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Syzygium aromaticum as a possible source of SARS-CoV-2 main protease inhibitors: Evidence from a computational investigation

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ABSTRACT

SARS-CoV-2, a new and fast circulating coronavirus strain, infected over 214 countries and territories worldwide and caused global health emergencies. The absence of appropriate medicines and vaccinations has further complicated the condition. SARS-CoV-2 main protease (M^{pro}) is crucial for its propagation, and it is considered a striking target. This study used several computational approaches to determine the probable antagonist of SARS-CoV-2 M^{pro} from bioactive phytochemicals of *Syzygium aromaticum*. A total of 20 compounds were screened through *in silico* approach. The molecular dynamics simulation studies were then carried out for further insights. We found crategolic acid, oleanolic acid, and kaempferol have considerable binding affinity and important molecular contacts with catalytic pocket residues, His41-Cys145. The pharmacological properties through ADMET analysis also showed that these compounds could be used as safe drug candidates. The molecular dynamics simulation study further confirmed these compound's stability with M^{pro}. However, further detailed *in-vitro* and *in-vivo* analyses are compulsory to evaluate the real potentiality of identified compounds.

INTRODUCTION

A novel coronavirus strain was stated in late 2019 in Wuhan, China, called SARS-CoV-2, linked to lethal respiratory sickness in the patients [1]. The SARS-CoV-2 infection is termed as coronavirus disease 2019, which has created severe health issues and separated countries from one another. This disease triggered a global medical emergency and severely affecting international travel, tourism, and trade [2]. SARS-CoV-2 lies in the beta Coronavirus family [3], which has a similar sequence identity with its descendants SARS-CoV [4, 5].

The beta-coronaviruses synthesize ~800 kDa polyproteins, which are enzymatically sliced to synthesize several proteins. The papain-like protease (PL^{pro}) and main protease (M^{pro}) causes the proteolysis of coronavirus proteins [6]. The M^{pro} slices the polyprotein and generates various polypeptides vital for viral replication, transcription, and translation [4, 7, 8]. The dynamic behavior of M^{pro} exemplifies its possibility to become a striking target for drug design. Moreover, SARS-CoV-2 M^{pro} is not similar to human homologous proteases [9]. The ligand-binding site of M^{pro} is placed into the groove of domains I and II comprising the crucial catalytic dyad His 41 and Cys 145 [10, 11].

Moreover, some recent studies demonstrated that M^{pro} could be the prominent target of SARS-CoV-2 infection [11-14].

Still today, there is no specific anti-SARS-CoV-2 drugs are available. However, several clinical trials are underway; and most of them are focused on relieving the symptoms [15]. Besides, the antiviral efficiency of several already existing drugs has been testified in several studies [16, 17]. However, repurposed drugs have proven effective, but their efficacy and safety are still ambiguous [18-20]. Besides, the recent coronavirus strain (B.1.351) found in South Africa possesses more infection rate, and it shows the ability to re-infect people. Recently, South Africa [21] and several European countries, including Austria, Estonia, Iceland, Italy, Lithuania, Luxembourg, Latvia, and Norway, has postponed the use of the AstraZeneca vaccine following reports of blood clots [22]. Nevertheless, the benefits outweigh the rare blood clot events, and the European Medical Agency, World Health Organization, and the International Society on Thrombosis and Hemostasis recommended taking the vaccine. However, it raises the urgency to find more specific drugs to inhibit SARS-CoV-2 infection with broader efficacy to overcome public concern.

Plant-derived compounds could be a great source of antiviral drug compounds as they possess low toxicity, have a more convenient biosynthesis process, and can be screened easily through computational biology techniques. Besides, most drug candidates used from the last four decades were derived from natural sources [23-25]. Also, plant synthesized compounds have shown antiviral activity against several viruses' including's Chikungunya [26], SARS [27], and SARS-CoV-2 [28].

This study was conducted to find out the potent natural anti-SARS-CoV-2 compounds from *Syzygium aromaticum* (clove). *Syzygium aromaticum* is commonly used as a spice, which contains many bioactive compounds and is cultivated worldwide [29, 30]. *S. aromaticum* has also been used as a traditional medicine for a long time [30]. Besides, *S. aromaticum* compounds have been shown to act against many viruses such as Hepatitis C virus, *Herpes simplex* virus [30], Feline calicivirus [31], and Adenovirus [32]. The potential antiviral activity of *S. aromaticum* against several RNA viruses raises the possibility to act against SARS-CoV-2. Thus, this study was projected to find out probable compounds against SARS-CoV-2 targeting M^{pro}.

METHODS AND MATERIALS

Isolation and preparation of ligands

In this study, initially, we built a compound dataset of *S. aromaticum* through related literature search on Scopus, Google Scholar, PubMed, and Web of science literature repository [33]. We curated 20 compounds of *S. aromaticum* from these databases and downloaded their three-dimensional structure from the Pubchem database [34]. The PyRx ligand preparation wizard was used to prepare compounds as ligands (Version Python prescription 0.8) [35] through Merck molecular force field (mmff94**)** [36], and the ligands were then converted into PDBQT format for further analysis.

Preparation of receptor

The M^{pro} 3D structure (PDB ID: 6LU7) [37] was extracted from the largest crystal structure repository, Protein Data Bank (https://www.rcsb.org/) [38]. Before molecular docking, the receptor was prepared using Chimera [39] and AutoDock tools integrated into PyRx [35].

ADMET analysis

The physicochemical properties of isolated ligands were evaluated by ADMET analysis. ADMET profiling analysis is a promising and cost-reductive approach that tells us about any compound's physicochemical properties, drug-likeness properties, potentiality, and effectiveness [40]. In silico studies have accelerated the velocity of drug design and are now widely used in pharmaceuticals, leading to finding novel compounds to combat various microorganisms [41]. Lipinski's rule of five is essential for determining a drug's probability with a particular pharmacological and biological activity [42]. Three or more violations do not follow the drug-likeness requirements and are not considered a drug for further study. ADMET properties were analyzed using the Schrodinger QikProp (QikProp, Schrödinger, LLC, New York, NY, USA) program [43]. The drug-likeness properties of the selected compounds were studied using Lipinski's "rule of five" [44].

Compound's screening

The virtual screening was conducted using AutoDock wizard integrated [35, 45] PyRx software (Version Python prescription 0.8) [35]. The ligands were kept as flexible, and the receptor was inflexible. The docking grid box (x = -13.09, y = 15.00, z = 69.32) was generated using Auto Grid engine in PyRx. The conformational root-mean-square deviation (RMSD) result of less than 1.0 A° was taken as perfect and bunched for later promising binding analysis. The highest negative score was considered as better binding. Here, α-ketoamide was considered as a control ligand [10]. The BIOVIA Visualizer (Discovery Studio v 4.5) was employed to observe molecular interactions [46].

Molecular dynamics simulation

The molecular dynamics simulation was conducted using the "WebGRO for Macromolecular Simulations (https://simlab.uams.edu/)" server utilizing the "GROMACS" macromolecular simulation system [47]. Initially, the ligand topology files were prepared by the "PRODRG" server [48]. In this study, the GROMOS96 43a1 force field was utilized, along with the SPC water model and NaCl (0.15 M) solvated cubic box. The energy was minimized using the steepest descent algorithm (5000 steps). For temperature control, NVT/NPT temperature (300 K) system was used in 1 bar pressure. Finally, we conducted a 50 ns simulation. The trajectory was used to calculate RMSD, Rg (Radius of gyration), RMSF (Root mean square fluctuation), SASA (Solvent accessible surface area), and Hydrogen bond analysis.

RESULTS

ADMET analysis

The QikProp ADME/Tox analysis protocol deciphered that all compounds follow "rule of 5" except bicornin (shown in Table 1). According to drug-likeness property analysis, the selected compounds' molecular weights were between the recommended range (≤500 g/mol) except for bicornin. The hydrogen bond acceptor and donor were also below the recommended range (≤10 and ≤5, respectively). The filtered 19 compounds were then employed for further investigation.

Table 1. ADMET properties of all compounds.

Compounds	Molecular weight	Number of hydrogen bond donors	Number of hydrogen bond acceptors	Octanol/water partition coefficient (QPlogPo/w)	QPlogHERG	QPPMDCK
Campesterol	402.702	1	1.7	7.005	-4.113	1880.296
Carvacrol	156.267	1	1.7	2.459	-2.281	2123.9
Crategolic acid	476.738	4	6.8	3.913	-3.435	194.373
Ellagic acid	318.323	6	13.6	-2.397	-0.896	7.154
Eugenin	216.277	2	6.8	0.186	-1.516	979.328
Eugenitin	230.303	2	6.8	0.54	-1.369	1235.933
Eugenol	172.267	1	3.4	1.338	-0.822	2082.283
Ferulic acid	204.266	3	6.8	-0.347	-1.427	201.381
Gallic acid	178.185	5	8.5	-1.903	-1.781	24.819
Kaempferol	302.367	5	10.2	-1.017	-0.99	38.682
Myricetin	334.366	7	13.6	-2.326	-1.542	5.655
Oleanolic acid	460.739	3	5.1	4.979	-3.669	529.115
Quercetin	318.366	6	11.9	-1.604	-0.908	16.301
Rhamnetin	332.393	5	11.9	-1.074	-2.853	54.578
Salicylic acid	146.186	3	5.1	-0.563	0.49	287.837
Stigmasterol	416.729	1	1.7	7.331	-4.075	1880.391
Vanillin	160.213	2	5.1	-0.081	-0.451	553.933
Bicornin	1088.763	5	25	-3.521	-6.386	0.038
Biflorin	364.392	7	15.3	-2.169	-3.051	18.469
Caffeic acid	190.239	4	6.8	-0.989	-1.8	57.264

Compound's library screening and interaction visualization

The binding affinity of all selected ligands is shown in Table 2. The top graded anti-M^{pro} hits were selected based on their interaction with the catalytic dyad His41 and Cys145 and higher binding affinity. We found three compounds, i.e., oleanolic acid, crategolic acid, and kaempferol having higher binding affinity -7.7 kcal/mol, -7.6 kcal/mol, and -7.6 kcal/mol, respectively. Besides, they have shown crucial molecular interactions; thereby chosen for further analysis.

Table 2. Binding affinity of *Syzygium aromaticum* compounds with M^{pro}

Ligand	Binding affinity (kcal/mol)
α-ketoamide (+ control)	-7.3
Oleanolic acid	-7.7
Crategolic acid	-7.6
Kaempferol	-7.6
Biflorin	-7.3
Ellagic acid	-7.3
Rhamnetin	-7.3
Myricetin	-7.3
Quercetin	-7.2
Stigmasterol	-7.1
Campesterol	-6.9
Eugenin	-6.0
Eugenitin	-6.0
Ferulic acid	-5.7
Caffeic acid	-5.6
Gallic acid	-5.4
Vanillin	-4.9
Salicylic acid	-4.9
Carvacrol	-4.8
Eugenol	-4.7

The molecular interaction analysis showed that all the selected compounds either interact with Cys145 and His41 or, at least with one of them. α-ketoamide is a positive control in this study that forms four H bonds with Gln189 residue and several alkyl

bonds with Cys145, Met49, Met165, Leu27 & His41 residues (Figure 1a). Crategolic acid comprises H bond with Thr25, Leu141, and Gly143 residues and pi-alkyl bonds with Cys145, His41, Met165, and Met49 residues (Figure 1b). Oleanolic acid comprises H-bond with Ser144 and pi-alkyl bonds with Cys145 and Met49 residues (Figure 1c). The last compound, kaempferol, comprises H-bond with His163, pi-alkyl bonds with Cys145 and Met165, and pi-stacked bond with His41 residue (Figure 1d).

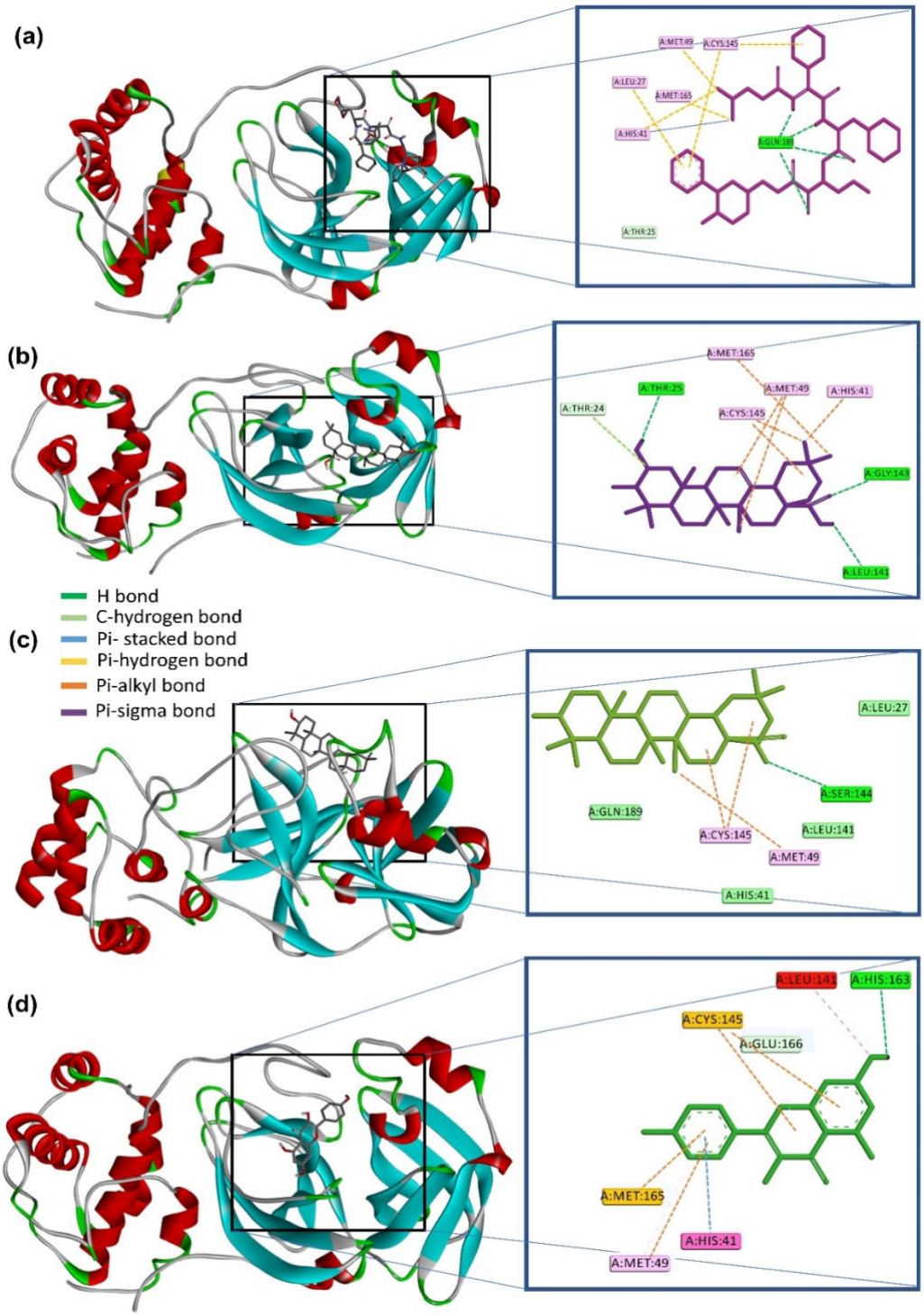

Figure 1. Molecular interactions of selected compounds with SARS-Cov-2 M^{pro} (a) Positive control α-ketoamide, (b) Crategolic acid, (c) Oleanolic acid, and (d) Kaempferol.

Molecular dynamics simulation

Molecular dynamics simulation was used to project the behaviour of projected compounds in the biological system. In this study, both control complex and newly selected compounds' behaviors were studied through RMSD, Rg, RMSF, SASA and H bond studies. In Figure 2a, it is seen that the selected compounds have lower RMSD values than the positive control α-ketoamide. Crategolic acid showed relatively lower RMSD values than other compounds. The average RMSD values of α-ketoamide, crategolic acid, oleanolic acid, and kaemferol were 2.07 Å, 1.51 Å, 1.55 Å, and 1.57 Å, respectively. Interestingly, crategolic acid, oleanolic acid, and kaempferol showed a more stable condition in simulation compared to the control. The radius of gyration demonstrates the compactness of the system over time. The average Rg values of crategolic acid, oleanolic acid, and kaempferol were 2.17 Å, 2.12 Å, and 2.13 Å, respectively (Figure 2b). Oleanolic acid and kaempferol have lower Rg values compared to the control 2.15 Å. The fluctuation pattern of each amino acid residue was calculated using RMSF (Figure 2c). Figure 2c showed notable fluctuations in the terminal regions for each complex, but fewer fluctuations were seen in the active site region. The average RMSF values of α-ketoamide, crategolic acid, oleanolic acid, and kaemferol were 1.25 nm^2, 0.97 nm^2, 0.97 nm^2, and 1.04 nm^2, respectively. The solvent-accessible surface area was also evaluated for each complex. The higher SASA value demonstrates the openness of the systems. The average SASA values of the selected complex were 139.02 nm^2, 139.45 nm^2, 135.05 nm^2, and 136.80 nm^2 for α-ketoamide, crategolic acid, oleanolic acid, and kaempferol, respectively (Figure 2d). The positive control and crategolic acid have almost similar SASA values, and interestingly oleanolic acid and kaemferol have lower SASA values than control. The intermolecular hydrogen bonds play a vital role in deciphering the proper functions of any small molecules with receptors. Thus, we also calculated the hydrogen bonds of our system (Figure 2e). The oleanolic acid has more hydrogen bonds than α-ketoamide, while the rest two compounds have relatively similar hydrogen bonds. The number of hydrogen bonds also in parallel with other calculations, which depicts the compactness of our simulated systems.

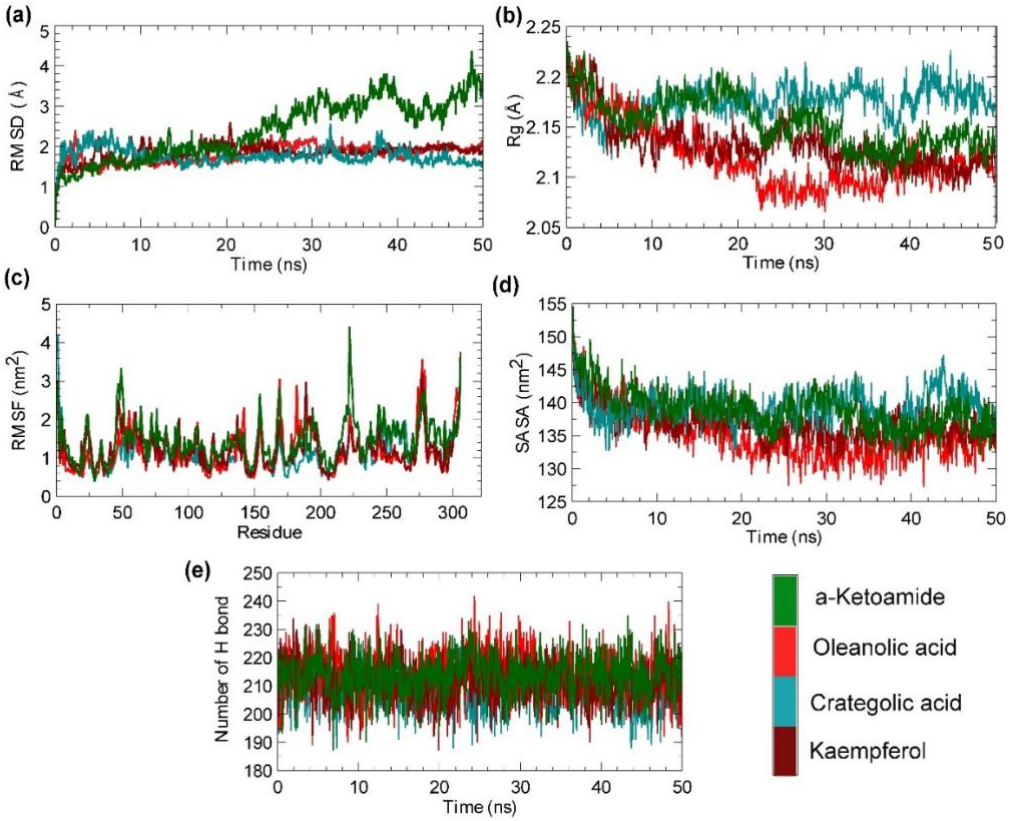

Figure 2. Molecular dynamics simulation studies. (a) Root Mean Square Deviation analysis; (b) Radius of gyration analysis, (c) Root Mean Square Fluctuation analysis, (d) Solvent accessible surface area analysis, (e) Number of Hydrogen bond analysis.

DISCUSSION

This research aimed to identify potential SARS-CoV-2 Mpro drug candidates from natural sources [37]. M^{pro} has been investigated as an effective target to restrain the expansion of SARS-CoV-2 contamination. We have considered *Syzygium aromaticum* because it contains several bioactive compounds [49, 50]. In addition, it was found to have antioxidant activity and a broad range of pharmacological efficiency [51, 52]. Besides, *Syzygium aromaticum* has traditional history to use as common spice around the world. Peoples consumes *Syzygium aromaticum* daily.

It has been shown that α-ketoamide interacts with the residues of $M^{pro,}$ namely His41, Gly143, Ser144, Cys145, His163, His164, Glu166, Pto168, and Gln189 [53]. Our study also found that α-ketoamide interacts with almost similar residues that rectify our methods for further study (shown in Figure 2b). Besides, the N3 (native ligand) of the chosen main protease (6LU7) interacts with His41 and Cys145 residues [11], which implies that His41 and Cys145 residues are crucial for SARS-CoV-2 M^{pro} inhibition. Moreover, recent studies showed that the phytochemicals form strong interactions with Leu27, His41, Met49, Cys145, Met165, Thr190 residues of SARS-CoV-2 M^{pro} [45, 54, 55]. In addition to the main protease, phytochemicals showed antiviral activity against SARS-CoV-2 envelope protein [56].

Among the studied 20 compounds of *Syzygium aromaticum,* only four compounds were used for more investigation considering their binding affinity and compared to the known antagonist α-ketoamide. All these compounds form hydrogen or hydrophobic interactions with the crucial residues His41-Cys145 of M^{pro}. In a previous study, oleanolic acid was described as an active compound against the hepatitis C virus (HCV)

[57]. Likewise, Kaempferol was suggested to be an excellent anti-coronavirus candidate [58]. Recently, Jo et al. showed the anti-SARS-CoV-2 activity of kaempferol [59]. Khan et al. also showed that kaempferol bonds with the essential active site residue, inhibiting SARS-CoV-2 [60]. Moreover, kaempferol was also shown in several other pharmacological activities [61]. Besides, the presented compounds (Table 1) showed considerable bio-activities in different *in-vitro* studies. For example, recently, Alhadrami et al. depicted that olive-derived phytochemicals inhibit SARS-CoV-2 main protease at IC_{50} = 3.22–14.55 μM [62]. Furthermore, Colunga Biancatelli and colleagues reported the possible synergistic benefits of quercetin and vit-C against COVID-19 [63]. In addition, a clinical study denoted that quercetin improves the patient's condition [64].

Our study found that all of our selected compounds follow Lipinski's rule of five except bicornin. The selected three compounds, crategolic acid, oleanolic acids, and kaempferol, showed considerable water solubility, whereas bicornin failed to fulfill these parameters also. Also, these compounds showed considerable in vitro hERG toxicity. However, the only bicornin violates the Lipinski rule of five (shown in Table 1).

Molecular dynamics simulation is an effective technique to understand the stability and dynamics of the protein-ligand complex [65, 66]. The lower RMSD and RMSF values indicate the higher stability of the complex [66, 67]. The compounds, crategolic acid, oleanolic acid, and kaemferol formed stable complex with SARS-CoV-2 M^{pro}, though crategolic acid showed a sudden surge initially, but overall, it showed stable binding. Nukoolkarn et al. (2008) conducted two ns simulations and found that inhibitor compound binds with His41 and Cys145 residues of SARS-CoV $3CL^{pro}$ [68]. Besides, several recent molecular simulation studies showed similar results [45, 67, 69]. Similarly, our identified compounds crategolic acid, oleanolic acid, and kaemferol also interacted with the active side residues His41 and Cys145 and formed stable conformation, which depicts their possible effectiveness over time.

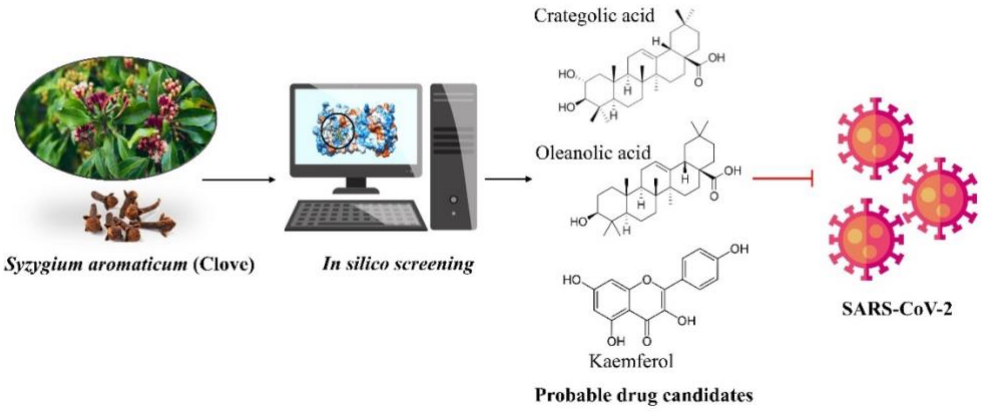

Figure 3. A graphical representation of the study. The screened compounds could potentially inhibit the activity of SARS-CoV-2.

CONCLUSION

The highly infectious nature of SARS-CoV-2 has possessed a devastating effect on human life all around the world. Therefore, SARS-CoV-2 antagonists are desperately needed to reduce the fast transmissibility of the virus. The major goal of this research was to find novel inhibitors for the SARS-CoV-2 main protease. This study employed

several computational approaches to identify the probable antagonist of SARS-CoV-2 M^{pro} from 20 bioactive phytochemicals of *Syzygium aromaticum*. Considering the outputs of ADMET analysis, molecular docking, and molecular dynamics simulation, three compounds, crategolic acid, oleanolic acids, and kaempferol, showed satisfactory results to inhibit SARS-CoV-2 infection targeting the main protease (Figure 3). The identified compounds can be considered as lead molecules to develop drugs against COVID-19. However, more studies are required to confirm their activity and efficacy.

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AUTHOR'S CONTRIBUTION

MAHMJ, and MCA conceived the plan of this research. MCA and MSR run the experiment. MCA, AJN, RAH, RAR, MAM, and MSK wrote the manuscript and analyzed the data. MCA prepared the final draft. MAHMJ, RAH, MSR, MKA, MAM, and MMR edited the manuscript. All authors revised and approved the manuscript for final submission.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

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