Prayogik Rasayan

Targeting COVID-19 (SARS-CoV-2) RNA dependent RNA polymerase through active phytochemicals of ayurvedic medicinal plants Limonia acidissima Linn. And Ocimum sanctum: A molecular docking study

Avishek Ghosh,* Putul Karan

Department of Chemistry, Midnapore City College

Kuturia, Bhadutala, Paschim Medinipur, West Bengal 721129, India.

Email: avishek.ghosh.mcc@gmail.com

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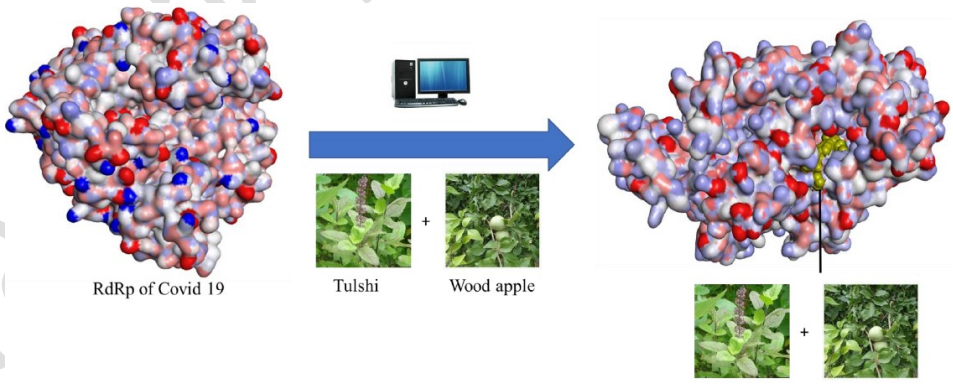
Abstract

Virtual screening of phytochemicals was performed through molecular docking to identify the potential hits that can inhibit the effects of SARS-CoV-2. Considering the published literature on medicinal importance, the phytochemicals from tulsi and wooden apple were selected to search potential inhibitors for RdRp (RNA dependent RNA polymerase) of SARS-CoV-2. The *in silico* computational results revealed that the phytochemicals such as Limonin, Obacunone, Rutaevin,(-)-(2S)-5,3'-Dihydroxy-4'-methoxy-6'',6''-dimethylchromeno-(7,8,2'',3'')-flavanone, Lupeol, 5-Hydroxy-2-(-hydroxyphenyl)-7-methoxy-6-(3-methylbut-2-enyl)chroman-4-one, Vitexin, Orientin, and Urosolic acid were found to be effective against the target RdRp (RNA dependent RNA polymerase) of SARS-CoV-2. The protein-ligand interaction study revealed that these phytochemicals bind with the amino acid residues at the active site of the target proteins with a higher binding affinity than remdesivir. Therefore, the core structure of these potential hits can be used for further lead optimization to design drugs for SARS-CoV-2. Also, the medicinal plants containing these phytochemicals like tulsi and wooden apple can be used to formulate suitable therapeutic approaches in traditional medicines.



Keywords: Coronavirus COVID-19, Molecular docking, tulsi, wood apple

1. Introduction

Covide-19 is a deadly viral infection where the disease is caused by the SARS-COV-2. The SARS-CoV-2 belongs to the family of Coronaviruses, which is an RNA virus, and after SARS and MERS, it is the third highest deadly human coronavirus reported in the 21st century [1]. The non-availability of medically proven effective drugs or vaccines is the main concern of the COVID-19 pandemic [2]. Therefore, effective measures like lockdown, rapid testing, social distancing, and use of face masks and hand sanitizer, etc. are taken to fight against this deadly virus [3-8]. To propose an effective and long-lasting solution, understanding the structure of the virus and its action is very important. The literature survey shows there are mainly two types of proteins that are characterized

by structural and non-structural proteins. Structural proteins comprise spikes, nucleocapsids, matrix, and envelope proteins whereas non-structural proteins include RNA-dependent RNA polymerase protein (RdRp) [9-11].

The RdRp protein plays a crucial role during this replication process. RdRp is the most significant gene in the virus genome which catalyses the synthesis of a complementary RNA strand using the virus RNA template and helps the virus for its multiplication into the human cell [12-16]. Literature studies reported that antiviral drug Remdesivir is a potent inhibitor to virus replication targeting the RNA-dependent RNA polymerase (RdRp) [17]. Despite some promising results further, clinical studies are required regarding efficacy and safety in the treatment of COVID-19 [18]. Thus, identification or discovery of new effective antivirals is urgently needed to fight the worldwide corona crisis [19]. Computer-aided drug discovery/design methods have played a major role in the development of therapeutically important small molecules for over three-decade, screening chemical virtual libraries using computational methods as molecular docking can save money and reduce time [20] consequently, speed up the identification of potential drug candidates [21-22]. Several research groups have come up with interesting strategies such as repurposing existing drugs or natural products to fight against COVID19 [23-25]. The current research work was focused to screen phytochemicals found mainly in the Indian medicinal plants with the important objectives to search new natural compounds that bind effectively at the active sites of RdRp of SARS-CoV-2 with no side effect. In this study, the biologically active components of Limonia acidissima Linn. (most commonly called wood apple) [26] and Ocimum sanctum Linn. (commonly called Tulsi) [27] are virtually screened against the RdRp (6M71) of SARS Co-V-2. to propose the potential hits against SARS-CoV-2.

2. Experimental

In this study, 3D structures of the phytochemicals are retrieved from the PubChem database. All the 3D structures are converted into PDBQT format before performing docking using Autodock Vina. Similarly, the 3D structures of RdRp (6M71) proteins are downloaded from the Protein Data Bank. Water molecules, ions, and other ligands present in the protein targets are removed and the structures are converted into PDBQT format for molecular docking analysis. A grid box with a size of (90,70,90) and the center of (119.721, 123.605, 120.300) are set to cover the entire protein. Visual inspection of docking poses and the analysis of protein-ligand interactions are performed using Biovia Discovery Studio Visualizer version 2016 (Dassault systems Biovia corp) (Dassault Syst_emes BIOVIA, 2016) and PyMOL 2.3 (Schrodinger L.L.C).

3. Result and discussion:

NSP12 chain of RdRp, the non-structural protein, is the major replication tool that plays a key role in the transcription cycle of the virus with the help of cofactors NSP7 and NSP8. So, the primary target of the RdRp is NSP12 chain [28]. In literature molecular docking study of phytochemicals of tulshi, and limonoids compounds has been performed on Mpro (Main protease) protein [29]. In this study, the biologically active components of *Limonia acidissima* Linn. (most commonly called wood apple) and *Ocimum sanctum* Linn. (commonly called Tulsi) are virtually screened against the NSP12 protein chain of RdRp of SARS Co-V-2. The biological active compounds Eugenol, Urosolic acid, Carvacrol, Linalool, Caryophylline, Estragol, Rosmarinic acid and Apigenin of Tulshi and Acidissiminol, (-)-(2S)-5,3'-Dihydroxy-4'-methoxy-6'',6''-dimethylchromeno-(7,8,2'',3'')-flavanone, Syringaldehyde, Syringaresinol, Yangambin, 5-(3-Acetoxypropenyl)-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-2,3-dihydroxybenzofuran-3-ylmethyl acetate, Physcion, 4-Methoxy-1-methyl-2-quinolone, Tanakamine, Tanakine, Tembamide, Hederatriol, Limonin, Lupeol, Obacunone, Rutaevin, Bergapten, Psoralen, Orientin, Saponarin, Vitexin, Stigmasterol, Aurapten, Dihydrosuberenol, Isopimpinellin, Marmesin, Osthenol, Osthol and Xanthotoxin, Gallacatechin from wood apple have been used to dock and the cut off value of docking score has been set >8.0 Kcal/mol because the docking score of Remdesivir against RdRp of SARS Co-V-2 is found -8.1 kcal/mol in our in-silico study. Limonin, Obacunone, Rutaevin,(-)-(2S)-5,3'-Dihydroxy-4'-methoxy-6'',6''-dimethylchromeno-(7,8,2'',3'')-flavanone, Lupeol, 5-Hydroxy-2-(-hydroxyphenyl)-7-methoxy-6-(3-methylbut-2-enyl)chroman-4-one, Vitexin, Orientin and Urosolic acid shows docking score -9.5 kcal/mol , -9.0 kcal/mol , -8.8 kcal/mol , -8.4 kcal/mol , -8.3 kcal/mol , -8.3 kcal/mol , -8.1 kcal/mol , -8.1 kcal/mol , -8.1 kcal/mol respectively which were more the docking score of Remdesivir against RdRp of SARS Co-V-2 . The data are summarized in Table-1.

Out of all biological active compounds, Limonin shows the highest binding affinity -9.5 kcal/mol against the protein of SARS Co-V-2. Limonin forms five hydrogen bonding interactions with Lys47A, Tyr129A, Lys714A, Lys780A, and Ser784A, eight hydrophobic interactions with Tyr32A, His133A, Asp135A, Asn138A, Ser709A, Thr710A, Asp711A, Gln773A amino acids of targeted protein chain (Fig 1). Similarly, based on binding energy the second-best compound in our series is Obacunone, the binding energy of which is found -9.0 kcal/mol and forms five pi-alkyl interactions with Leu271A, Pro328A, Val330A, Val398A, and Met666A and Twelve hydrophobic interactions with Leu270A, Lys272A, Tyr273A, Ser325A, Thr324A, Phe326A, Gly327A, Leu329A, Pro378A, Ala379A, Ala382A and Ala383A amino acids (Fig 2). The third best compound is Rutaevin. The binding energy against NSP12 chain

of SARS Co-V-2 is -8.8 kcal/mol and it binds with protein chain by forming four hydrogen-bonding interactions (Tyr32A, Ser709A, Lys714A and Asn781A) and ten hydrophobic interactions (Ala46A, Lys47A, Tyr129A, His133A, Asn705A, Ala706A, Thr710A, Asp711A, Gln773A, and Lys780A) (Fig 3). Next best compound (-)-(2S)-5,3'-Dihydroxy-4'-methoxy-6'',6''-dimethylchromeno-(7,8,2'',3'')-flavanone binds with protein chain, the binding energy of which is -8.4 kcal/mol and forms a pi-cationic interaction with Lys47A, Two hydrogen bonding interactions with Asp140A, Thr141A and twelve hydrophobic interactions with Tyr32A, Ala46A, Tyr129A, Ala130A, His133A, Asp135A, Asn138A, Cys139A, Leu142A, Ser709A, Asn781A, and Ser784A amino acids (Fig 4). Lupeol forms two hydrogen-bonding interactions with Tyr32A, and Lys714A, two pi-alkyl interactions with His133A and Lys 780A, ten hydrophobic interactions with Lys47A, Tyr129A, Phe134A, Asp135A, Asn138A, Ser709A, Thr710A, Asp711A, Asn781A, and Ser784A and its shows binding energy -8.3 kcal/mol (Fig 5). 5-Hydroxy-2-(-hydroxyphenyl)-7-methoxy-6-(3-methylbut-2-enyl)chroman-4-one interacts against protein chain with binding energy is -8.3 kcal/mol using three pi-sigma interactions (Phe35A, Ile37A, and Val204A), two pi-alkyl interactions (Phe48A and Lys50A), and ten hydrophobic interactions (Asp36A, Tyr38A, Asn39A, Thr206A, Asp208A, Asn209A, Asp221A, Ser236A, Tyr728A and Arg733A) (Fig 6). The Vitexin, binding energy -8.1 kcal/mol, binds with RdRp using five hydrogen bonding interactions with Lys47A, Ser709A, Gln773A, and Lys780A amino acids, ten hydrophobic interactions with Tyr32A, Ala46A, Tyr129A, Ala706A, Thr710A, Asp711A, Ile715A, Gly774A, Ser778A and Asn781A amino acids and one pi-alkyl interactions with Lys714A amino acid. (Fig 7). The binding energy of Orientin with protein chain is -8.1 kcal/mol and it interacts through four hydrogen-bonds (His133A, Ser709A, Lys714A, and Asn781A), one pi-alkyl interaction (Lys47A), one pi-stacking interaction (Tyr129A) and ten hydrophobic interactions (Tyr32A, Ala46A, Ala130A, Phe134A, Asp135A, Asn138A, Ala706A, Thr710A, Asp711A, and Gly774A) with targeted protein chain. (Fig 8). The last one is Urosolic acid, the binding energy of which is -8.1 kcal/mol with RdRp. It forms one hydrogen-bond with Asp218A, one pi-sigma interaction with Phe35A, three pi-alkyl interactions with Ile37A, Lys50A, Val204A and six hydrophobic interactions with Tyr38A, Asn39A, Thr206A, Asp208A, Asn209A, and Asp221A amino acids (Fig 9). The details analysis of protein-Ligand interactions with various phytochemicals of ayurvedic medicinal plants Limonia acidissima Linn. and Ocimum sanctum with NSP12 chain of RdRp of SARS-CoV-2 shows compounds 1,3,4,5,7 and 8 surrounded by Tyr32A, Lys47A, Tyr129A, Ser709A, Thr710A, Asp711A and Lys714A (binding site -1). Compounds 6 and 9 interact with binding site 2 which contain Phe35A, Ile37A, Leu372A, Ala375A, Phe506A, Trp509A, Leu514A and Tyr515A amino acids. Compound 2 binds with protein chain with completely different binding site namely binding site 3 (Leu270A, Leu271A, Lys272A, Tyr273A, Thr324A, Ser325A, Phe326A, Gly327A, Pro328A, Leu329A, Val330A, Pro378A, Ala379A, Ala382A and Ala383A, Val398A and Met666A amino acids (Figure 10).

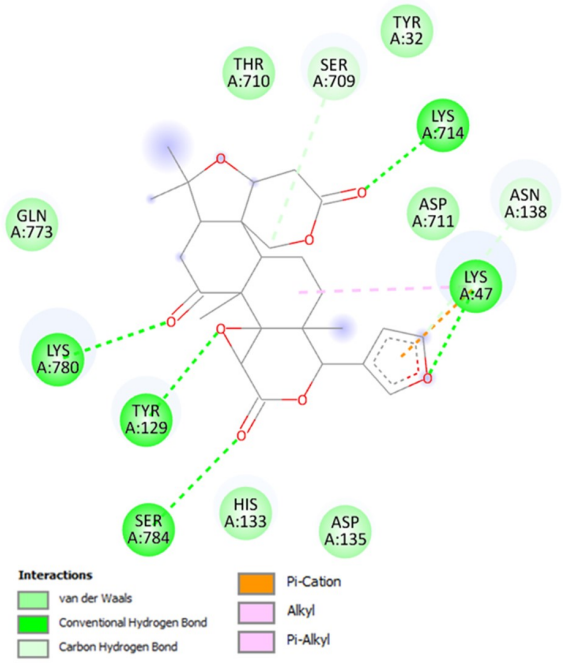


Figure 1. 2D representation of Ligand (Limonin) interaction against RNA-dependent RNA polymerase of SARS-CoV-2

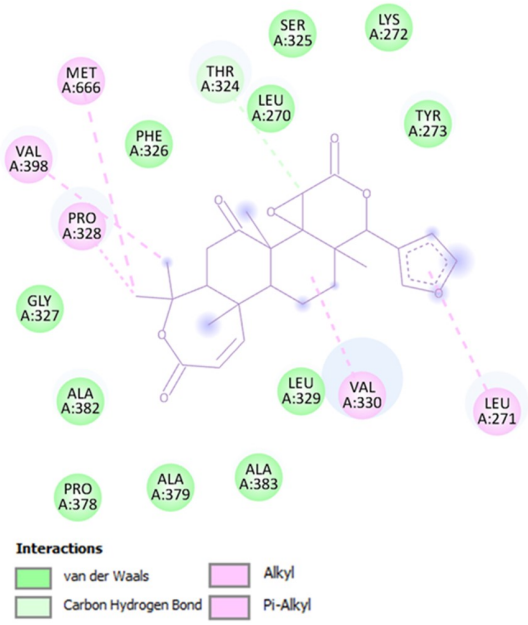


Figure 2. 2D representation of Ligand (Obacunone) interaction against RNA-dependent RNA polymerase of SARS-CoV-2.

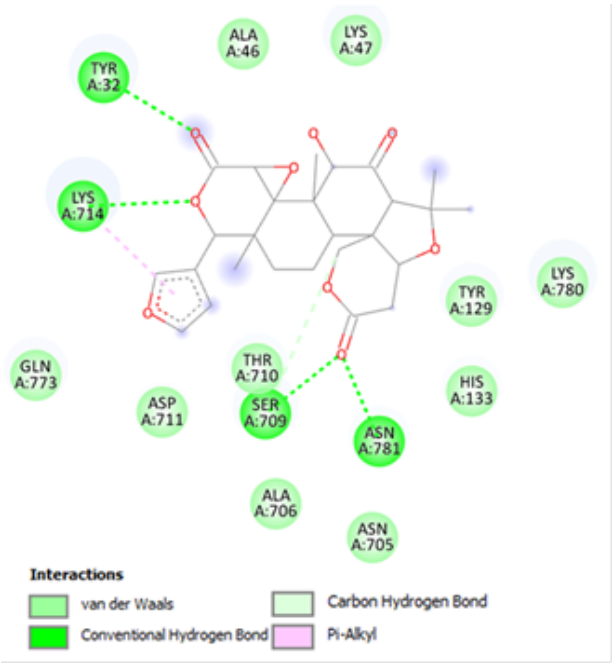


Figure 3. 2D representation of Ligand (Rutaevin) interaction against RNA-dependent RNA polymerase of SARS-CoV-2.

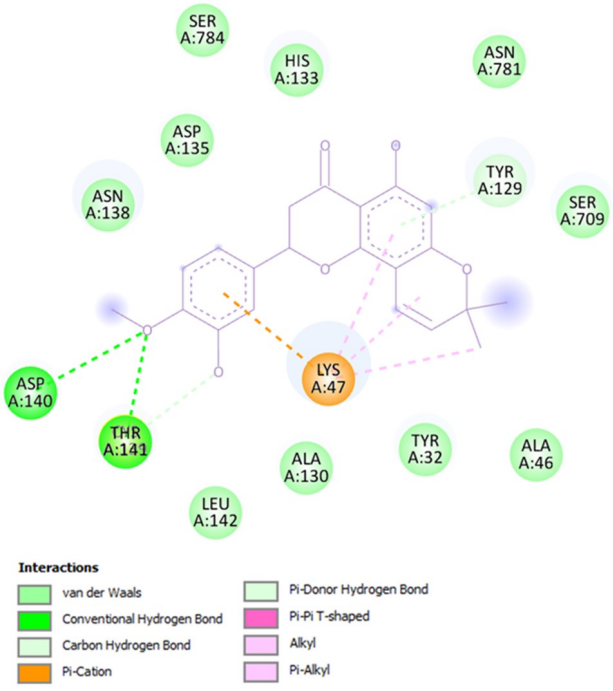


Figure 4. 2D representation of Ligand ((-)-(2S)-5,3'-Dihydroxy-4'-methoxy-6'',6''-dimethylchromeno-(7,8,2'',3'')-flavanone) interaction against RNA-dependent RNA polymerase of SARS-CoV-2.

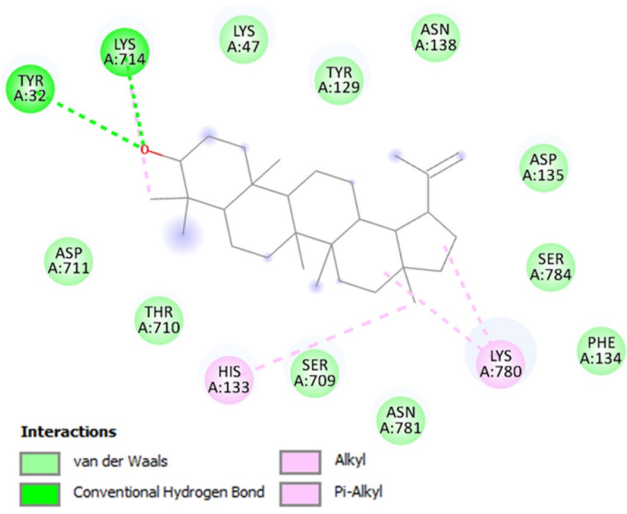


Figure 5. 2D representation of Ligand (Lupeol) interaction against RNA-dependent RNA polymerase of SARS-CoV-2.

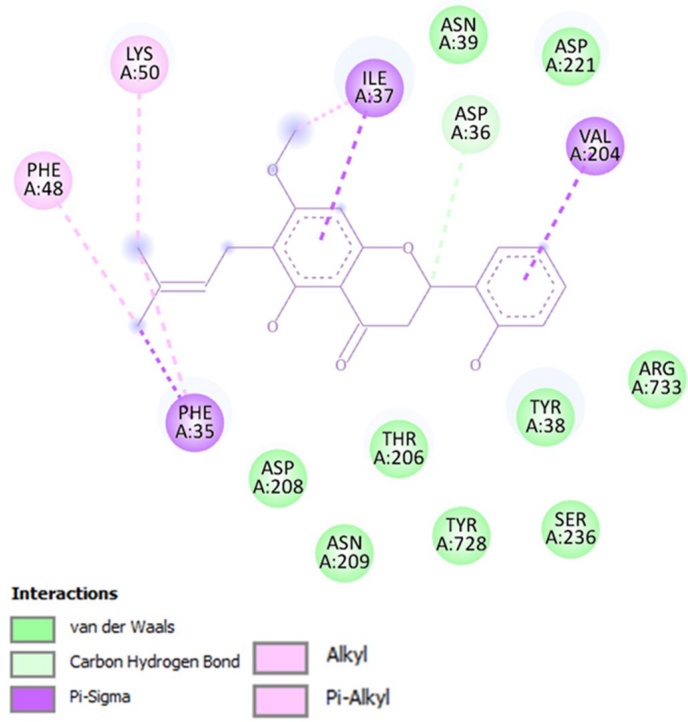


Figure 6. 2D representation of Ligand (5-Hydroxy-2-(-hydroxyphenyl)-7-methoxy-6-(3-methylbut-2-enyl)chroman-4-one) interaction against RNA-dependent RNA polymerase of SARS-CoV-2.

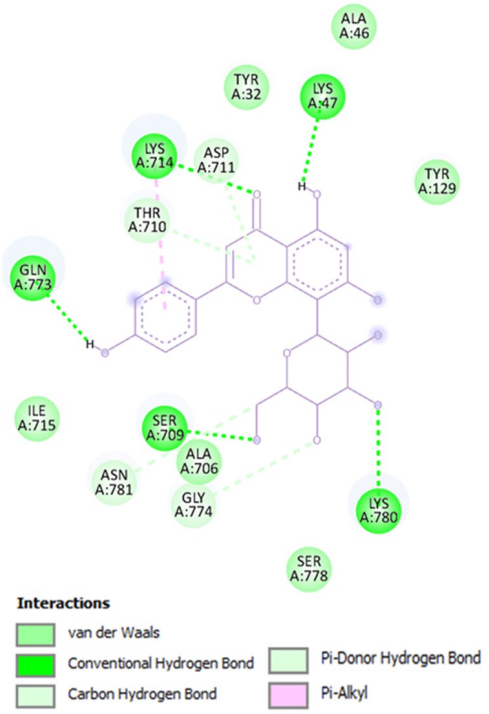


Figure 7. 2D representation of Ligand (Vitexin) interaction against RNA-dependent RNA polymerase of SARS-CoV-2.

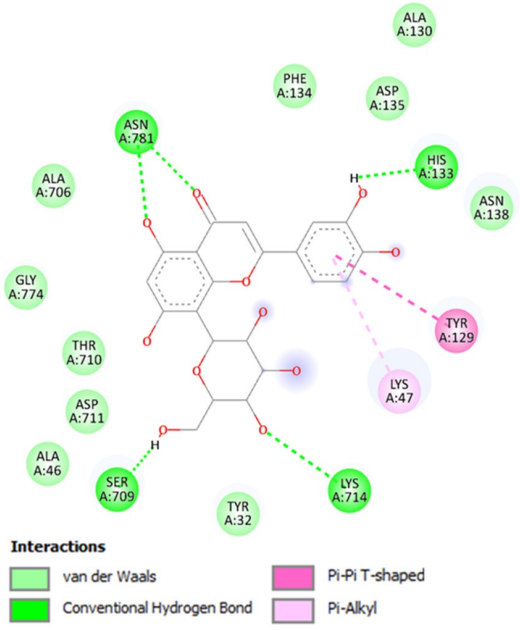


Figure 8. 2D representation of Ligand (Orientin) interaction against RNA-dependent RNA polymerase of SARS-CoV-2.

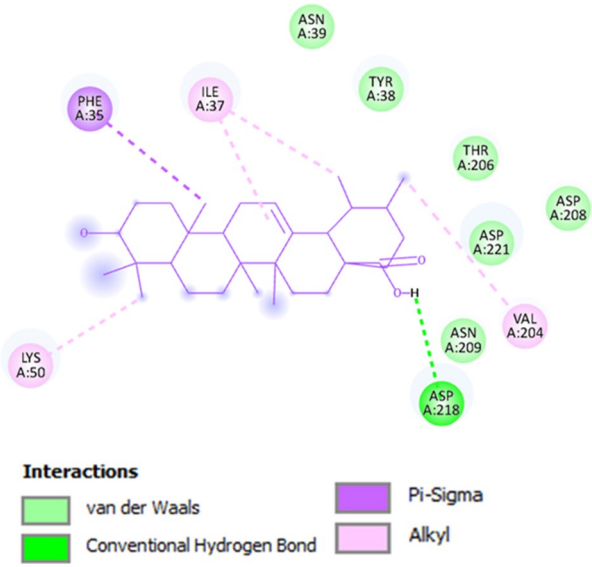


Figure 9. 2D representation of Ligand (Urosolic acid) interaction against RNA-dependent RNA polymerase of SARS-CoV-2.

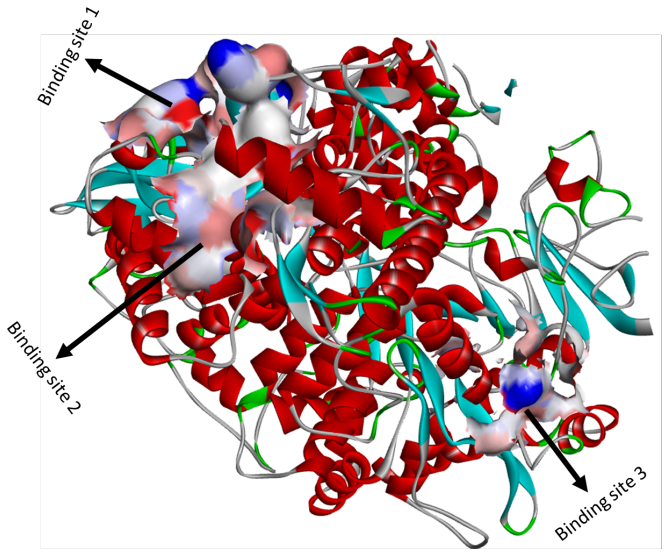


Figure 10: Binding sites of with NSP12 chain of RdRp of SARS Co-V-2

Table: 1- The summarized docking score

Table-1.

Name	Binding energy [kcal/mol]	Hydrogen bonds	Other interactions
Limonin	-9.5	LYS(47A),TYR(129A), LYS(714A),LYS(780A), SER(784A)HIS(133A),ASP(711A)	TYR(32A),HIS(133A),ASP(135A), ASN(138A), SER(709A), THR(710A),ASP(711A), GLN(773A)
Obacunone	-9.0	LEU(271A), PRO(328A),VAL(330A), VAL(398A), MET(666A),	LEU(270A), LYS(272A),TYR(273A), THR(324A),Ser(325A),PHE(326A),Gly(327A),LEU(329A), PRO(378A),ALA(379A) ALA(382A),ALA(383A
Rutaevin	-8.8	TYR(32A),SER(709A),LYS(714A),ASN(781A)	ALA(46A), LYS(47A),TYR(129A),HIS(133A),ASN(705A),ALA(706A),THR(710A), ASP(711A),GLN(773A), LYS(780A)
Flavanone compound	-8.4	ASP(140A),THR(141A),	TYR(32A),ALA(46A),LYS(47A),TYR(129A),ALA(130A),HIS(133A),ASP(135A),ASN(138A),CYS(139A),LEU(142A),SER(709A),ASN(781A),SER(784A)
Lupeol	-8.3	TYR(32A), LYS(714A),	LYS(47A),TYR(129A),HIS(133A),PHE(134A),ASP(135A),ASN(138A),SER(709A),THR(710A),ASP(711A),LYS(780A),ASN(781A),SER(784A)
Chroman Compound	-8.3		PHE(35A),ASP(36A),ILE(37A),TYR(38A),ASN(39A),PHE(48A),LYS(50A),VAL(204A),THR(206A),ASP(208A),ASN(209A),ASP(221A),SER(236A),TYR(728A), ARG(733A)
Vitexin	-8.1	LYS(47A),SER(709A), GLN(773A),LYS(780A)	TYR(32A),ALA(46A),TYR(129A),ALA(706A),THR(710A), ASP(711A),LYS(714A),ILE(715A),GLY(774A),SER(778A),ASN(781A)
Orientin	-8.1	HIS(133A),SER(709A), LYS(714A),ASN(781A)	TYR(32A),ALA(46A),LYS(47A),TYR(129A),ALA(130A),PHE(134A),ASP(135A),ASN(138A),ALA(706A),THR(710A),ASP(711A),ASN(781A)
Urosolic acid	-8.1	(Asp218A)	PHE(35A),ILE(37A),TYR(38A),ASN(39A),LYS(50A),VAL(204A), THR(206A),ASP(208A), ASN(209A),ASP(221A)
Ramdisivir	-8.1		

4. Conclusions

In summary, we have screened phytochemicals obtained from tulsi and wood apple by molecular docking and selected 9 phytochemicals to propose the potential hits against the RdRp of SARS-CoV-2 in comparison to remdesivir. The phytochemicals Limonin, Obacunone, Rutaevin,(-)-(2S)-5,3'-Dihydroxy-4'-methoxy-6'',6''-dimethylchromeno-(7,8,2'',3'')-flavanone, Lupeol, 5-Hydroxy-2-(-hydroxyphenyl)-7-methoxy-6-(3-methylbut-2-enyl)chroman-4-one, Vitexin, Orientin, and Urosolic acid are found to bind at the active site of RdRp with higher binding affinity. In which Limonin which is a phytochemical obtained from a wooden apple tree shows the highest binding affinity. We believe the outcomes will be useful in formulating therapeutic strategies using traditional medicines, and also the potential hits can be used for further lead optimization for drug discovery against COVID-19.

5. Acknowledgements

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6. Notes and References

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7. About the author(s)

Dr. Avishek Ghosh is an Assistant Professor at the Department of Pure and applied science, Midnapore City College since July 2018. Dr. Ghosh received his PhD in Chemistry from the National Institute of Technology, Rourkela, Odisha, India in January 2019. His research fields of interest are Synthesis and Characterization of organic and organometallic compound and their applications in different fields.

Miss. Putul Karan is a research scholar of Midnapore City College. She received her M.Sc degree from Midnapore City College in 2020.