In silico screening of *Pueraria tuberosa* (PTY-2) for targeting COVID-19 by countering dual targets M\(^{\text{PRO}}\) and TMPRSS2

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**ABSTRACT**

COVID-19 pandemic was started in Wuhan city of China in December 2019; immensely affected global population. Herein, an effort was made to identify potential inhibitors from active phytochemicals of *Pueraria tuberosa* (PTY-2) via molecular docking study. Our study showed five potential inhibitors (Robinin, Genistin, Daidzin, Hydroxytuberosone, Tuberostan) against M\(^{\text{PRO}}\) and five inhibitors (Robinin, Anhydrotuberosin, Daidzin, Hydroxytuberosone, Stigmasterol) against TMPRSS2. Out of these, Robinin, Daidzin and Hydroxytuberosone were common inhibitors for M\(^{\text{PRO}}\) and TMPRSS2. Among these, Robinin showed the highest binding affinity, therefore, tested for MD simulation runs and found stable. ADMET analysis revealed the best-docked compounds are safe and follow the Lipinski Rule of Five. Thus, it could be suggested that phytochemicals of PTY-2 could serve as potential inhibitors for COVID-19 targets.

**HIGHLIGHTS**

- Application of active phytoconstituents of *Pueraria tuberosa* (PTY-2) for the repurposing in the management of COVID-19.
- Promising effect of Robinin as a multifocal inhibitor of virus-host interaction including main protease (M\(^{\text{PRO}}\)) and TMPRSS2 with highest binding energy through molecular docking and molecular dynamics simulations studies.
- Robinin acts as common inhibitor against M\(^{\text{PRO}}\) and TMPRSS2.

**Impact statement**

The present work aims to provide an alternative treatment for COVID-19, a pandemic with the help of natural phytoconstituents found in *Pueraria tuberosa* (PTY-2). The current strategy, worldwide is developing a vaccine and alternatively repurposing the medicine, for example, Hydroxychloroquine, Lopinavir and Dexamethasone to manage COVID-19. The natural products can play a critical role by providing an additional benefit of minimum or no toxicity. As time is a crucial factor in the current pandemic, our work utilizes the virtual analysis of the phytochemicals via molecular docking and molecular dynamic simulations approach by targeting the till-to-date available macromolecular targets the virus-host interaction. Our study also clarifies the ADME profile of the phytochemicals and follows the Lipinski rule for drug palatability. We hope with this study, one could focus towards nature for fighting one of the gravest viral infections of this century.

**1. Introduction**

On December 31, 2019, China informed the World Health Organization (WHO) regarding a cluster of cases of atypical pneumonia in Wuhan city in Hubei Province, now known as COVID-19 or coronavirus disease 2019. As of this paper is being drafted, COVID-19 globally has affected 116,061,296 cases, including 2,580,050 deaths as of 7:43 pm CEST, February 28th, 2021, as per the WHO situation report (WHO, 2020a), these figures on the COVID-19 spread and related casualties are rapidly becoming out-dated, suggesting extreme and rapid transmission rate. On January 31, 2020, the WHO declared it a public health emergency of international concern and pandemic on March 12, 2020 (Cascella et al., 2020). As for India, it once hold the largest number of confirmed cases in Asia (Times of India, 2020), and globally had recorded the second-highest number of confirmed cases after the US (Johns Hopkins Coronavirus Resource Center, 2020). The causative agent is known by many names, such as Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), 2019 novel coronavirus (2019-nCoV), and is responsible for COVID-19 that belongs to the *Sarbecovirus* sub-genus, genus *Betacoronavirus*, family *Coronaviridae*. It has shown 79.6% and 96.2% nucleotide sequence identity to SARS-CoV and bat coronavirus (BatCoV RaTG13) (Zhou et al., 2020).
1. Binding of the viruses to the host membrane.
2. Fusion and transmission of the viruses.
3. Manipulation of the host immune system.

SARS-CoV-2 embraces more than 30,000 nucleotides containing replicase gene at pp1a and pp1b (polypeptide) (L. Zhang et al., 2020) essential for replication and rapid transmission, a 38-KDa enzyme known as main protease (M\textsuperscript{pro}) or 3C-like main protease(3CLM\textsuperscript{pro}) (Liu & Wang, 2020) digest the protein on at least eleven conserved sites causing proteolysis and releasing functional viral pp1a and pp1b that helps in the formation of non-structural proteins required for viral translation (Sheree et al., 2020) signifying the role of M\textsuperscript{pro} in viruses life cycle and serving as a curious target for an antiviral drug.

A study done by Shulla and colleagues identified that trans membrane serine proteases are somehow related to respiratory infections of viral origin. The proteases expedite the process of viral entry into the pulmonary region (Shulla et al., 2011). Recently a study performed by Hoffman et al. (2020) observed that TMPRSS2 (a member of the serine protease transmembrane family type II) initiate the cleavage of the coronavirus fusion Spike glycoprotein. TMPRSS2 facilitates the merging of viruses and host cell membrane, thereby easing the entry of SARS-CoV-2 in the lungs, suggesting that blocking TMPRSS2 may provide a treatment option by blocking the tissue tropism of the virus and thereby preventing further extension and complications. Many in silico study on Mpro and TMPRSS2 has been going on against COVID-19 targets using commercially available drugs. In our work, we have focused on searching naturally available phytoconstituents from medicinal plants' treasure against these targets.

Natural products have provided an answer for various ailments from time to time, including respiratory system disorders. Ayurveda has always viewed the human being as a sacred entity and has used a holistic approach in treating the disorder and working on creating a balanced environment within the body to prevent any disease or disorder. Pueraria tuberosa (Roxb. ex Willd.) DC. is a perennial herb commonly known as ‘vidarikandaa’ distributed in the tropical parts of India. The tubers of Pueraria have abundant flavonoids and isoflavones namely puerarin (8.31%), daidzein(1.70%), genistein(1.37%), robinin, daidzin, genistin, tuberostan, tuberin, 4’-methoxypuerarin, quercetin, hydroxypterobosone, biochanin A, biochanin B, irisolidon, glycocside (C-glycoside 4’:6-diacetyl), puerarone and tectoridin (Maji et al., 2014; Rastogi et al., 2013). Reported pharmacological activities of vidarikand includes its anti-inflammatory (Y. Tripathi et al., 2013), antioxidant (Nagwani & Tripathi, 2010; Y. Tripathi et al., 2013), antidiabetic properties (Srivastava et al., 2017, 2018; Y. B. Tripathi et al., 2017), immunomodulatory (Sawale et al., 2013; Shukla et al., 2017) and nootropic activity (’NOPR: Nootropic activity of tuber extract of Pueraria tuberosa (rxb), n.d.). In vivo and in vitro studies offered various bioactive phytochemicals in Pueraria tuberosa, mostly isoflavonoids support immune-boosting activity. In one of the recent studies aqueous extract of tuber amplified the phagocytic ability of macrophages and enhanced the level of IgG and IgA antibodies in serum sample of mice (Sawale et al., 2013). In numerous other studies upturn antibody production, phagocytosis suppression of delayed-type hypersensitivity reaction, hindering the production of TNF-alpha, NF-kB, MIP-2 and the expression of iNOS, COX-2 and CRP (Cooke et al., 2006; Maji et al., 2014; R. Zhang et al., 1997). Vidarikand or PTY-2 helps reduce the symptoms of the Flu. In the ancient textboks of Ayurveda, Flu or influenza is known as Vata Shleshmika Jwara, a viral infection of the upper respiratory tract. According to Ayurveda, Vata, Pitta and Kapha dosha go out of balance during the seasonal changes, resulting in Flu. Pueraria tuberosa lessens the symptoms of Flu and fights against seasonal changes due to its Rasayana (rejuvenating) properties (Jackson III & AHS, 2020; Tsang et al., 2017), suggesting PTY-2 as a potent immunomodulator which can be repurposed for treatment against COVID-19.

In the current work, we have tested the bioactive phytochemicals of PTY-2 for identifying their potential inhibitors for SARS-CoV-2 main protease (M\textsuperscript{pro}) and Transmembrane protein receptor TMPRSS2 for the treatment of COVID-19 infection through molecular docking approach (Figure 1). The docked compounds’ stability with the macromolecular
targets were assessed by molecular dynamics simulation studies and drug-likeness, ADMET profile analysis were simultaneously carried out.

2. Materials and methods

2.1. Protein preparation

Three dimensional structure of SARS-CoV-2 main protease (MPro) (PDB ID: 7BQY) was retrieved from RCSB Protein Data Bank (https://www.rcsb.org/). MPro has one chain of total 306 amino acids with a resolution of 1.7 Å. Preparation of proteins was done with the help of ‘Prepare protein’ protocol of BIOVIA Discovery studio 4.5 (DS 4.5) at physiological pH 7.4 and water molecules, other heteroatoms were removed from the structure.

2.2. Ligands selection

For identification of potential inhibitors, total 25 active phytochemicals gathered from LC-MS analysis of Pueraria tuberosa (PTY-2) (Table S1), were taken from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in 2D SDF format and conversion of all small molecules in 3D PDB format, geometry minimization was done via DS 4.5.

2.3. Homology modelling

The crystal structure of TMPRSS2 has not been resolved yet, which is found to be the entry point for coronavirus spike protein. There is a need to build a model for which we have used SWISSMODEL server for homology modelling. Target sequence of TMPRSS2 has been downloaded from Uniprot database (https://www.uniprot.org/) with Uniprot ID O15393. Template search for the target sequence has been done using SWISS MODEL server (https://swissmodel.expasy.org/interactive) (Schwede et al., 2003) and found 50 templates (https://swissmodel.expasy.org/interactive/ZnD5rx/templates/). Out of these we have select template of Serine protease hepsin (PDB ID: 5CE1) having 33.82% sequence identity, 38% sequence similarity and 0.53 GMQE value. Alignment of target-template sequence and model building was done through this server. Energy minimization of created model was done through ‘clean geometry’ protocol of DS 4.5. Additionally ‘prepare protein’ protocol of DS 4.5 was used for created model preparation at physiological pH 7.4. Further verification of created homology model was carried out using web-based tools ProSA (Wiederstein & Sippl, 2007) and PROCHECK (Laskowski et al., 1993) to evaluate the quality of homology model structure.

2.4. Molecular docking

Selected compounds from PTY-2 were docked with SARS-CoV-2 main protease (PDB ID: 7BQY) and homology Model 01 for TMPRSS2 using Autodock Vina-based YASARA software (Krieger et al., 2014; Trott & Olson, 2010). For complete molecular docking study, prepared ligand files and receptors were used to set the target and play macro. A command ‘Macro file dockrun.mcr’ was used to estimate interaction energy between receptor and selected ligands independently. Using YASARA, 25 VINA docking runs of the ligand object 2 to the receptor object 1 was performed. Further docked complexes were saved in PDB file format for 2D-3D interactive visualization study. The result log files sorted based of binding energy [kcal/mol] and dissociation constant [pM]. The compound having more positive binding energies indicates stronger binding, and negative energies denote no binding.

2.5. Molecular dynamic simulations

In recent times, MD simulations have been a remarkable approach for identifying protein-ligand stability, structural transformations, binding energies change in complexes and so on. We have also examined the binding stability of the compound Robinin with the selected two receptors MPro and TMPRSS2 of SARS-CoV-2 for 20 ns simulation period. For this purpose, the Gromacs simulation package was employed using Gromos54A7 force field for the calculation of simulation parameters (Berendsen et al., 1995). PRODRG webs server was utilized for generating ligand topology (van Aalten et al., 1996). The simulation setup was prepared using SPC water model for aqueous environment and 0.15 M salt concentration for proper electrostatic distribution in a cubic simulation box. Charge neutralization was done by adding counter ions. For energy minimization, 50,000 steps of steepest descent algorithm were executed with Verlet cut-off scheme to calculate the neighbouring interactions. Further, equilibration process under NPT and NVT conditions for 1 ns was done Parrinello-Rahman and V-rescale methods for pressure and temperature coupling respectively. LINCS algorithm was used for calculating bond parameters (Hess et al., 1997). Particle Mesh Ewald (PME) for long-range electrostatics with Fourier spacing of 0.16 was used for production MD. Finally, production MD run for all systems was performed in periodic boundary conditions for 20 ns each. Further, MD analysis consisting Root Mean Square Fluctuation (RMSF), Root Mean Square Deviation (RMSD), Radius of Gyration (Rg) of C-α atoms were done using gmx rms, rmsf and gyrate commands. For binding energy calculation, g_mmpbsa tool was implemented (Hou et al., 2011).

2.6. Drug likeness and ADMET prediction

Compounds with significant binding energy were further analyzed for their drug-like property and safety profile assessment by using web-based server Lipinski rule of five (http://www.scribio-ltdt.res.in/software/drugdesign/lipinski.jsp) (Jayaram et al., 2012; Lipinski, 2004) and admetSAR server (http://lmmd.ecust.edu.cn/admetsar1/predict/) (Cheng et al., 2012), respectively.

3. Results and discussion

3.1. Homology modelling

Out of 50 templates for target sequences we selected template of Serine protease hepsin with PDB ID: 5CE1 for
Active phytochemicals obtained from *Pueraria tuberosa* (PTY-2) LC-MS analysis and theirStructure retrieval from PubChem Compound Database

M\textsubscript{pro} protein structure from Protein Data Bank and Homologymodelling of TMPRSS2 (model 01) from Swiss-model

Structure-Ligand based drug designing

Molecular docking (YASARA)

Binding energy < 8.0 kcal/mol

Rejected

Binding energy > 8.0 kcal/mol

MD simulation (GROMACS)

Drug-likeness prediction andADMET profile

From PTY-2, seven active phytochemicals identified against SARS-CoV-2 M\textsubscript{pro} (PDB ID:6LU7) and TMPRSS2 (model 01).

Figure 1. Schematic representation of work flow for identifying inhibitors against M\textsubscript{pro} and TMPRSS2 via structure-ligand-based computational analysis.

3.2. Molecular docking

Based on YASARA scoring system, molecular docking studyidentifies seven different phytochemicals from PTY-2 with high binding affinity with viral and host macromolecular targets. Table 1 shows the list of the active phytochemicals which showed significant binding affinity (>8.0 kcal/mol) with SARS-CoV-2 main protease (M\textsubscript{pro}) and Transmembrane receptor TMPRSS2.

3.3. Potential inhibitors for SARS-CoV-2 mainprotease (M\textsubscript{pro})

Molecular docking study revealed that out of 25 active phytochemicals of PTY-2, five phytochemicals namely Robinin, Genistin, Daidzin, Hydroxytuberosone and Tuberostan have highest binding affinity with M\textsubscript{pro}. Robinin was found tohave highest binding affinity of 8.87 kcal/mol. Robinin is a Kaempferol derived glycosyloxyflavone and dihydroxyflavone, reported to have antibacterial and antifungal
Robinin formed numerous conventional and carbon hydrogen bonding with the residues Glu 288, Lys 5, Lys 137, Asp 197, Thr 199 and Tyr 237, two $\pi$-alkyl interactions were formed with the residues Leu 286 and Leu 287, a $\pi$-anion bond was formed with Asp 289 and remaining residues showed few van der Waals interactions (Figure 5(a,b)). Genistin was found to be the second inhibitor of Mpro with binding energy 8.18 kcal/mol. It is an isoflavone derived from Genistein, reported to have antiviral activity (Donovan et al., 2009) and estrogenic activity (Allred et al., 2001). Molecular docking study showed Genistin forming different ligand-protein interactions, including conventional and $\pi$-donor hydrogen bonding with the residues Gln 192, Glu 166, His 41 and Gln 189, $\pi$-sulfur and $\pi$-alkyl interactions was formed with Met 165, $\pi-\pi$ T-shaped interaction was formed with the residue His 41 and the residue Thr 26 showed unfavourable donor-donor and unfavourable acceptor-acceptor interaction and multiple van der Waals interactions were formed with the receptor protein (Figure 5(c,d)). In continuation to this, Daidzin was found to be the third inhibitor of Mpro with binding affinity 8.15 kcal/mol. Daidzin is an isoflavone glycoside derived from Daidzein with tumour preventive and antidipsotropic activity (Keung & Vallee, 1998). Different interactions shown by Daidzin which includes conventional, carbon and $\pi$-donor hydrogen bonding with the residues Gln 192, His 41, Glu 166 and Gln 189, $\pi$-alkyl interaction was formed with the residues Pro 168 and Met 165, a $\pi-\pi$ T-shaped interaction was formed with the residue His 41, a $\pi$-sulfur interaction was made with the residue Met 165 and many van der Waals interactions were indicated by remaining residues (Figure 5(e,f)). Fourth inhibitor was Hydroxytuberosone with binding affinity 8.09 kcal/mol. Interactions shown by Hydroxytuberosone involves conventional hydrogen bonds with residues Asp 295, Gln 110 and Thr 111, $\pi$-alkyl interactions with Tyr 154 and Arg 298 and van der Waals interactions showed by remaining residues (Figure 5(g,h)). Tuberostan was reported to be the fifth inhibitor for Mpro with predicted binding energy 8.01 kcal/mol. It showed conventional hydrogen bonding with the residue Arg 131, a $\pi$-anion interaction was formed with the residue Asp 289, alkyl and $\pi$-alkyl interactions with the residues Leu 286, Tyr 239, Leu 272 and Leu 287 and few van der Waals interactions were also formed with the receptor protein (Figure 5(i,j)). It was deduced from the molecular docking study that significant binding energies of Robinin, Genistin, Daidzein, Hydroxytuberosone and Tuberostan could block the active site of Mpro enzyme.

### 3.4. Potentials inhibitors for TMPRSS2

Robinin bound to our model 01 with binding affinity of 8.84 kcal/mol. It formed many conventional and carbon hydrogen bonding with the residues Val 280, Gln 438, Gly 464, Ser 436, His 296 and Gln 389, amide-$\pi$ stacked bonds with the residues Trp 461 and Cys 437 and multiple van der Waals interactions were formed with the receptor protein (Figure 6(a,b)). Anhydrotuberosin was found to be second
inhibitor, having binding energy of 8.30 kcal/mol. It shows different interactions including conventional and carbon hydrogen bonding with the residues Arg 240, Thr 287 and Cys 241, a π-σ bond with Ile 242, π-π T-shaped bonds with Phe 357 and Phe 194, alkyl and π-alkyl interactions with Ala 243 and Pro 288. Remaining residues formed van der Waals interactions (Figure 6(c,d)). Daidzein was found to be third, having binding energy of 8.24 kcal/mol. It showed conventional hydrogen bonding with the residues Glu 289, Asn 193 and Arg 182, a π-cation bond with Arg 240, π-π stacked and π-π T-shaped interactions with the residues Phe 357 and Phe 194, π-alkyl interaction were formed with the residues Ala 243, Pro 288 and Ile 242 and some van der Waals interactions were also formed by remaining residues (Figure 6(e,f)). Fourth inhibitor was found to be Hydroxytuberosone with binding energy 8.03 kcal/mol. It

**Figure 4.** Homology modelling (Model 01) validation: (a) z-score plot represent model 01 (black spot) within the range of native structure (b) Local energy plot (c) Ramachandran plot.

**Table 1.** List of phytochemicals with binding energy >8.0 kcal/mol for COVID-19 targets molecules.

| Compounds          | Target molecules; Binding energy (kcal/mol) (>8.0 kcal/mol) |
|--------------------|-------------------------------------------------------------|
|                    | SARS-CoV-2 main protease (PDB ID:7BQY) TMPRSS2-homology model 01 |
| Native ligand (N3) | 5.41                                                        |
| Phytochemicals     |                                                             |
| Robinin            | 8.87 8.48                                                   |
| Genistin           | 8.18                                                        |
| Daidzin            | 8.15 8.24                                                   |
| Hydroxytuberosone  | 8.09 8.03                                                   |
| Tuberoxan          | 8.01                                                        |
| Anhydrotuberosin   |                                                             |
| Stigmasterol       | 8.30 8.01                                                   |
Figure 5. 3D–2D interactions of the best docked compounds. \( M^{\text{pro}} \) complexes; (a, b) Robinin, (c, d) Genistin, (e, f) Daidzin, (g, h) Hydroxytuberosone, (i, j) Tuberostan.
Figure 6. 3D–2D interactions of the best docked compounds. TMPRSS2 complex; (a, b) Robinin, (c, d) Anhydrotuberosin, (e, f) Daidzin, (g, h) Hydroxytuberosone, (i, j) Stigmasterol.
showed a π-π stacked interaction with the residue Phe 194, a π-alkyl interaction was formed with the residue Ile 242 and remaining residues formed van der Waals interactions (Figure 6(g,h)). Stigmasterol was found to be the fifth inhibitor, having binding affinity of 8.01 kcal/mol. It is a steroid that maintains the cell membranes’ structure and physiology (Ferrer et al., 2017), reported to have anti-angiogenic, anti-inflammatory and anti-cancerous activity (Kangsamaksin et al., 2017). It formed a π-donor hydrogen bond with Phe 357, alky Cys145 and π-alkyl interactions with residues Ala 243, Pro 288, Ile 242 and Phe 194, and many van der Waals interactions were also formed by remaining residues (Figure 6(i,j)). Strong binding affinities of Robinin, Anhydrotuberosin, Daidzin, Hydroxytuberosone and Stigmasterol with TMPRSS2 could block its active sites to reanimate SARS-CoV-2 for reattaching ACE2, thus preventing COVID-19 infection.

As per molecular docking study, from YASARA scoring system, it had been observed that Robinin, Genistin, Daidzin, Hydroxytuberosone, Tuberostan, Anhydrotuberosin and Stigmasterol from PTY-2, can act as probable inhibitors of SARS-CoV-2 Mpro and TMPRSS2. Structures of best docked phytochemicals were shown in Figure 7.

4. Molecular dynamics simulations

To further analyze the stability of binding, interactions of the best-docked compound, that is, ROBININ, we performed MD simulations for two setups upto 150 ns. These complexes are formed by the compound Robinin with proteins Mpro and TMPRSS2. We have also calculated binding energy for the complexes from simulation trajectories.

4.1. Mpro-Robinin

The docked complex of main protease with high scoring and best interacting compound Robinin was investigated for its binding stability and found to be stable upto 150 ns with minor fluctuations within the range of 0.2–0.4 nm. As shown in Figure 8, the RMSD plot showed quite stable trend with an average value of approx. 0.32 nm. The slight fluctuations were observed near 11–14 ns, 150–180 ns and in last 10 ns with minimum varying values 0.3–0.36 nm. Similar trends were observed with radius of gyration where the average value of Rg was 2.19 nm. A few fluctuations were seen in the Rg value upto 80 ns and afterward, the plot became stable at 2.18 nm. However, the RMSF plot showed little fluctuations in the favourable range. The catalytic site residues, including His41 and Cys145 are found to be least fluctuating throughout the simulation period. Further, for the last 20 ns of simulation trajectory, the binding energy was also observed to correlate with other parameters (Figure 9(A)). The average binding energy was $-31.9 \pm 27.2$ kJ/mol for the protein-ligand complex.

4.2. TMPRSS2-Robinin

The complex of serine protease TMPRSS2 with Robinin was simulated till 150 ns using GROMOS54a7 forcefield. As shown in Figure 10, the complex has experienced very little fluctuations and has gained an average of RMSD 0.43 nm. In terms of compactness, the radius of gyration of the protein upon binding with complex is reduced (average Rg is 2.14 nm) which shows attained stability by the protein with inhibitor. Also, the RMSF plot was observed to be varying between 0.2 to 0.5 nm throughout the simulation. Lastly, the binding energy values were calculated and found to be in decreasing trend for the last 20 ns (Figure 9(B)). However, there were heavy fluctuations observed in last 8 ns time of simulation. Overall, the binding energy for last 20 ns was calculated to be $-50.4 \pm 33.6$ kJ/mol.

5. Drug likeness and ADMET prediction

Drug-likeness of our best-docked compounds was predicted using Lipinski rule of five which states for any ligand to be considered as drug-like, a molecule should follow five parameters: molecular weight <500 Dalton, number of H-bond donors <5, number of H-bond acceptors <10, LogP <5 and molar refractivity between 40–130. It should follow 2 or more of its constraints; consequently, all of our best-docked compounds from our study followed this rule and subsequently deliberated as drug-like compounds. Further, admetSAR server was used for the prediction of the ADMET profile of the best-docked compounds. Moreover, our compounds were found to have good optimal oral bioavailability, human intestinal absorption, Caco-2 permeability, non-carcinogenic effect (within the reference range), considered safe and harmless (Table 2).

The current rising scenario of COVID-19 pandemic demands its resolution to save the global population. At present, both individual drugs and in combination of antimalarial, antiviral and corticosteroids are being utilized as a treatment strategy in the modern medicine. In recent time WHO is recommending Dexamethasone (WHO, 2020b). Glenmark an Indian-based pharmaceutical has introduced antiviral drug Favipiravir (‘Glenmark launches Covid-19 drug at Rs 103 per tablet - India News - Hindustan Times’, 2020) for treating mild to moderate COVID-19 patient has got regulatory approval for Phase III assessment. Drug repurposing will save the time and in silico analysis of the natural phytoconstituents is one of the initial and crucial steps in this direction. Mpro and TMPRSS2 are shown to be chief targets for curbing COVID-19 infection. In the contemporary work, molecular docking analysis revealed seven different active phytochemicals from PTY-2 (Robinin, Genistin, Daidzin, Hydroxytuberosone, Tuberostan, Anhydrotuberosin and Stigmasterol) as potential inhibitors of Mpro and TMPRSS2. Robinin, Daidzin and Hydroxytuberosone act as common inhibitor for Mpro and TMPRSS2. From these, Robinin has significant binding affinity for Mpro and TMPRSS2 and showed structural stability, thereby acting as a dual target inhibitor against COVID-19. Overall, the analyzed active phytoconstituents of PTY-2 attacks on Mpro prevent its attachment to host receptor ACE2 and prevent its attachment to TMPRSS2 which activates SARS-CoV-2 for reattachment to ACE2 responsible for COVID-19 infection.
Figure 7. Structures of best docked compounds for Mpro and TMPRSS2.
These active phytochemicals were portrayed as drug-like compounds as per Lipinski rule and exhibited safe ADMET properties, supporting developing more efficient and potent COVID-19 inhibitors.

6. Conclusion

In current investigation, the active phytoconstituents of Ayurvedic medicinal plant *Pueraria tuberosa* (PTY-2) have multi-targeted potency to counteract the infection of COVID-19. Our study identified seven potential inhibitors (Robinin, Genistin, Daidzin, Hydroxytuberosone, Tuberostan, Anhydrotuberosin and Stigmasterol) against COVID-19 molecular targets. From these Robinin, Daidzin and Hydroxytuberosone were found to be common inhibitors for M\textsuperscript{pro} and TMPRSS2. Robinin with the highest binding affinity for M\textsuperscript{pro} and TMPRSS2 acts as a common inhibitor. During trajectory analysis, Robinin showed significant stability with M\textsuperscript{pro} and TMPRSS2, thus reducing the strength of the SARS-CoV-2 virus against the host. It also has a drug-like property with a harmless ADMET profile suggesting in development of improved potent COVID-19 inhibitors.

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Disclosure statement

Authors declare no conflict of interest.

Author’s contribution

Study conception and design by Prof. Yamini Bhusan Tripathi and Priya Shree. Acquisition, analysis and interpretation of data were done by Priya Shree. Drafting of manuscript were done by Priya Shree, Priyanka Mishra and Harsh Pandey. Prateek Kumar and Rajanish Giri performed the MD simulations and contributed to writing of the manuscript. Critical revision was done by Prof. Yamini Bhusan Tripathi, Dr. Radha Chaube and Dr. Neha Garg.

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