JOURNAL OF BIOMOLECULAR STRUCTURE AND DYNAMICS
https://doi.org/10.1080/07391102.2020.1780947

Natural compounds from *Clerodendrum* spp. as possible therapeutic candidates against SARS-CoV-2: An *in silico* investigation

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Communicated by Ramaswamy H. Sarma

ABSTRACT

The COVID-19 pandemic caused by the novel coronavirus SARS-CoV-2 has rattled global public health, with researchers struggling to find specific therapeutic solutions. In this context, the present study employed an *in silico* approach to assess the inhibitory potential of the phytochemicals obtained from GC-MS analysis of twelve *Clerodendrum* species against the imperative spike protein, main protease enzyme M^{pro} and RNA-dependent RNA polymerase (RdRp) of SARS-CoV-2. An extensive molecular docking investigation of the phytocompounds at the active binding pockets of the viral proteins revealed promising inhibitory potential of the phytochemicals taraxerol, friedelin and stigmasterol. Decent physicochemical attributes of the compounds in accordance with Lipinski's rule of five and Veber's rule further established them as potential therapeutic candidates against SARS-CoV-2. Molecular mechanics-generalized Born surface area (MM-GBSA) binding free energy estimation revealed that taraxerol was the most promising candidate displaying the highest binding efficacy with all the concerned SARS-CoV-2 proteins included in the present analysis. Our observations were supported by robust molecular dynamics simulations of the complexes of the viral proteins with taraxerol for a timescale of 40 nanoseconds. It was striking to note that taraxerol exhibited better binding energy scores with the concerned viral proteins than the drugs that are specifically targeted against them. The present results promise to provide new avenues to further evaluate the potential of the phytocompound taraxerol *in vitro* and *in vivo* towards its successful deployment as a SARS-CoV-2 inhibitor and combat the catastrophic COVID-19.

ARTICLE HISTORY
Received 14 May 2020
Accepted 4 June 2020

KEYWORDS
SARS-CoV-2; *Clerodendrum* spp; molecular docking; drug-likeness; MM-GBSA; taraxerol; molecular dynamics simulations

1. Introduction

The catastrophic coronavirus disease 2019 (COVID-19) pandemic caused by a novel coronavirus referred to as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has brought the world to a standstill and has afflicted global public health (Abraham et al., 2020; Bhardwaj et al., 2020; Elasnaoui & Chawki, 2020; Paniri et al., 2020; Wu et al., 2020). The virus has infected over six million people and has ruthlessly claimed nearly four hundred thousand lives till date (as per updates on June 2, 2020) (https://www.worldometers.info/coronavirus/). COVID-19 has surpassed the two other coronavirus-related outbreaks associated with severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS) that occurred in recent past in terms of the frequency of infected individuals and the number of deaths (Lu et al., 2020), though the overall case-fatality rate remains lower than both SARS and MERS (Wu & McGoogan, 2020). Patients infected with COVID-19 are diagnosed with mild-to-severe respiratory illness and symptoms like high fever, cough, dizziness and shortness of breath which might further advance to pneumonia and acute respiratory distress causing death (Lu et al., 2020; Wu & McGoogan, 2020).

SARS-CoV-2 is a positive-stranded RNA virus that represents the genus *Betacoronovirus* and belongs to the family *Coronaviridae* (Benvenuto et al., 2020; Sarma et al., 2020). The novel coronavirus is significantly distant from SARS-CoV (around 79% identify) and MERS-CoV (around 50% identity) (Lu et al., 2020). Despite the novelty of SARS-CoV-2, significant advancements have been made in elucidating the complex genomic features (Wu et al., 2020), understanding the codon usage signatures and evolutionary enigma (Andersen et al., 2020; Tort et al., 2020) and unraveling the riddles of infectivity and epidemiology (Lu et al., 2020; Rothan & Byrareddy, 2020) of the intimidating virus. The viral genome encodes several structural and non-structural proteins (nsp) that play crucial roles in attaching the virus to host cellular receptors, regulating viral replication and facilitating subsequent infection (Gupta et al., 2020; Tai et al., 2020; Wu et al., 2020). The spike (S) protein is a vital structural protein component that forms prominent spikes on the outer surface of the virus and helps in viral attachment, successful fusion and subsequent entry into the host cells (Elfiky, 2020; Sinha et al.,

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Supplemental data for this article can be accessed online at https://doi.org/10.1080/07391102.2020.1780947.

2020; Tai et al., 2020). The imperative main protease enzyme M^{pro} (also referred to as 3 C-like protease) of SARS-CoV-2 plays pivotal role in proteolytic cleavage and processing of the large viral polyprotein orf1ab in combination with papain-like proteases and facilitates viral replication (Al-Khafaji et al., 2020; Gyebi et al., 2020; Islam et al., 2020; Jin et al., 2020; Joshi et al., 2020; Khan et al., 2020; Mittal et al., 2020). Efficient replication and the spread of SARS-CoV-2 in host cells are largely dependent on proper functioning of the RNA-dependent RNA polymerase (RdRp) (Elfiky, 2020; Ziebuhr, 2005). Non-structural protein 12 (nsp12), the catalytic subunit of RdRp, mediates viral replication and enhances template binding and processivity in combination with nsp7 and nsp8 (Subissi et al., 2014; Te Velthuis et al., 2010). The pivotal roles of these viral proteins in the attachment of the virus to host cell receptors and subsequent replication and infection establish them as promising drug and vaccine candidates (Aanouz et al., 2020; Adeoye et al., 2020; Das et al., 2020; Kumar et al., 2020; Lobo-Galo et al., 2020; Mahanta et al., 2020; Pant et al., 2020; Sk et al., 2020; Tai et al., 2020; Yin et al., 2020). Several methods like drug repurposing (Ciliberto & Cardone, 2020; Elmezayen et al., 2020), administration of convalescent plasma transfusion (Shen et al., 2020) and usage of SARS-CoV and MERS-CoV antibodies (Huang et al., 2020) are presently being employed to combat COVID-19. A combination of hydroxychloroquine and azithromycin has been observed to be effective in the treatment of the disease (Gautret et al., 2020). Hydroxychloroquine, an anti-malarial drug, facilitates endosomal acidification and blocks viral entry and fusion by inhibiting glycosylation of the host cellular receptors that bind with the viral proteins (Sanders et al., 2020). Though hydroxychloroquine efficiently arrests viral growth, toxic side effects and concerns over drug poisoning remain the major bottlenecks in using it in COVID-19 therapy (Enmozhi et al., 2020). Drugs like arbidol targeted against the SARS-CoV-2 spike protein (Sanders et al., 2020) and lopinavir and ritonavir targeted against SARS-CoV-2 M^{pro} (Li et al., 2020) have been reported to be effective against the menacing pathogen. Antiviral drugs like remdesivir and favipiravir that inhibit RdRp activity (Hendaus, 2020; Li et al., 2020) ; Nejadi Babadaei et al., 2020have been observed to arrest SARS-CoV-2 replication and are currently in clinical trials. Despite relentless attempts, researchers and medical practitioners are struggling to limit the rapid transmission and combat the calamitous health hazards posed by SARS-CoV-2 due to the lack of specific effective therapeutics and prophylactics. Though several drugs and vaccines are in clinical trials all over the world, an effective therapeutic remedy specifically targeted to treat and cure COVID-19 is yet to be achieved.

Since time immemorial, phytochemicals and natural compounds have been employed as medicinal agents towards the effective treatment of grave ailments. In the quest of natural therapeutic remedy against COVID-19 several studies have been designed to explore the inhibitory prospects of phytochemicals from various important and conventionally used medicinal plants and herbs all over the world (Boopathi et al., 2020). *Clerodendrum*, a perennial shrub and a member of Lamiaceae family, is prevalent in the tropical regions of Asia including India, Myanmar, Bangladesh, Malayasia, Indonesia, Thailand, Bhutan, Nepal and also in temperate Tibet (Chathuranga et al., 2019). Various medicinally important species of *Clerodendrum* are frequently used to treat respiratory ailments like common cold, bronchitis and pneumonia (Chathuranga et al., 2019; Patel et al., 2014). A crude extract of *Clerodendrum trichotomum* has been reported to actively inhibit Respiratory syncytial virus (RSV) without any cytotoxic effects towards normal tissues (Chathuranga et al., 2019).

Considering the fact that *Clerodendrum* spp. often finds widespread applications in treating respiratory disorders (Chathuranga et al., 2019; Patel et al., 2014), an in house repertoire of the phytochemicals obtained from GC-MS analysis of twelve *Clerodendrum* species were assessed to explore their inhibitory prospects against the imperative spike, M^{pro} and RdRp proteins of SARS-CoV-2 employing an *in silico* molecular interaction-based approach. Present work also involves a comprehensive investigation of the physicochemical features of the screened phytocompounds and their accordance with the general rules of drug-likeness viz., Lipinski's rule of five (RO5) and Veber's rule, with a purpose to assess their potential drug-likeness.

2. Materials and methods

2.1. Sample collection

The leaves of twelve species of *Clerodendrum* were collected during 2019 (Table S1, Supplementary material) from different localities in North Bengal (Garden of the medicinal plants of Department of Botany, University of North Bengal and Jalpaiguri) and roadside sites from Azra, Guwahati, Assam. The plants were identified by plant taxonomist of Department of Botany, University of North Bengal and were deposited at the Herbarium of the same department.

2.2. Chemicals and reagents

All the reagents and chemicals used in the present study were purchased from Hi-Media Laboratories Pvt. Ltd. (Mumbai, India), Merck (Mumbai, India) and Sigma-Aldrich (USA). Milli-Q ultrapure water from the departmental facility was used in all the experiments. All the chemicals were of analytical grade.

2.3. Preparation of plant extracts

Fresh and disease free leaves of selected plants were washed twice with double distilled water, shade dried at room temperature for 21 days and pulverized into fine powder by using a mechanical grinder. Powdered leaves of selected plants (10 g each) were extracted in a Soxhlet apparatus using absolute methanol (the ratio of plant material to solvent was 1:10 m/v) for 6-7 h. The extracts were then concentrated under reduced pressure and controlled temperature (40°-50 °C) using a rotary evaporator (Buchi Rotavapor R-3,

Switzerland). The extracts were further lyophilized using an Eyela Freeze Dryer (FDU-506, USA) to obtain dry powder and stored at 4 °C until further use.

2.4. Gas chromatography-mass spectrometry (GC-MS) analysis

All the lyophilized methanolic extracts were centrifuged at 12,000 rpm for 15 min. Clear supernatants thus, obtained were utilized for GC-MS analysis to identify the different phytochemical compounds. Thermo Scientific make 'Trace 1300' GC, attached to a TG-5MS column (30 $m \times 0.25$ mm $\times$ 0.25 μm) and equipped with ISQ-QD (Thermo Scientific) single quadrupole mass analyzer conditioned at ion trap 230 °C, transfer line 290 °C, electron energy of 70 eV (vacuum pressure- 2.21e-0.5 Torr) was employed for the present study (Kar et al., 2016). One microlitre of the sample was injected through a Thermo Scientific AI-1310 auto-sampler in splitless mode (split flow 50 ml/min) with a splitless time of 0.80 min. Helium was selected as the carrier gas with a constant flow of 1 ml/min.

All samples were run thrice to minimize the error and the results were plotted only with the compounds identified in all three runs. The essential compounds were identified by their retention times and mass fragmentation patterns using the reference of parent compound using MS interpreter Version 2.0 and also by matching with the reference database of National Institute of Standards and Technology (NIST) with a MS library version 2011. No external standards were used in this study.

2.5. Retrieval and refinement of protein and ligand structures

The most prominent and active compounds present in the selected species of *Clerodendrum* (detected through GC-MS analysis) were subjected to robust molecular docking analysis against the imperative SARS-CoV-2 proteins that currently serve as promising targets for effective drug development namely, the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein, SARS-CoV-2 main protease enzyme M^{pro} and SARS-CoV-2 RdRp (Tai et al., 2020). The high-resolution X-ray diffraction crystal structures of the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein (PDB ID: 6LZG Chain B; 2.50 Å resolution) (Wang et al., 2020), SARS-CoV-2 main protease enzyme M^{pro} (PDB ID: 6LU7 Chain A; 2.16 Å resolution) (Jin et al., 2020) and SARS-CoV-2 RdRp (PDB ID: 7BV2 Chain A; 2.50 Å resolution) were retrieved from PDB (Yin et al., 2020). The inhibitor and water molecules were removed from the PDB files prior to analysis. Polar hydrogen atoms and Kollman charges were added to the corresponding PDB files using AutoDock tools. The structures of the concerned phytocompounds were retrieved from NCBI PubChem (https://pubchem.ncbi.nlm.nih.gov/) and respective three-dimensional structures were generated through the Open Babel software (O'Boyle et al., 2011). Energy-optimization of the structures was achieved using the PRODRG server (Schüttelkopf & Van Aalten, 2004). Gromos 96 force field was applied for the process of energy minimization. Gasteiger charges were added to the corresponding ligand structures using AutoDock tools.

2.6. Molecular docking

Molecular docking was achieved using the AutoDock Vina software (Naidoo et al., 2020; Trott & Olson, 2010). Thorough information about the active site residues of the RBD of SARS-CoV-2 spike, SARS-CoV-2 M^{pro} and SARS-CoV-2 RdRp proteins was obtained from recent reports on their structure and interaction/inhibition binding pockets (Andersen et al., 2020; Jin et al., 2020; Wang et al., 2020; Yin et al., 2020). The ligands were docked at the active binding pockets of the concerned proteins employing a grid-based docking method opting a rigid protein receptor and flexible ligand docking protocol (Naidoo et al., 2020). Grid boxes of coordinates 30, 30 and 30 in x, y and z dimensions involving the active site residues were generated to carry out molecular docking at the binding pockets of the viral proteins (Naidoo et al., 2020). The DINC server was used to further validate the results of AutoDock Vina (Antunes et al., 2017). Ligand-receptor complexes with the lowest binding scores and RMSD $<$ 2.0 Å (Naidoo et al., 2020) were used for robust interaction profiling employing the PyMol software (version 1.7.4) and the Protein-Ligand Interaction Profiler (PLIP) web-server (Salentin et al., 2015). The compounds displaying binding energy scores ≤ -7.0 kcal/mol were selected for the next phases of analysis (Naidoo et al., 2020).

2.7. Assessment of physicochemical features and potential toxicity of the phytocompounds

Physicochemical features of the selected phytocompounds were thoroughly estimated using the SwissADME server and the results were validated with the pkCSM server (Daina et al., 2017; Pires et al., 2015). Pan-Assay Interference Structures (PAINS) medicinal chemistry analysis was performed employing the SwissADME and FAF-Drugs4 (Lagorce et al., 2017) servers. Toxicity parameters like mutagenicity (AMES mutagenesis) and cytotoxicity of the phytochemicals were assessed using the ProTox-II web-server (Banerjee et al., 2018) and validated with the vNN-ADMET server (Schyman et al., 2017).

2.8. Prime MM-GBSA (molecular mechanics-generalized born surface area) calculation

Molecular mechanics-generalized Born surface area (MM-GBSA) was used to estimate the free energies of binding (ΔG_{bind}) of the selected protein-ligand complexes employing the default parameters of the Prime MM-GBSA modules (Vijayakumar et al., 2014) implemented in the Schrödinger software. Prime MMGBSA is an approach that uses the optimized potential for liquid simulations (OPLS) force field in combination with molecular mechanics energies (EMM), surface generalized Born (SGB) solvation model for polar solvation (GSGB) and a non-polar solvation term (GNP) for the calculation purpose (Al-Khafaji et al., 2020; Mittal et al., 2020;

Table 1. Binding energy scores and interaction profile of the phytochemicals of *Clerodendrum* spp. with the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein.

Ligands	Binding energy scores (kcal/mol) RBD of the SARS-CoV-2 spike protein (6LZG)	Interacting residues
Taraxerol	−7.5 ± 0.01	*Leu455, Glu484, Tyr489*
Friedelin	−7.3 ± 0.02	*Tyr449, Asn450,* **Lys444**
Stigmasterol	−7.2 ± 0.01	*Tyr449, Phe490*
Arbidol (Drug)	−6.2 ± 0.01	*Ile468,* Glu471#

Hydrophobic interactions are marked in italics, hydrogen bonds are highlighted in bold and salt bridges are displayed with #.

Sarma et al., 2020). The total free energy of binding of each concerned protein-ligand complex was calculated as:

$$\Delta Gbind = \Delta Gcomplex - (\Delta Gprotein + \Delta Gligand)$$

2.9. Molecular dynamics simulations

Molecular dynamics (MD) simulations allow proper validation of molecular docking results and offer reliable assessment of potential stability of a protein-ligand complex (Khan et al., 2020; 2020; Kumar et al., 2020; Muralidharan et al., 2020). The complexes of the ligands displaying the best interaction (in terms of binding energy values) with each SARS-CoV-2 spike, M^{pro} and RdRp proteins were subjected to MD simulation for a timescale of 40 nanoseconds (ns) using GROMACS software (version 2019) (Abraham et al., 2015). The MD simulations were executed as per the protocols opted by Khan et al. (2020). The equilibration steps were set with constant pressure and temperature (NPT) ensemble (Elfiky & Azzam, 2020; Enayatkhani et al., 2020; Umesh et al., 2020) The MD simulations were carried out at standard temperature of 300 K and pressure level of 1.013 bar (Umesh et al., 2020). The MD trajectories were estimated by analyzing the root mean square deviation (RMSD) and the root mean square fluctuation (RMSF) of the complexes for a timescale of 40 ns (Khan et al., 2020). MD simulations were followed by detailed interaction profiling of the protein-ligand complexes using the LIGPLOT software (Wallace et al., 1995).

3. Results and discussion

3.1. Investigation pertaining to gas chromatography-mass spectrometry (GC-MS)

The current study was intended at the identification of active compounds present in the twelve species of *Clerodendrum* using a GC-MS method. It was evident from a thorough analysis of the GC-MS chromatograms of the twelve concerned *Clerodendrum* spp. (Figures S1-S12) that among the 68 commonly shared phytocompounds (Table S2, Supplementary material), 29 were unique. Accordingly, molecular docking analysis was performed employing these 29 unique phytocompounds (Tables 1–3 and S3 [Supplementary material]) against the concerned SARS-CoV-2 proteins. Taraxerol, friedelin and betulin, as identified by the GC-MS analysis, are triterpenoid chemical compounds mainly found in various higher groups of plants. The compounds have been shown to possess anti-inflammatory, anti-carcinogenic, anti-obesity and antimicrobial activities with significant therapeutic potential (Giweli et al., 2012; Takasaki et al., 1999; Tang et al., 2011; Yao et al., 2013). Fatty acids like linoleic acid (LA) and hexadecanoic acid, profiled in present study, are some of the essential fatty acids that human beings require from their diet (Kar et al., 2016). A deficiency in linolenic acid has been reported to be associated with infertility, skin, kidney degeneration and abrupt changes in the fatty acid composition of lipids (Dobryniewski et al., 2007). In addition, squalene and stigmasterol, as identified by the present GC-MS analysis, have been reported to be potent antioxidants and effective against oxidative stress related disorders (Amarowicz, 2009; Yoshida & Niki, 2003).

Table 2. Binding energy scores and interaction profile of the phytochemicals of *Clerodendrum* spp. with SARS-CoV-2 main protease enzyme M^{pro}.

Ligands	Binding energy scores (kcal/mol) SARS-CoV-2 main protease enzyme M^{pro} (6LU7)	Interacting residues
Taraxerol	−8.4 ± 0.01	*Glu166, Pro168, Ala191*
Friedelin	−7.9 ± 0.02	*Pro168*
Stigmasterol	−7.7 ± 0.01	*Pro168*
Michael acceptor inhibitor N3	−7.1 ± 0.02	*Val3, Thr25, Thr26,* **Gly143, Ser144, His163, His164, Glu166**
Lopinavir (Drug)	−8.1 ± 0.01	*Thr25, Asn142,* ***Glu166,*** *Gln189,* **Gly143**
Ritonavir (Drug)	−7.5 ± 0.01	*Leu27,* ***Glu166,*** *Pro168, Gln189*

Hydrophobic interactions are marked in italics and hydrogen bonds are highlighted in bold.

Table 3. Binding energy scores and interaction profile of the phytochemicals of *Clerodendrum* spp. with SARS-CoV-2 RdRp.

Ligands	Binding energy scores (kcal/mol) SARS-CoV-2 RdRp (7BV2)	Interacting residues
Taraxerol	−7.4 ± 0.02	*Lys545, Arg555*
Friedelin	−7.1 ± 0.01	*Asp623*
Stigmasterol	−7.0 ± 0.03	*Lys545, Arg555, Thr687, Ala688*
Remdesivir (Drug)	−6.3 ± 0.01	**Asp623,** Lys551#, Arg553#
Favipiravir (Drug)	−3.6 ± 0.01	**Arg553, Arg555, Ser682**

Hydrophobic interactions are marked in italics, hydrogen bonds are highlighted in bold and salt bridges are displayed with #.

3.2. Molecular docking of the phytocompounds with the receptor-binding domain of the SARS-CoV-2 spike protein

SARS-CoV-2 infection initiates with the attachment of its spike protein to the human receptor angiotensin converting enzyme 2 (ACE2) via the receptor-binding domain (RBD) (Abdelli et al., 2020; Basit et al., 2020; Nejadi Babadaei et al., 2020;; Tai et al., 2020; Wahedi et al., 2020). Molecular docking of the phytochemicals of *Clerodendrum* spp. was performed at the active pocket of the RBD of the SARS-CoV-2 spike protein (PDB ID: 6LZG), in light of prior knowledge about the active site residues that bind with human receptor ACE2 (Andersen et al., 2020; Hasan et al., 2020; Tai et al., 2020; Wan et al., 2020). The detailed binding energy scores of all the concerned phytocompounds have been provided in Table 1 (compounds displaying binding energy scores ≤

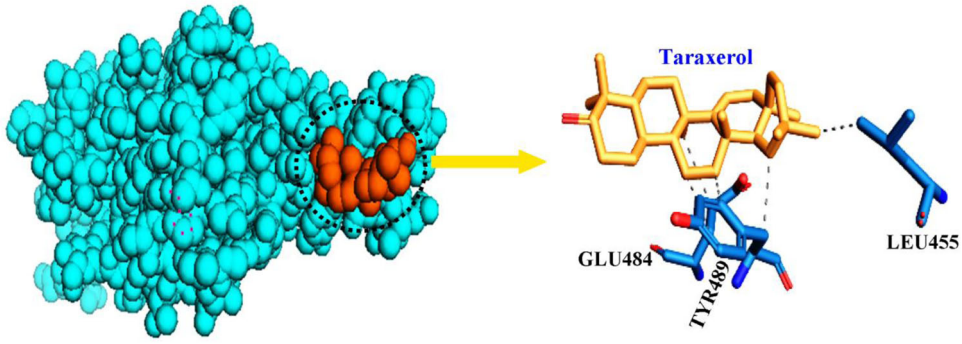

Figure 1. Mode of interaction of taraxerol with the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein. Cyan sphere represents the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein. Taraxerol is represented as an orange sphere. Hydrophobhic interactions have been represented as grey dashed lines.

−7.0 kcal/mol) and Table S3 [Supplementary material] (compounds displaying binding energy scores > −7.0 kcal/mol). The compounds taraxerol, friedelin and stigmasterol were found to exhibit the best binding energy scores of -7.5 (± 0.01) kcal/mol, −7.3 (± 0.02) kcal/mol and -7.2 (± 0.01) kcal/mol, respectively, among the concerned phytochemicals (Table 1). A thorough inspection revealed that taraxerol interacted with Leu455, Glu484 and Tyr489 residues of the RBD (Figure 1 and Table 1), all of which have been inferred to play vital roles in the attachment of the SARS-CoV-2 spike protein with human ACE2 receptor (Andersen et al., 2020; Ortega et al., 2020; Wan et al., 2020). Molecular interactions of friedelin involved the residues Lys444, Tyr449 and Asn450, whereas, stigmasterol interacted with the residues Tyr449 and Phe490 of the receptor-binding domain of the SARS-CoV-2 spike protein (Table 1). In the present study, the mode of binding of the drug arbidol which is currently being used in targeting the SARS-CoV-2 spike protein to inhibit viral attachment with human ACE2 receptor (Sanders et al., 2020) was also analyzed and compared with the binding patterns of the concerned phytochemicals. Arbidol displayed a binding energy score of -6.2 (± 0.01) kcal/mol with the RBD of the SARS-CoV-2 spike protein (Table 1). It was striking to note that the compounds taraxerol, friedelin and stigmasterol exhibited significantly higher ($p < 0.01$) binding potential relative to the drug arbidol, as evident from the binding energy scores (Table 1). Our observations provide opportunities to further validate the inhibitory potential of the compounds *in vitro* and *in vivo* towards the development of effective therapeutics targeted against SARS-CoV-2 spike protein.

3.3. Molecular docking of the phytocompounds with SARS-CoV-2 main protease enzyme m^{pro}

Proper inhibition of SARS-CoV-2 M^{pro} arrests viral replication (Jin et al., 2020). Recently, Michael acceptor inhibitor N3 has been observed to exhibit significant inhibitory efficacy against SARS-CoV-2 M^{pro} and has been proposed as a promising drug lead compound (Jin et al., 2020). A robust molecular docking of our in-house phytochemicals of *Clerodendrum* spp. revealed interesting facts regarding their inhibitory prospects. A detailed account of the binding energy scores has been provided in Table 2 (compounds displaying binding energy scores ≤ −7.0 kcal/mol) and Table S3 [Supplementary material] (compounds displaying binding energy scores > −7.0 kcal/mol). Compounds taraxerol, friedelin and stigmasterol were found to display significantly higher ($p < 0.01$) binding potential, with binding energy scores of -8.4 (± 0.01) kcal/mol, −7.9 (± 0.02) kcal/mol and -7.7 (± 0.01) kcal/mol respectively (Table 2), than the inhibitor N3 (-7.1 ± 0.02 kcal/mol) at the active binding pocket of SARS-CoV-2 M^{pro}. It has been reported that Glu166 and Pro168 residues of SARS-CoV-2 M^{pro} are crucial in the binding of the inhibitor N3 (Jin et al., 2020). Interestingly, taraxerol also involved these two imperative residues in its interaction (Figure 2 and Table 2) which indicated towards its potential inhibitory efficacy against the SARS-CoV-2 M^{pro} enzyme. The compounds friedelin and stigmasterol interacted with the residue Pro168 at the active binding pocket of SARS-CoV-2 M^{pro} (Table 2). The binding efficacy of the drugs lopinavir and ritonavir that are currently being employed to inhibit SARS-CoV-2 M^{pro} activity (Sanders et al., 2020) were estimated through molecular docking and the binding energy scores were observed to be -8.1 (± 0.01) kcal/mol and -7.5 (± 0.01) kcal/mol for lopinavir and ritonavir respectively. It was interesting to note that taraxerol exhibited significantly higher ($p < 0.01$) binding energy score with SARS-CoV-2 M^{pro} than both the currently used drugs (Table 2) and present observations confer scopes to further evaluate the inhibitory efficacy of taraxerol *in vitro* and *in vivo* against SARS-CoV-2.

3.4. Molecular docking of the phytocompounds with SARS-CoV-2 RdRp

The vital catalytic component nsp12, in combination with nsp7 and nsp8, of SARS-CoV-2 RNA-dependent RNA polymerase (RdRp) mediates viral replication in host cells (Subissi et al., 2014; Ziebuhr, 2005). Proper inhibition of RdRp activity has been reported to arrest SARS-CoV-2 replication and thus, limit its infective prowess (Sanders et al., 2020; Yin et al., 2020). The binding energy scores of the phytocompounds of *Clerodendrum* spp. at the active binding pocket of SARS-CoV-2 RdRp (Yin et al., 2020) have been detailed in Table 3 (compounds displaying binding energy scores ≤ −7.0 kcal/mol) and Table S3 [Supplementary material] (compounds

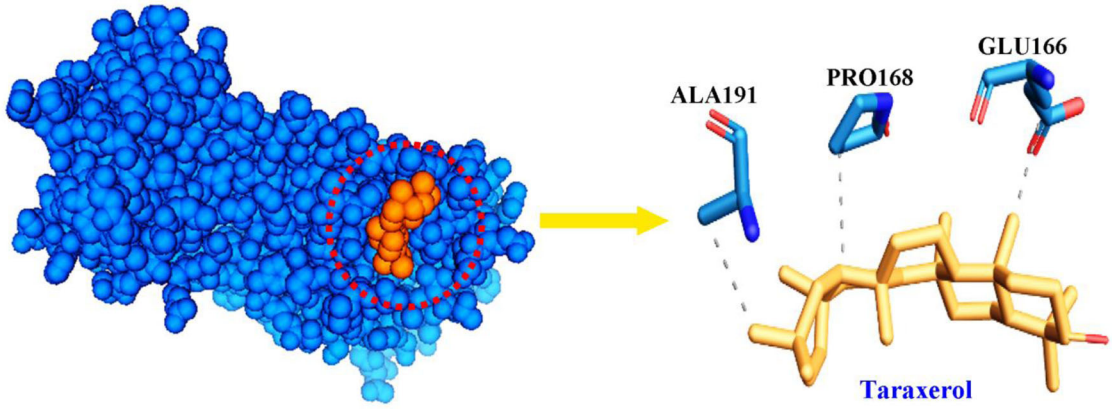

Figure 2. Mode of interaction of taraxerol with SARS-CoV-2 main protease enzyme M^{pro}. Blue sphere represents SARS-CoV-2 main protease enzyme M^{pro}. Taraxerol is represented as an orange sphere. Hydrophobhic interactions have been represented as grey dashed lines.

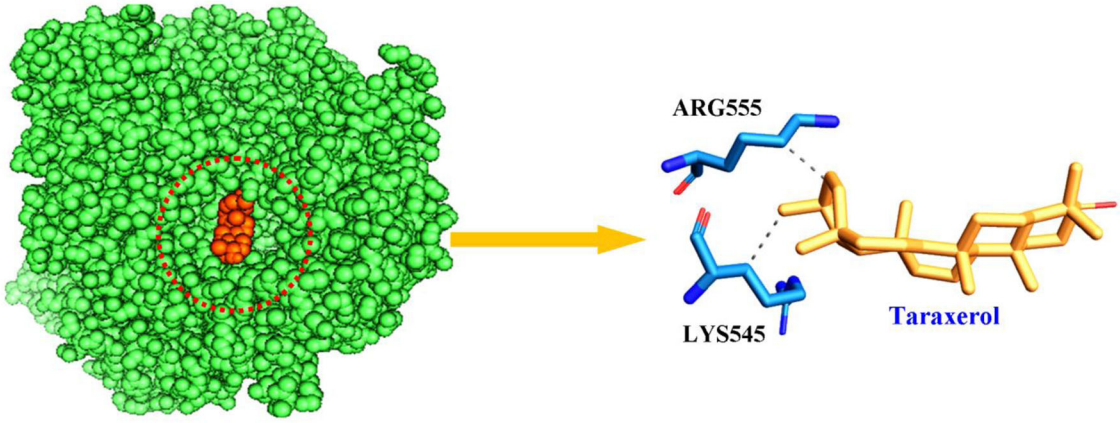

Figure 3. Mode of interaction of taraxerol with SARS-CoV-2 RNA-dependent RNA polymerase (RdRp). Green sphere indicates SARS-CoV-2 RdRp. Taraxerol is represented as an orange sphere. Hydrophobhic interactions have been represented as grey dashed lines.

displaying binding energy scores > -7.0 kcal/mol). The compounds taraxerol, friedelin and stigmasterol displayed the highest binding potential with scores -7.4 (± 0.02) kcal/mol, −7.1 (± 0.01) kcal/mol and -7.0 (± 0.03) kcal/mol, respectively, with SARS-CoV-2 RdRp (Table 3). Taraxerol was observed to involve the residues Lys545 and Arg555 (Figure 3 and Table 3), whereas, friedelin interacted with SARS-CoV-2 RdRp through the residue Asp623 (Table 3). Stigmasterol was found to interact with the residues Lys545, Arg555, Thr687 and Ala688 of SARS-CoV-2 RdRp (Table 3). Remdesivir and favipiravir are antiviral drugs that inhibit viral RdRp activity and arrest their growth and replication (Sanders et al., 2020). Both these drugs have been reported to exhibit significant viral inhibition and are presently in clinical trials with prospects of effective treatment against COVID-19 (Li et al., 2020;; Sanders et al., 2020). Accordingly, the binding patterns of these drugs with SARS-CoV-2 RdRp were compared with the modes of binding of the concerned phytocompounds of *Clerodendrum* spp. Remdesivir and favipiravir were noted to display binding energy scores of -6.3 (± 0.01) kcal/mol and -3.6 (± 0.01) kcal/mol respectively, with SARS-CoV-2 RdRp (Table 3). It was intriguing to note that the compounds taraxerol, friedelin and stigmasterol exhibited significantly higher ($p < 0.01$) binding energy values than both the drugs (Table 3). Thus, the present findings provide opportunities to further explore the inhibitory potential of the concerned phytocompounds *in vitro* and *in vivo* towards their effective deployment as inhibitors against SARS-CoV-2.

3.5. Physicochemical features and accordance with the rules of drug-likeness

An accurate account of the physicochemical features of a therapeutic compound in compliance with the general rules of drug-likeness is an imperative step in the initial phases of drug development (Paul Gleeson et al., 2011). Decent binding affinities (binding energy scores ≤ -7.0 kcal/mol) of the compounds taraxerol, friedelin and stigmasterol against the SARS-CoV-2 spike, M^{pro} and RdRp proteins conferred an appropriate scope to further evaluate their physicochemical attributes related to drug-likeness. Lipinski's rule of five proposes that a drug candidate with logarithm of partial coefficient (log P) $\leq$ 5, molecular weight $\leq$ 500 Dalton, number of hydrogen bond acceptors $\leq$ 10 and number of hydrogen bond donors $\leq$ 5 exhibits decent gastrointestinal absorption and enhanced membrane permeability (Lipinski et al., 2001). A thorough investigation revealed that all the three compounds (Table 4) were in accordance with Lipinski's rule of five (RO5), with slightly higher log P (logarithm of partial coefficient) values

Table 4. Physicochemical properties and drug-likeness features of the selected phytochemicals from various *Clerodendrum* spp.

Phytocompound	Molecular weight (g/mol)	Number of Hydrogen bond acceptor (HBA)	Number of Hydrogen bond donor (HBD)	log P	TPSA (Å^2)	Number of rotatable bonds	Bioavailability Score	PAINS Structural alert	Mutagenicity (AMES mutagenesis)	Cytotoxicity
Taraxerol	426.72	1	1	8.1689	20.23	0	0.55	0 alert	No	No
Friedelin	426.72	1	0	8.457	17.07	0	0.55	0 alert	No	No
Stigmasterol	412.69	1	1	7.8008	20.23	5	0.55	0 alert	No	No
Arbidol	477.41	4	1	5.177	80.00	8	0.55	1 alert	No	No
Lopinavir	628.80	5	4	4.3281	120.00	17	0.55	0 alert	No	No
Ritonavir	720.94	7	4	5.9052	202.26	22	0.17	0 alert	No	No
Remdesivir	602.58	12	4	2.31218	213.36	14	0.17	0 alert	No	No
Favipiravir	157.10	4	2	−0.9921	88.84	1	0.55	0 alert	No	No

TPSA- Topological Polar Surface Area; log P- Logarithm of partial coefficient; PAINS- Pan-Assay Interference Structures.

Table 5. Free energies of binding (ΔG_{bind} MM-GBSA) of the concerned protein-ligand complexes.

	ΔG_{bind} MM-GBSA (kcal/mol)		
Ligands	RBD of the SARS-CoV-2 spike protein (6LZG)	SARS-CoV-2 protease M^{pro} (6LU7)	SARS-CoV-2 RdRp (7BV2)
Taraxerol	−45.19	−58.53	−43.72
Friedelin	−42.22	−51.11	−39.24
Stigmasterol	−41.25	−48.15	−37.78

than the optimum. In this pretext, it is worth mentioning that most of the oral drugs frequently used in the treatment of cancer have log P values higher than the optimum range proposed by Lipinski's rule of five (O'neill & Twelves, 2002). Veber's rule suggests that a potential therapeutic molecule with the number of rotatable bonds $\leq$ 10 and topological polar surface area (TPSA) $\leq$ 140Å^2 tends to display decent oral bioavailability (Veber et al., 2002). The compounds taraxerol, friedelin and stigmasterol were noted to be in agreement with the Veber's rule and our observation correlated well with their decent bioavailability score of 0.55 (Table 4) (Naidoo et al., 2020). The physicochemical features of the compounds were further compared with the relevant drugs like arbidol, lopinavir, ritonavir, remdesivir and favipiravir that are presently being employed against SARS-CoV-2, with a motive to infer about the potential druggability of the compounds in relevance to the drugs. It was striking to note that the drugs lopinavir (molecular weight $>$ 500 dalton; number of rotatable bonds $>$ 10), ritonavir (molecular weight $>$ 500 dalton; number of rotatable bonds $>$ 10; TPSA $>$ 140Å^2) and remdesivir (molecular weight $>$ 500 dalton; number of rotatable bonds $>$ 10; TPSA $>$ 140Å^2) violated the rules of drug-likeness. The compounds taraxerol, friedelin and stigmasterol were predicted not to possess any structural alerts associated with toxic consequences on PAINS toxicophore estimation (Table 4) (Naidoo et al., 2020; Pouliot & Jeanmart, 2016). However, the drug arbidol was observed to possess 1 structural alert. The phytocompounds were found to display relatively better physicochemical and drug-likeness features compared to the drugs that are presently being employed against SARS-CoV-2. An *in silico* toxicity estimation revealed that all the compounds were potentially non-mutagenic and non-cytotoxic (Table 4) and our observations were found to be consistent with previous reports demonstrating their non-toxic nature (Al-Snafi, 2016; Ayaz et al., 2019; Boldrin et al., 2013; Borges et al., 2013; Subash-Babu et al., 2017; Surapaneni & Prakash, 2018). Thus, the concerned phytocompounds exhibited promising drug-like features and provide further scopes to be validated *in vitro* and *in vivo* towards the development of effective therapeutics against COVID-19 (Blomme & Will, 2016).

3.6. Investigation of MM-GBSA binding free energies of the protein-ligand complexes

Decent binding energy scores ($\leq$ −7.0 kcal/mol) of the compounds taraxerol, friedelin and stigmasterol against the SARS-CoV-2 spike, M^{pro} and RdRp proteins offered scopes to further estimate the free energies of binding of the concerned protein-ligand complexes using the MM-GBSA method. It has been suggested that MM-GBSA provides apt estimates of free energies of binding of protein-ligand complexes and a more negative value indicates stronger binding (Al-Khafaji et al., 2020). A detailed investigation of the MM-GBSA binding free energy scores (ΔG_{bind}) of the selected protein-ligand complexes revealed that the compound taraxerol displayed higher binding potential with the respective SARS-CoV-2 proteins in comparison to the compounds friedelin and stigmasterol ($p < 0.01$), as evident from the ΔG_{bind} MM-GBSA scores of taraxerol with the SARS-CoV-2 spike (-45.19 kcal/mol), M^{pro} (-58.53 kcal/mol) and RdRp (-43.72 kcal/mol) proteins (Table 5). Our observations signified a better binding efficacy of taraxerol with the concerned SARS-CoV-2 proteins relative to friedelin and stigmasterol.

3.7. Analysis of molecular dynamics simulations

The compound taraxerol exhibited better binding potential with the SARS-CoV-2 spike, M^{pro} and RdRp proteins in terms of binding energy (Tables 1–3) and ΔG_{bind} MM-GBSA scores (Table 5) in comparison to the phytochemicals friedelin and stigmasterol. Accordingly, the complexes of taraxerol with the respective SARS-CoV-2 proteins were selected for MD simulations along a timescale of 40 ns to validate the results of molecular docking and assess their conformational stability. The taraxerol-RBD SARS-CoV-2 spike protein complex displayed fluctuations in RMSD values of Cα atoms until 31 ns and attained stability thereafter (Figure 4). The taraxerol-SARS-CoV-2 M^{pro} complex was noted to experience initial fluctuations in RMSD values of Cα atoms until 25 ns after which it attained equilibrium and remained stable (Figure 4). The taraxerol-SARS-CoV-2 RdRp complex exhibited significant

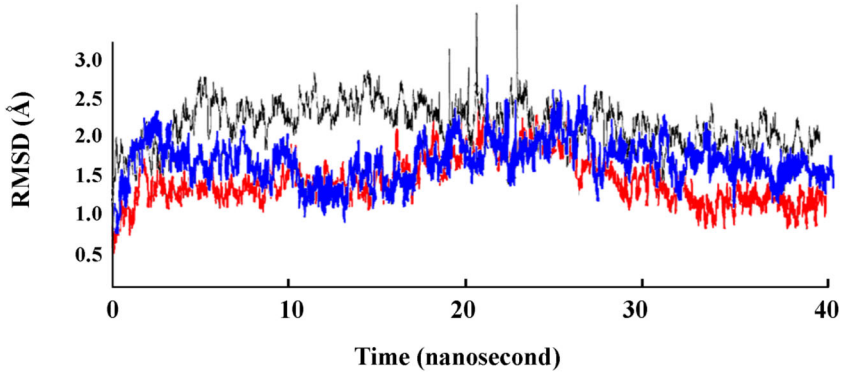

Figure 4. RMSD analysis of the complexes taraxerol-RBD SARS-CoV-2 spike protein (red), taraxerol-SARS-CoV-2 M^{pro} (black) and taraxerol-SARS-CoV-2 RdRp (blue) along a timescale of 40 ns.

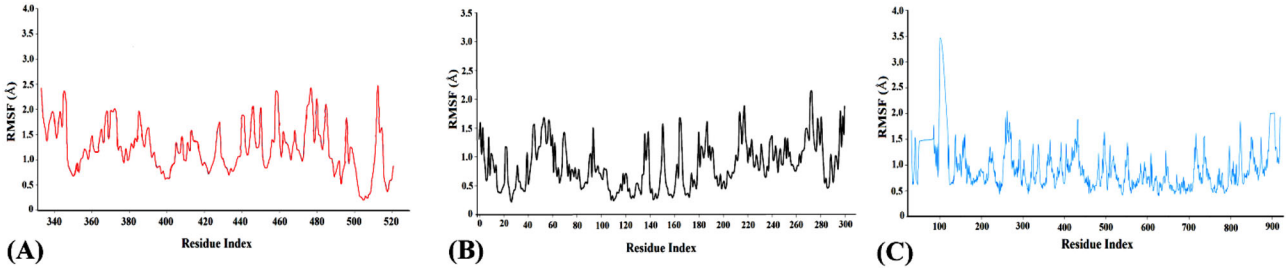

Figure 5. RMSF analysis of the complexes **(A)** taraxerol-RBD SARS-CoV-2 spike protein (red) **(B)** taraxerol-SARS-CoV-2 M^{pro} (black) and **(C)** taraxerol-SARS-CoV-2 RdRp (blue).

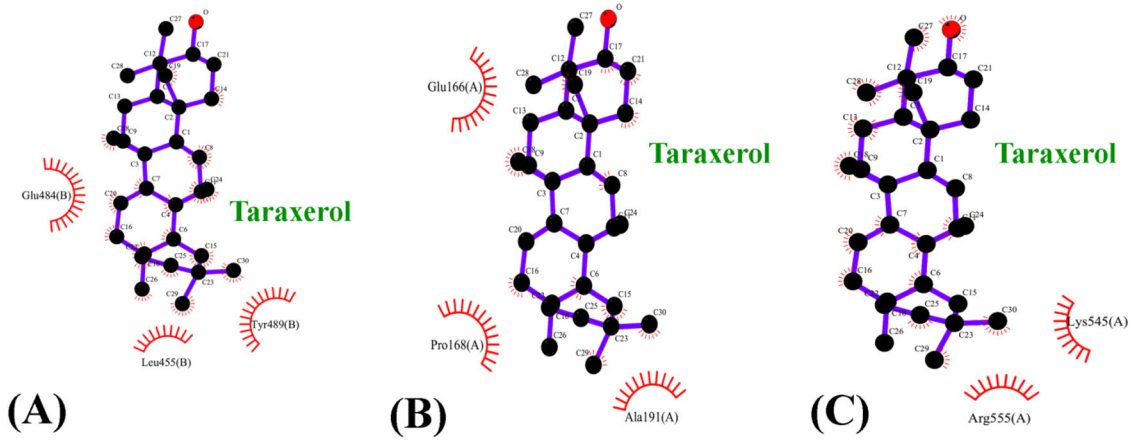

Figure 6. Interaction profile (Ligplot image) of the complexes **(A)** taraxerol-RBD SARS-CoV-2 spike protein **(B)** taraxerol-SARS-CoV-2 M^{pro} **(C)** taraxerol-SARS-CoV-2 RdRp. Amino acid residues displaying hydrophobic interactions with taraxerol in the respective figures have been marked in red.

dynamicity in RMSD values of Cα atoms in the initial phases of MD simulation. However, it attained considerable stability after 27 ns and remained in equilibrium (Figure 4). Our observations reflected the conformational stability of the complexes and indicated towards the decent binding potential of taraxerol against the SARS-CoV-2 spike, M^{pro} and RdRp proteins (Khan et al., 2020).

Analysis of root mean square fluctuation (RMSF) of the residues of a protein-ligand complex is effective in addressing local changes along a protein chain and deciphering its conformational stability (Muralidharan et al., 2020). Extensive analysis of RMSF pertaining to the complexes revealed that the average RMSF values were 1.9 Å, 1.3 Å and 1.6 Å for the complexes taraxerol-RBD SARS-CoV-2 spike protein (Figure 5A), taraxerol-SARS-CoV-2 M^{pro} (Figure 5B) and taraxerol-SARS-CoV-2 RdRp (Figure 5C) respectively. The peaks in the RMSF plot depict the residues which experience major fluctuations during the MD simulations (Muralidharan et al., 2020). It was evident from our analysis that the residues in the active binding pockets of the SARS-CoV-2 proteins that interacted with taraxerol (Tables 1–3) were relatively stable (Figures 5A-C) throughout the course of the MD simulations and thus, reflected the conformation stability of the respective complexes.

Conformational stability of a protein-ligand complex is inferred when there are no considerable changes in the interaction patterns of a complex prior to and after MD simulations (Khan et al., 2020). Extensive analysis of interaction profiles of the complexes taraxerol-RBD SARS-CoV-2 spike protein, taraxerol-SARS-CoV-2 M^{pro} and taraxerol-SARS-CoV-2 RdRp after MD simulations for 40 ns revealed that they did not suffer any change in their binding patterns. The interacting residues prior to and after the MD simulations of 40 ns were found to be the same (Figure 6A–C and Tables 1–3). Thus, it was evident that the complexes were conformationally stable and supported the initial observations of the molecular docking studies.

4. Conclusion

COVID-19 pandemic has afflicted human population worldwide with its calamitous impact, and rapid spread, transmission and infectivity. There has been an utmost demand for the development of specific drugs and vaccines targeted against SARS-CoV-2, the causal agent of COVID-19. The present research venture aimed at exploring the inhibitory prospects of the phytocompounds from *Clerodendrum* spp. revealed that taraxerol was indubitably the most promising inhibitory candidate against the SARS-CoV-2 spike, M^{pro} and RdRp proteins, followed by friedelin and stigmasterol, as evident from the binding energy scores. The fact that taraxerol displayed decent binding potential against all the concerned SARS-CoV-2 proteins included in the present analysis might provide a foundation for it to be deployed as a universal target against SARS-CoV-2 with further *in vitro* and *in vivo* validations required to draw a final inference.

Acknowledgements

PK acknowledges University of North Bengal for providing fellowship to execute this work. AR, NRS and BS acknowledge Lovely Professional University, India for providing the necessary infrastructure facility. AS and PK acknowledge University of North Bengal, India for the infrastructure support to the work. The authors thank Dr. Devashan Naidoo (Centre for Algal Biotechnology, Faculty of Natural Sciences, Mangosuthu University of Technology, South Africa) for critical reading of the manuscript and thorough language correction. We also thank the anonymous reviewers for their suggestions which helped to improve the manuscript.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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