalon O, Fairhurst R M and Menard D 2013 Lancet Infect. Dis. 13 1043–9
[34] Earlia N, Muslem, Suhendra R, Amin M, Prakoeswa C R S, Khairan and Idroes R 2019 Sci. World J. 2019 1–7
[35] Earlia N, Rahmad R, Amin M, Prakoeswa C, Khairan K and Idroes R 2019 Sains Malaysiana 48 1019–24
[36] Misra B B, Yin Z, Geng S, de Armas E and Chen S 2016 Sci. Rep. 6 35778
[37] Tian W, Chen C, Lei X, Zhao J and Liang J 2018 Nucleic Acids Res. 46 W363–7
[38] Maiti S and Banerjee A 2020 Drug Dev. Res. ddr.21730
[39] Sen D, Debnath P, Debnath B, Bhauvik S and Debnath S 2020 J. Biomol. Struct. Dyn. 1–22
[40] ul Qamar M T, Alqahtani S M, Alamri M A and Chen L-L 2020 J. Pharm. Anal 10(4) 313-319.
[41] Goyal B and Goyal D 2020 ACS Comb. Sci. 22 297–305
[42] Nimgampalle M, Devanathan V and Saxena A 2020 J. Biomol. Struct. Dyn. 1–13