Computational studies reveal piperine, the predominant oleoresin of black pepper (*Piper nigrum*) as a potential inhibitor of SARS-CoV-2 (COVID-19)

Prassan Choudhary[1], Hillol Chakdar[1,*], Dikchha Singh[1], Chandrabose Selvaraj[2], Sanjeev Kumar Singh[2], Sunil Kumar[3] and Anil Kumar Saxena[1]

[1]ICAR-National Bureau of Agriculturally Important Microorganisms, Kushmaur, Mau 275 103, India
[2]Department of Bioinformatics, Alagappa University, Karaikudi 630 003, India
[3]Centre for Agricultural Bioinformatics, ICAR-Indian Agricultural Statistics Research Institute, New Delhi 110 002, India

In this study, we screened 26 bioactive compounds present in various spices for activity against SARS-CoV-2 using molecular docking. Results showed that piperine, present in black pepper had a high binding affinity (–7.0 kCal/mol) than adenosine monophosphate (–6.4 kCal/mol) towards the RNA-binding pocket of the nucleocapsid. Molecular dynamics simulation of the docked complexes confirmed the stability of piperine docked to nucleocapsid protein as a potential inhibitor of the RNA-binding site. Therefore, piperine seems to be potential candidate to inhibit the packaging of RNA in the nucleocapsid and thereby inhibiting the viral proliferation. This study suggests that consumption of black pepper may also help to combat SARS-CoV-2 directly through possible antiviral effects, besides its immunomodulatory functions.

Keywords: Binding affinity, black pepper, COVID-19, homology modelling, piperine.

SARS-CoV-2 (COVID-19) is a novel human coronavirus belonging to *Betacoronaviruses* which originated from Wuhan Province in China[1,2]. Since its outbreak around November 2019, it has created havoc in more than 200 countries infecting about two million people and leading to 1.5 lakh deaths globally[3]. Throughout the world, scientists are engaged in the development of a vaccine in order to curb its viral action. According to Li *et al.*[2], a total 73 vaccines are at preclinical or exploratory stages, while five candidate vaccines have entered phase-I clinical trial[4]. Most of the lead candidates have structural spike protein or the main protease (M^{pro}, $3CL^{pro}$) as their drug target[5–7]. There are several limitations to these drug targets as mutations in the S protein can help the virus elude the therapeutic target and also lead to changes in host-cell receptor binding conformations[8]. Inhibitors of protease have the risk of causing severe side effects as they can inhibit the cellular homologous proteases non-specifically[9]. Whole genome sequencing (WGS) has played a crucial role in paving the way for exploration of novel drug targets[10]. The GISAID database has undertaken a global initiative and currently holds WGS of approximately 9300 different isolates of SARS-CoV-2, characterizing the epidemiology and functional annotation of this virus genome (https://www.gisaid.org/).

Nucleocapsid (NC) is a highly conserved zinc finger structural protein which plays a crucial role in viral replication[11,12]. This multimeric protein encapsulates the viral genome while also facilitating entry into human cells through the ACE2 receptors[13]. NC along with Nsp3 plays a pivotal role in the coronavirus life cycle by controlling the replication–transcription complexes[14]. More importantly, NC is necessary for viral RNA packaging in the early stages of viral infection[15]. These properties of NC make it a suitable drug target for a first generation of anti-NC drugs. With therapeutic vaccines not available as early as 2021 (ref. 16), there is an urgent requirement to promote complementary and alternative medicine (CAM) practices in order to combat this sudden outbreak till any concrete therapies/vaccines are available globally[17]. Moreover, alternative medicines are essential for developing countries which cannot bear the cost of vaccines[18]. Lack of enough testing kits and efficient outreach programmes for the promotion of such vaccines also greatly hinders the cause.

The Ministry of Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homoeopathy (AYUSH), Government of India (GoI) has issued an advisory on immunity-boosting measures which can help to fight SARS-CoV-2 infection. They have outlined in detail the use of spices like clove, ginger, cinnamon, black pepper, dalchini, cumin, ajwain, etc. to boost immunity (https://www.mohfw.

*For correspondence. (e-mail: hillol.chakdar@gmail.com)

gov.in/pdf/ImmunityBoostingAYUSHAdvisory.pdf). Spices and other Ayurvedic remedies are known to contain diverse bioactive compounds and well documented to possess immunomodulatory properties[19–21]. It is also known that spices like ginger, turmeric, black pepper, etc. also have antimicrobial activities[21].

In this study we examined whether such spices can really be effective against SARS-CoV-2 apart from boosting immunity. Briefly, we predicted the 3D structure of N-terminal RNA-binding domain of NC protein of SARS-CoV-2 Wuhan-Hu-1 using homology modelling. Molecular docking has been employed to screen predominant bioactive compounds found in spices commonly used in households and as advised by the Ministry of AYUSH, GoI. We performed molecular dynamic simulation which provided evidence to validate our findings. The study throws light on the significance of these natural compounds in the fight against COVID-19.

Methods

Data retrieval

The annotated sequence of NC protein sequence of SARS-CoV-2 Wuhan-Hu-1 was obtained from the National Center for Biotechnology Information (NCBI; protein id: YP_009724397.2). BLASTp and CDD were used to determine the N-terminal domain conserved site of the protein[22]. The sequence was curated to locate the NTD, eliminating the rest of the amino acids from the study. The truncated protein (200 amino acids) was used for further analysis.

The 3D structures of 26 natural compounds from seven different spices and few other drugs, including known synthetic anti-HIV analogues targeting NC used in the study were directly imported from Pubchem database using UCSF Chimera v1.13.1 (Table 1)[23]. The structures of adenosine monophosphate (AMP) and three synthetic analogues were also imported to examine their affinity to SARS-CoV-2 NC structure (Table 1). The imported ligand structures were prepared using Dock Prep tool of Autodock Vina, as reported previously[24]. Charges were computed using ANTECHAMBER with AMBER ff14SB charges allotted to standard residues and Gasteiger charges to other residue types, as reported in previous studies[25]. The receptor protein was prepared following the same protocol, barring the computation of charges step. All the prepared files were stored in .mol2 format for further evaluation and docking .analysis.

Homology modelling and Ramachandran plot analysis

Modeller v9.20 was used for homology modelling of the protein sequence using Python script[26]. The PDB ID of templates along with their percentage identity were: (i) 6M3M (100%), (ii) 6YI3 (99.28%), (iii) 1SSK (92.03%), (iv) 6VYO (100%), and (v) 2OFZ (92.06%). The best template (6YI3) was chosen based on high-resolution (1 Å), query coverage (69%) and percentage identity. On the basis of the lowest DOPE score, the final model was selected. RAMPAGE was used to carry out Ramachandran plot analysis and was represented using Discovery Studio module[27,28]. Expresso tool of T-COFFEE server was used for sequence alignment of the query sequence with the templates using default parameters[29]. The modelled structure was superimposed onto the template 6YI3 using PYMOL software package[30].

Molecular docking and interaction studies of SARS-CoV-2 NC

Autodock Vina module of UCSF Chimera was used for the docking studies on a Windows 10 platform[23]. The prepared .mol2 files of receptor and ligands were imported and a search volume allotted to the receptor molecule for each docking study keeping all other parameters constant[31]. The software uses Opal web service for docking and the files were allotted executable location on the local host computer. The best Autodock Vina score with the suitable energetically favoured conformations was used for further analyses. The interaction of compounds with amino acid residues was analysed and represented using Discovery Studio Client.

Molecular dynamics simulation

The apo protein of Npro (N protein) and the ligand complexes (Npro-AMP, Npro-piperine) were prepared, hydrogen bond-optimized, and the final complexes were minimized till the root mean square deviation (RMSD) value reached 0.30 Å (ref. 32). The prepared complex was subjected to molecular dynamics simulation to understand the molecular stability of protein and protein–ligand complex using the Desmond MD package, as described earlier[33,34].

Results and discussion

Homology model of SARS-CoV-2 NC

The 3D structure of NC protein had a sequence identity of 99.28% with the template (PDB : 6YI3), with a query coverage of 69% (Figure 1 *a*). Two pairs of anti-parallel β-sheets (β-hairpin) with a β-core were found in the structure. Ramachandran plot analysis showed 96% of the residues in the favoured region and 2% in the allowed region, making it a robust structure (Figure 1 *b*). The modelled structure was used for docking and interaction

Table 1. Bioactive natural compounds used in the study along with their sources

Plant species		Important chemical constituents	Reference
Scientific name	Common name		
Syzygium aromaticum	Clove	Eugenol (~94.4% of essential oils in clove oil) β-caryophyllene (~3.56% of essential oils)	47, 48
Cinnamomum zeylanicum	Cinnamon	Cinnamaldehyde (65–80% of essential oils in bark), cinnamic acid and eugenol (5–10% of essential oils in bark)	49
Piper nigrum	Black pepper	δ-3-Carene (~2% of essential oils), limonene (~19% of essential oils), β-caryophyllene (~15% of essential oils), sabinene (~16% of essential oils), β-pinene (~11% of essential oils) α-pinene (6% of essential oils), piperine and its isomers chavicine, isochavicine and isopiperine (35–55% of oleoresins).	35, 50
Nigella sativa	Black cumin	Thymoquinone (30–48% of active compounds of seeds)	51
Ocimum sanctum	Basil/tulsi	Eugenol (67–72% of essential oils) β-elemene (~11% of essential oils) and β-caryophyllene (7–8% of essential oils)	52
Cuminum cyminum	Cumin	Cuminaldehyde (~23% of essential oils), γ-terpinene (~20% of essential oils) *p*-cymene (~19% of essential oils), β-pinene (~16% of essential oils) and 1-phenyl-1,2-ethanediol (~14%)	53
Foeniculum vulgare	Fennel	Anethole (70.1% of essential oils), fenchone (6.9% of essential oils) and methyl chavicol (4.8% of essential oils)	54
Zingiber officinale, *Boesenbergia rotunda* (Zinger family)	Ginger	Gingerol (6.2–6.3%), zingerone (9.25%) and chalcone (12%)	55–57
Synthetic analogues			
CMPD-1			39
CMPD-8			39
Baricitinib			37

studies. Supplementary Figure 1 *a* shows the structural superimposition of the predicted structure with the template 6YI3. All the protein sequences were aligned and conservation profile of the residues have been marked as shown in Supplementary Figure 1 *b*.

Docking and interaction studies

To understand which amino acid residues bind to RNA while packaging, AMP was docked against NC 3D model. AMP had a binding affinity of –6.4 kCal/mol. A total of seven amino acid interactions were found for AMP: three amino acids, viz. SER51 (2.89 Å from O6, 2.73 Å from H13), PHE53 (2.48 Å from H13), ARG149 (2.14 and 2.36 Å from O7, 4.27 Å from P1) interacted with the phosphate group; two amino acids, viz. TYR109 (1.28 Å from H8) and GLU174 (2.52 Å with H7) interacted with the ribose sugar, and two other amino acids, ALA155 (5.18 Å), ALA156 (2.08, 2.32, 3.85 and 4.8 Å) interacted with the nitrogen base. ALA149 was found to have close interactions with the phosphate group of the AMP structure (Figure 2).

Twenty-six compounds were docked onto the SARS-CoV-2 NC, out of which six compounds had a binding affinity of ≥–6.0 kCal/mol (Table 2). Six natural compounds, viz. piperine (Pubchem ID: 638024), chavicine (Pubchem ID: 1548912), isochavicine (Pubchem ID: 1548914), isopiperine (Pubchem ID: 1548913), β-caryophyllene (Pubchem ID: 5281515) and chalcone (Pubchem ID: 637760) had binding affinities of –7.0, –6.8, –6.8, –6.6, –6.4 and –6.0 kCal/mol respectively. Piperine and β-caryophyllene are found abundantly in black pepper (*Piper nigrum*) (Table 1)[35]. The amino acid interactions of piperine were: ALA50 (5.10 Å from benzene ring), ARG88 (2.32 Å from O3), ARG92 (3.95 Å from benzene ring II), TYR109 (4.83 Å from benzene ring II), ARG149 (2.22 Å from O1) and ARG156 (2.46 Å from O1, 4.03 Å from benzene ring I) (Figure 3). Piperidine (Pubchem ID: 8082) and piperic acid (Pubchem ID: 5370536) formed as a result of acid or alkali hydrolysis were also docked against NC and had binding affinities of –3.3 and –5.9 kCal/mol respectively[36]. The remaining natural compounds had a binding energy less than –6.0 kCal/mol and hence was excluded from further analysis. Three potential synthetic analogues were also used for the study. Two of the synthetic anti-HIV analogues targeting NC, i.e. CMPD-1 (Pubchem ID: 26532231) and CMPD-8 (Pubchem ID: 26541579) had binding affinity of –7.0 and –6.8 kCal/mol respectively (Supplementary Figure 2 *a* and *b*). Recently, Baricinitib has been suggested as a drug analogue of SARS-CoV-2 and showed good interactions with SARS-CoV-2 NC having a binding affinity of –7.0 kCal/mol (Supplementary Figure 2 *c*)[37].

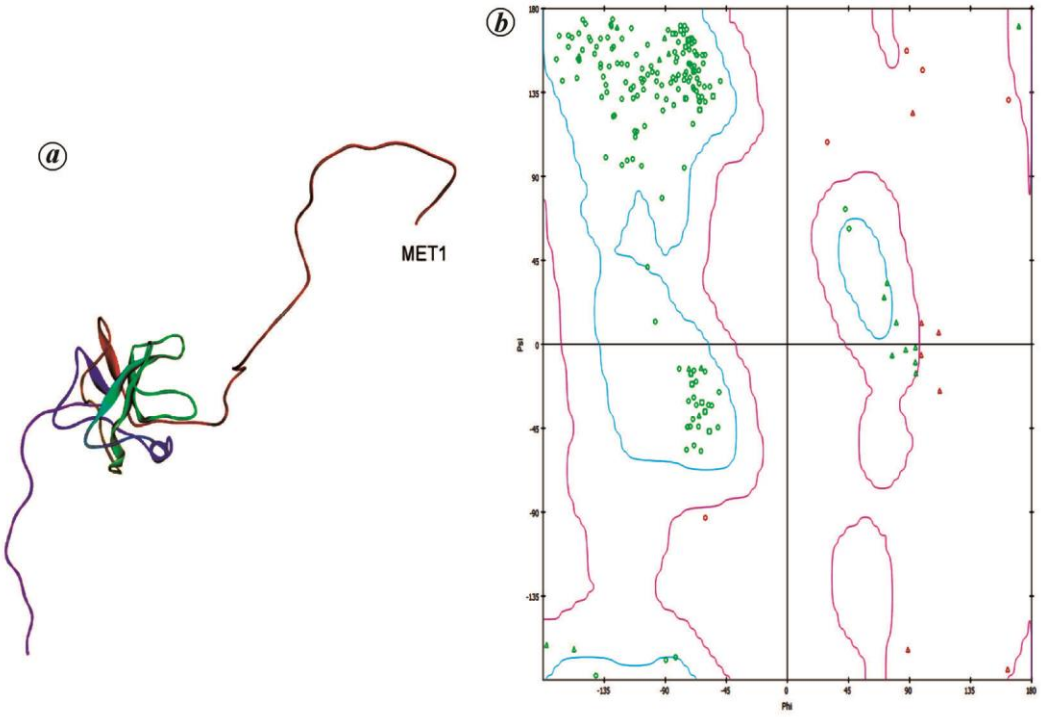


Figure 1. ***a***, Three-dimensional modelled structure of NT-domain of SARS-CoV-2 nucleocapsid (NC). ***b***, Ramachandran plot for the predicted structure depicting the amino acid residues in favoured, allowed and outlier regions.

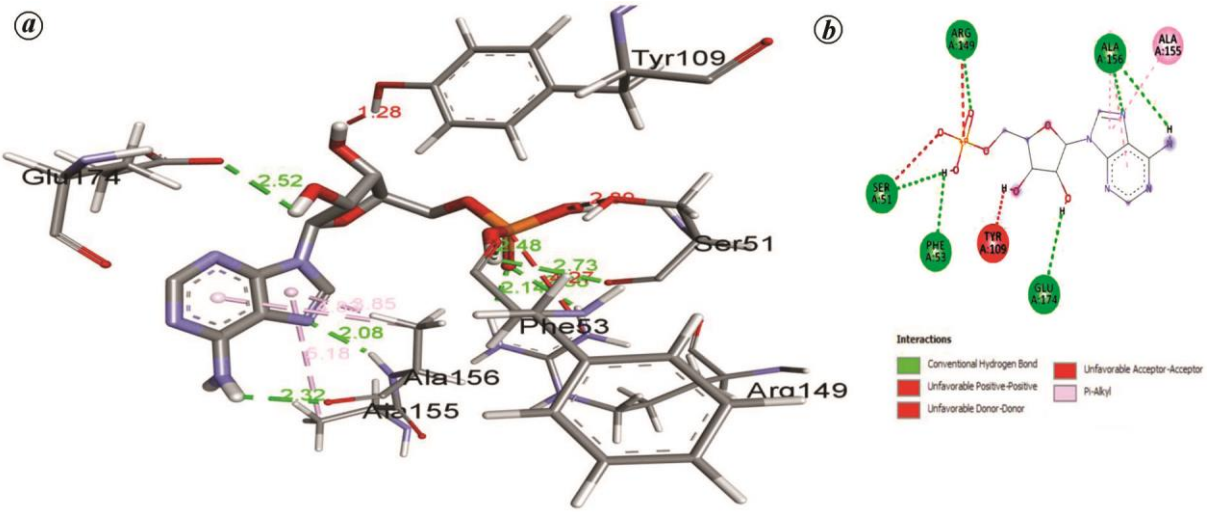
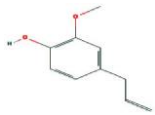
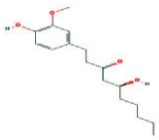
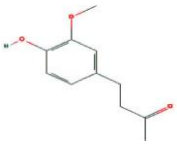
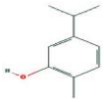
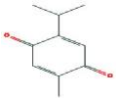
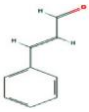
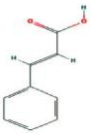
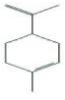
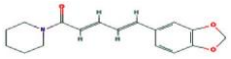
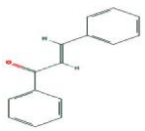


Figure 2. ***a***, Three-dimensional representation of docked adenosine monophosphate (AMP) with SARS-CoV-2 NC. ***b***, Two-dimensinal visualization along with bond types of the interacting residues in the SARS-CoV-2 NC/AMP complex.

As the aim of the study was to examine the potential of natural bioactive compounds present in various spices and herbs, synthetic analogues of piperine were not analysed; rather isomers and related compounds (like isopiperine, chavicine, isochavicine, piperidine and piperic acid) were tested through molecular docking, which established piperine as a potential compound which could have antiviral activities. Therefore, molecular dynamics simulation was also performed.

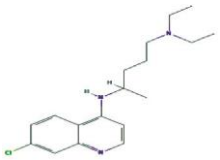
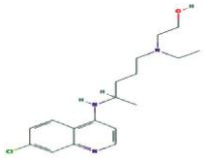
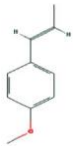
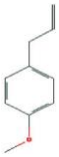
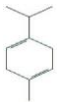
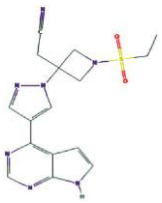
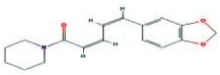
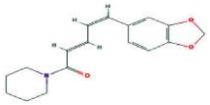
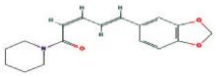
The Apo protein and the other two protein–ligand complexes were simulated and RMSD values were noted with the reference value to its initial position, for understanding the structural deviations in the dynamic environment for the timescale of 50 ns. The values were calculated from 0 to 50 ns and plotted (Figure 4 *a*). The Apo protein showed initial deviation between ~1 and ~2 Å till the 5th ns (Figure 4 *a*). Thereafter, the deviations were limited, attaining a stable position till the

Table 2. List of natural compounds from spices and few synthetic antiviral compounds with their binding affinity, 2D structures and interacting residues

Compound	Pubchem ID	2D structure	Binding affinity (kcal/mol)	Interacting residues (three-letter code)
Eugenol	3314		–4.9	LEU56, LEU159, LEU161, LEU167, ALA173
Gingerol	442793		–5.2	ARG88, TYR109, TYR111, THR115, GLY116
Zingerone	31211		–5.2	LEU161, LEU167
Carvacrol	10364		–5.2	ALA50, SER51, TYR109, TYR111, PRO151, ALA156
Thymoquinone	10281		–5.5	ALA50, SER51, TYR109, PRO151, ALA156
Cinnamaldehyde	637511		–4.7	ALA50, ARG88, ARG149, PRO151, ALA156
Cinnamic acid	444539		–5.3	ALA50, ARG88, TYR109, ALA156
α-Pinene	440968		–5.1	LEU56, LEU159, LEU161, LEU167, ALA173
Sabinene	18818		–4.9	LEU56, LEU159, LEU161, LEU167, ALA173
β-Caryophyllene	5281515		–6.4	LEU161, LEU167
δ-3-Carene	26049		–5.0	LEU56, LEU159, LEU161, LEU167, ALA173
Limonene	22311		–5.0	LEU56, LEU159, LEU161, LEU167, ALA173
β-Pinene	440967		–5.1	LEU161, LEU167, ALA173
Piperine	638024		–7.0	ALA50, ARG88, ARG92, TYR109, ARG149, ALA156
Calcone	637760		–6.0	LEU161, THR166, LEU167, ALA173

(*Contd*)

Table 2. (*Contd*)

Compound	Pubchem ID	2D structure	Binding affinity (kcal/mol)	Interacting residues (three-letter code)
Chloroquine	2719		–5.3	LEU167
Hydroxychloroquine	3652		–5.7	LEU159, LEU161, PRO162, THR165, ALA173
Anethole	637563		–4.8	ALA50, TYR109, ARG149, PRO151, ALA156
Fenchone	14525		–5.1	ALA50, SER51, TYR109, ALA156
Methyl chavicol	8815		–4.7	LEU56, LEU159, LEU161, LEU167, ALA173
Cuminaldehyde	326		–5.1	ALA50, ARG88, TYR109, ALA156
γ-Terpinene	7461		–5.0	LEU159, LEU161, LEU167, ALA173
p-Cymene	7463		–5.0	LEU56, LEU159, LEU161, LEU167, ALA173
Baricitinib	44205240		–7.0	THR49, ARG88, ARG92, TYR109
Chavicine	1548912		–6.8	ALA50, ARG92, ARG149, ALA155, ALA156
Isochavicine	1548914		–6.8	LEU161, LEU167
Isopiperine	1548913		–6.6	ALA173
Piperidine	8082		–3.3	ALA123,ALA138

(*Contd*)

Table 2. (*Contd*)

Compound	Pubchem ID	2D structure	Binding affinity (kcal/mol)	Interacting residues (three-letter code)
Piperic acid	5370536		–5.9	LEU161, LEU167, PHE171
CMPD-1	26532231		–7.2	LEU161, LEU167, ALA173, TYR172, ARG177
CMPD-8	26541579		–6.8	ALA50, TYR109, PRO151, ALA156
Adenosine 5′-mono phosphate	6083		–6.4	SER51, PHE53, TYR109, ARG149, ALA155, ALA156, GLU 178

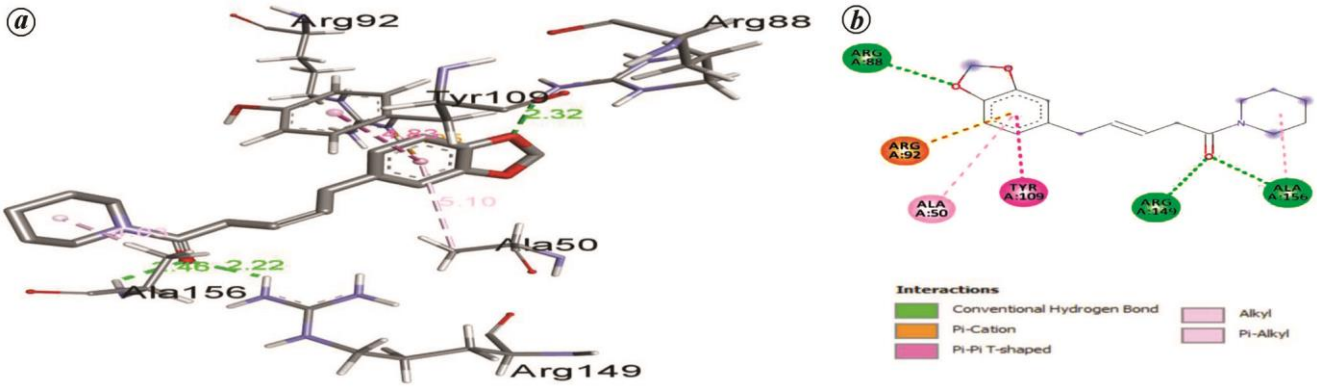


Figure 3. ***a***, Three-dimensional representation of docked piperine with SARS-CoV-2 NC. ***b***, Two-dimensional visualization along with bond types of the interacting residues in the SARS-CoV-2 NC/piperine complex.

35th ns. Then we could see fluctuations due to the loop regions functioning as the high deviating regions. Overall, the Apo protein remained stable beyond 35th ns till 50th ns. While keeping the Apo reference, as it was noticed that the ligand complex did not suit the phenomenon, as both the ligand complexes were reacting in different manner due to ligand binding. The Npro complexed with AMP showed significant stability throughout the simulation due to proper attachment of AMP to the binding pocket, that made prominent in the MD simulation to be stable throughout the simulations. The 5th to 40th ns seemed to be a stable position and at 40th ns, the ligand binding gained interactions with the loop regions, and thus a slight deviation occurred in the 40th to 50th ns. In the measurement, AMP complexed with Npro was positioned in ~2.6 to 3.6 Å levels for the whole simulation time of 50 ns.

The piperine-bound Npro complex showed initial stability till the 35th ns and deviations occurred thereafter. Overall, in this simulation, the loops played important role in the stability and ligand binding. For understanding the reliability of interactions, the hydrogen-bonds were calculated for each 10 ns average intervals and plotted (Figure 4 *b*). The results showed that the variations were clearly visible for 0–30th ns and the 30–50th ns. Both the ligands showed prominent binding throughout the 50 ns of the MD simulations. The ligand molecule AMP dominated in the H-bond formation, rather than piperine. On an average AMP formed 1.8 hydrogen bonds throughout the MD simulations, while piperine could form 1.5 hydrogen bonds in the 50 ns of the MD simulations. AMP and piperine were well adopted to form strong hydrogen-bonding interactions with Npro and were able to adjust with the loop regions, and thus showed prominence

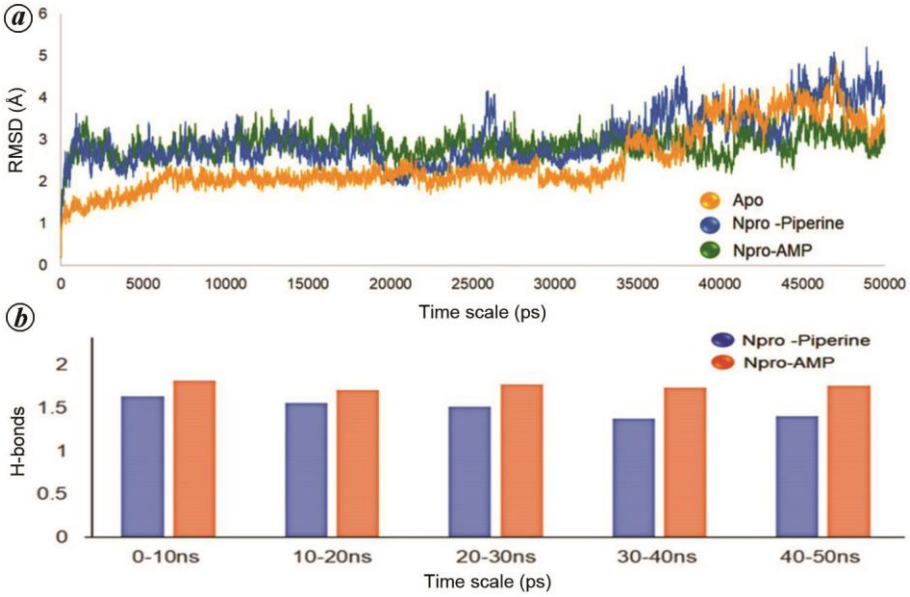


Figure 4. ***a***, RMSD (Å) values of Npro (Apo), Npro complexed with piperine and AMP for the time-scale of 50 ns. ***b***, H-bond interactions of Npro with AMP and piperine for each 10 ns interval.

in binding in the dynamic environment. Overall, molecular dynamics simulation revealed that binding of piperine could be as stable as that of AMP.

NC is a well-established drug target for major viral diseases like acute immunodeficiency syndrome (AIDS), Middle East respiratory syndrome coronavirus (MERS-CoV), chikungunya, swine fever virus, etc.[12,38–40]. It is a well-conserved protein with key roles in the replication and life cycle of SARS-CoV-2. Increasing efforts are being made to search for lead molecules in order to fight against this pandemic[6,7]. Spices like *Syzygium aromaticum*, *P. nigrum*, *Cinnamomum zeylanicum*, *Nigella sativa*, etc. have an abundance of natural compounds possessing antimicrobial properties. The Indian subcontinent is well-known for the production and export of spices worldwide, these are household consumables of the country[41,42].

The interactions of AMP with the NC domain revealed the RNA-binding domain of the protein, where ARG149 was an important amino acid due its close interaction with the phosphate group. Upon molecular docking of the natural compounds, it was found that piperine had a strong binding affinity towards SARS-CoV-2 NC and also interacted strongly with ARG149. The binding affinity of piperine and its isomers was higher than that of AMP. While β-caryophyllene and chalcone also showed favourable binding to the NC structure, their binding energy was lower than that of AMP. The results clearly indicate that piperine has the potential to block the RNA binding pocket of SARS-CoV-2 NC. Three of the pocket residues, viz. ARG149, TYR109 and ALA155 were occupied by piperine as well as AMP, which clearly proves that piperine with a binding affinity more than that of AMP is more likely to occupy this RNA binding pocket. Molecular dynamics simulation also revealed that the binding of piperine to the N-terminal of NC was quite stable. Interestingly, the binding affinity of piperine was found to be equivalent to that of synthetic analogues like CMPD-1, CMPD-8 and Baricitinib targeting NC[37,39].

Piperine is the predominant oleoresin of black pepper responsible for its pungency[43]. This compound is widely known for its antihypertensive, anti-asthmatic, antidepressant, antitumour and anti-carcinogenic properties[43,44]. However, antiviral properties have not been extensively and exclusively reported. In a study published in 2010, it was shown that a food supplement made up of black pepper, garlic and ginger (1 : 16 : 4) had a curative effect against chikungunya epidemic in Kerala during 2006–09. Our findings clearly indicate that black-pepper extracts containing piperine may be an effective means to control the proliferation of viral particles inside the human body due to its potential to block RNA packaging inside the capsid protein. Piperine has also been reported for its bioavailability-enhancing effects[45]. For example, Kasibhatta and Naidu[46] reported increased bioavailability of nevrapine used against HIV/AIDS. Therefore, use of black pepper in daily foods or incorporation of piperine with other drugs can be an effective means to combat the SARS-CoV-2 pandemic.

Conclusion

The results of the present study highlight piperine as a potential natural compound targeting NC of SARS-CoV-2 and possibly blocking the RNA packaging in the

protein. Therefore, intake of black pepper or piperine can help control viral proliferation. However, specific laboratory-based and clinical studies are required to substantiate the findings of this study. Nevertheless, the advisory issued by the Ministry of AYUSH, GoI should be followed to combat the SARS-CoV-2 pandemic, as the results of this study also indicate the possible anti-SARS-CoV-2 role of black pepper.

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