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Indian red rice phenolic metabolites as potential natural inhibitors of SARS-CoV-2 main protease: A metabolomic and *in silico* study

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Abstract

COVID-19 is a viral respiratory infectious disease caused by the novel strain of SARS-CoV-2 (Severe acute respiratory syndrome coronavirus 2). SARS-CoV-2 main protease (M^{pro}) is associated with viral replication and transcription, thereby making it a potent target for combating COVID-19. Natural compounds from plants are being widely explored for viral inhibitors due to their safety aspect. Therefore, the present study aimed at predicting the antiviral property of red rice phenolic metabolites (RRPM) from three Indian rice varieties against SARS-CoV-2 M^{pro} through metabolomics and *in silico* studies. The Indian red rice cultivars, *i.e.*, Veethi vadankan, Samba mosanam and Kuzhiadichan, were subjected to Q-TOF-LC-MS analysis and eighteen abundant polyphenols were selected using hierarchical cluster analysis. The abundant RRPM were virtually screened using Swiss ADME, a web-based tool. All the abundant polyphenols obeyed the Lipinski's rule except for hesperetin 3'-O-glucuronide, thereby exhibiting drug likeliness. Based on ADME analysis, fifteen of the RRPM showed good oral bioavailability, high GI absorption and acceptable overall bioavailability score. The 15 RRPM were further subjected to molecular docking analysis using Auto dock 1.5.6 against SARS-CoV-2M^{pro}. The analysis revealed 7, 4'-dihydroxy flavone and eriodictyol to have strong binding affinity (>10 kcal/mol) higher than nelfinavir (– 8.53 kcal/mol). Eight other compounds also proved to be potential natural inhibitors of SARS-CoV-2 M^{pro}. The present study has revealed Kuzhiadichan as a good source of eight potent natural inhibitors of SARS-CoV-2 M^{pro} and as a potential functional food for COVID-19 management.

1. Introduction

Coronavirus disease is a viral respiratory infectious disease caused due to the novel strain of SARS-CoV-2 (Severe acute respiratory syndrome coronavirus 2) which is responsible for symptoms like common cold, middle east respiratory syndrome and severe acute respiratory syndrome, *etc*. The pandemic has brought a serious health crisis affecting millions of people across the globe (Shah *et al*., 2020; Nupur, 2020). As per the recent updates provided by World Health Organization as on 21st March 2021, around 223 countries have been found to be affected by COVID-19 disease with 1,22,524,424 confirmed cases and the total number of confirmed deaths accounting up to 2,703,620 (WHO, 2021).

SARS-CoV-2 are positive stranded RNA viruses belonging to the family *Coronaviridae*, order *Nidovirals* and genus *Beta coronavirus*. The word 'corona' refers to 'crown', *i.e.*, the virus particle having crown like structure (Singh *et al*., 2020). Technically, SARS- CoV-2 consists of 30000 nucleotides with the complex structure comprising of four major structural proteins, *i.e.*, nucleocapsid protein, membrane protein, spike protein and envelope protein. The proteolytic processing of the SARS- CoV-2 proteins are responsible for transcription, translation, and replication of the virus. This process is mediated by the proteases - papain-like cysteine protease (PL^{pro}) and 3 chymotrypsin like protease ($3CL^{pro}$) also called as the main protease (M^{pro}). The main protease catalyzes the cleavage of the viral protein at multiple cleavage sites. Hence, SARS-CoV-2 M^{pro} is considered as a potent target for designing and formulating drugs against COVID-19 (Boopati *et al*., 2020).

Natural compounds from plants have a huge structural diversity and many of them possess drug like potency against certain diseases (Perez, 2003). Studies report that pharmacologically active compounds from plants are promising inhibitors of viral proteases with no toxic complications and are equally effective in regulating host immune response compared to the modern drugs (Paraiso *et al*., 2020; Bellik *et al*., 2020).

Among the phytochemicals, phenolic compounds present in different plants and foods have been reported to show defense mechanisms against viral pathogens like human immunodeficiency virus, poliovirus type 2, hepatitis B and C virus, adenoviruses, influenza virus, *etc*. (Zakaryan, 2017). Recent studies are focusing on the possibilities of using phenolics from different plant and food sources in the management of COVID-19 pandemic (Chojnacka *et al*., 2020). Several pigmented rice varieties, *i.e.*, red, purple, black, brown, *etc*., from countries such as China, Japan and Korea have been reported as good sources of phenolic compounds such as phenolic acids, flavonoids, anthocyanins, and their derivatives (Deng *et al*., 2013). Few recent studies have also reported the phenolic profile in some Indian pigmented rice varieties (Choudhury *et al*., 2020; Haldipur and Srividya, 2021). However, the phenolic

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profiles of several other Indian pigmented rice varieties remain unexplored. Moreover, the potent antiviral property of red rice phenolics against SARS-CoV-2 has not been investigated earlier to the best of our knowledge.

Therefore, in the present investigation, the phenolic profiling of three unexplored traditional Indian pigmented rice varieties: Veethi vadankan, Samba mosanam and Kuzhiadichan was carried out using an advanced metabolomic technique. The red rice phenolics identified were further screened for possible antiviral action against COVID-19 by targeting the inhibition of SARS-CoV-2 M^{pro} through *in silico* studies.

2. Materials and Methods

2.1 Sample procurement

The three Indian red rice varieties, namely: Veethi vadankan (VV), Samba mosanam (SM) and Kuzhiadichan (KC) were procured from the farms of Tamil Nadu, India. The colored grains were cleaned free from dirt and foreign materials manually. ground, sieved through 0.5 mm mesh. The powdered samples were freeze dried (Lyodel freeze dryer, India), followed by vacuum packaging and stored at –20°C for further analysis. The experimental design of the study is shown in Figure 1.

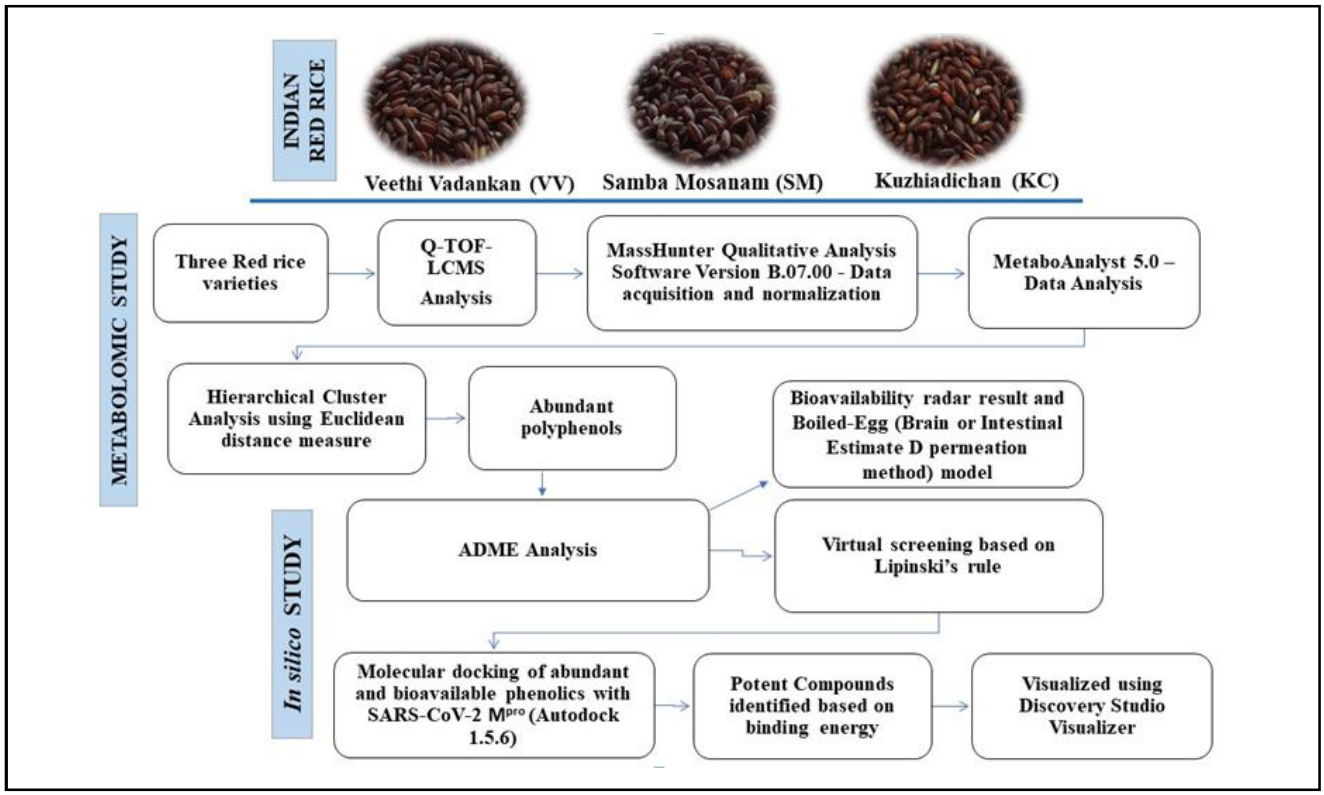

Figure 1: Experimental design of the study.

2.2 Metabolomic analysis of red rice varieties

2.2.1 Extraction protocol

Phenolic extraction was carried out by the method of Bordiga *et al.* (2014). Red rice flours (0.1 g) were extracted with 2.5 ml of solvent mixture of methanol: water: formic acid (50:48:5:1.5 v/v). The mixture was ultrasonicated (Bransonic Digital Bath 5800, USA) for 10 min at 50°C, followed by centrifugation (temperature maintained at 5°C) (Sorvall, ST 8R, Thermo Fisher Scientific, Germany) at 2,500 g for 5 min. Two more extractions were carried out from the resulting pellets. The extract aliquots were combined and concentrated using vacuum concentrator (miVac Duo concentrator, USA). The extracts were stored at –20°C until analysis. The sample extracts were spiked with a mixture of 30 µl of two internal standards: Epigallocatechin- 2,3,4- $^{13}C_3$ and Ferulic acid-1,2,3- $^{13}C_3$ (5 µg/ml) for normalization and to combat any disparities during the ionization process and mass analysis.

2.2.2 Instrument details and operating conditions

The phenolic profiling of the red rice extracts was carried out using an analytical metabolomic platform: Q-TOF- LC-MS (Agilent 1290 Infinity LC system, Agilent Technologies, Santa Clara, CA, USA) provided with quaternary pump (G4204A), autosampler (G4226A), autosampler thermostat (G1330B) and column compartment thermostat (G1316C). The LC system was coupled to Agilent 6550 iFunnel Quadrupole Time-of-Flight (QTOF) with Agilent Jet Stream Thermal Gradient Technology.

The column involved in metabolites separation was Agilent Zorbax RRHD Eclipse XDB-C18 (2.1 x 100 mm, 1.8 µm, 1200 bar). The composition of mobile phases used were acetonitrile: (A), and 10 mM ammonium acetate in water adjusted to pH 5 and formic acid (B). The operating parameters was as described earlier by Haldipur and Srividya (2021).

2.2.3 Data acquisition and analysis

The data were obtained using an extended dynamic range mode (2 GHz) and the full scan mass range of 50 to 1500 Da. Data acquisition and deconvolution was carried out using a software called as Agilent Mass Hunter Qualitative Analysis B.07.00 (Mass Hunter Qual, Agilent Technologies). ChemSpider, Phenol-Explorer, Pubchem and FoodB were used for obtaining the structures of phenolic compounds to create a custom phenolics database using Agilent Personal Compound Database and Library (PCDL). The database was uploaded in Mass Hunter Qual software's algorithm for data mining and analysis was further carried by using a freely available, streamlined metabolomics analysis tool- MetaboAnalyst 5.0.

2.3 *In silico* studies

2.3.1 ADME analysis

The ADME related parameters of the eighteen abundant red rice phenolic metabolites (RRPM) identified through metabolomics analysis were predicted using Swiss ADME (http://www.swissadme.ch/), a free web - based tool. The RRPM were screened based on the Lipinski's rule (molecular weight < 500 Da, lipophilicity (Log P) value less than 5, H-bond donor < 5 and H-bond acceptor < 10) for drug likeliness. The ADME analysis also predicts the oral bioavailability and yields an over all bioavailability score based on the six physicochemical properties, *i.e*., solubility, size, polarity, lipophilicity, flexibility, and saturation. The gastrointestinal absorption was predicted using the Boiled-Egg model (Daina *et al*., 2017).

2.3.2 Molecular docking analysis

Molecular docking is a useful *in silico* model to predict protein-ligand interaction. The molecular interaction of fifteen abundant bioavailable RRPM with the viral protein, SARS-CoV-2 main protease (M^{pro}) was analyzed using the software Auto dock 1.5.6. (Garg *et al*., 2020; Adem *et al*., 2020)

2.3.2.1 Optimization of SARS-CoV-2 M^{pro} structure

The crystalline structure of SARS-CoV-2 M^{pro}, complexed with an inhibitor N3 with a resolution of 2.1 Å, was selected as a target protein for the study. The 3-dimensional structure of the protein with PDB ID: 6LU7 was retrieved in the PDB format form RCSB PDB database (https://www.rcsb.org/structure/6LU7). The protease consists of 306 amino acid. The optimization of protein structure was done prior to the actual docking which involved removal of the complexed inhibitor and water molecules, followed by the addition of Kollman charges, Gasteiger charges and assigning up of AD4 type atom, respectively.

2.3.2.2 Optimization of phenolic ligands

The abundant RRPM obeying Lipinski's rule, with good oral availability and bioavailability score were chosen as ligands for the study. The 3D structures of the compounds were obtained from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in SDF format which were later converted to PDB format using PyMOL, Schrodinger with the addition of H-bonds.

2.3.2.3 Determination of active sites and docking

The active sites of the optimized target protein (6LU7) were selected depending on the amino acid residues associated with it. The amino acid residues involved in the active sites of the protein were selected based on the literature available: THR24, THR25, THR26, HIS41, MET49, PHE140, LEU141, ASN 142, GLY143, SER144, CYS145, HIS163, HIS164, MET165, GLU166, HIS172, ASP187, ARG188, GLN189 and THR190 (Jin *et al*., 2020). A grid of dimension 126 x 78 x 116 with 0.375 Å spacing was prepared around the active site of the protein to ensure that ligands fit into the active sites. While performing auto dock, the genetic algorithm was set as the search parameter, other docking parameters were set as default and the output file was obtained in DLG format utilizing Lamarckian genetic algorithm. Out of the 10 different protein confirmations, the one with the least binding energy (kcal/mol) was selected for information on ligand efficiency, intermolecular energy, *etc*. PyMOL and BIOVIA discovery studio visualizer software packages were used to visualize the structure of the docked complexes and to understand the types of interactions involved.

3. Results

3.1 Phenolic profiling of red rice varieties

A total of seventy-nine metabolites were identified in the three red rice samples through Q-TOF- LC-MS analysis. The most abundant class of metabolites were flavonoids with twelve flavones, eight isoflavonoids, six flavanones, five flavanols, four flavonols, two dihydroflavonols, and one anthocyanidin. About thirty-two phenolic acids were identified belonging to different subclasses such as hydroxycinnamic acid (15), hydroxybenzoic acid (9), hydroxyphenylpropanoic acid (2), hydroxyphenylacetic acid (3). Similarly, twelve other polyphenols were obtained from different subclasses, *i.e*., alkylphenols (1), hydroxybenzaldehyde (1), hydroxycinnamaldehyde (1), hydroxycoumarin (4) and hydroxyphenylpropene (1) and others were *p*-cresol, *o*-cresol, 2-methyl citric acid and malic acid.

3.1.1 Relative quantification of phenolic metabolites

The identified RRPM were subjected to hierarchical cluster analysis: Euclidean distance measure and Ward clustering algorithm using MetaboAnalyst 5.0 to generate a heatmap representing the top thirty phenolic compounds in the experimental samples (Figure 2). The dark red colored regions in the heat map represent the most abundant RRPM. Overall, eighteen such metabolites were identified.The abundant RRPM identified in Samba mosanam (SM) were 5, and included coumarin (other polyphenol), 3'-O-methylcatechin (flavanols), *p*-hydroxybenzoic acid (phenolic acid), gallic acid (phenolic acid) and dihydroxyphenylacetic acid (other polyphenol). Five RRPM were abundant in Veethi vadankan (VV), *i.e*., esculetin (other polyphenol), hesperetin 3'-O-glucuronide (flavanones), coumaroyl glucose (phenolic acid), ferulaldehyde and feruloylquinic acid (phenolic acids). The red rice variety-Kuzhiadichan (KC) contained a more diverse range of phenolics with 14 abundant RRPM such as 2, 4 dihydroxybenzoic acid (phenolic acid), eriodictyol (flavanones), pinocembrin (flavanones), caffeic acid 4-O-glucoside (phenolic acid), 3'-hydroxyequol (isoflavonoids), 3'-hydroxydaidzein (isoflavonoids), 3'-hydroxy-O-desmethylangolensin (isoflavonoids), 4-hydroxybenzaldehyde (other polyphenols), 7,4'-dihydroxyflavone (flavones), malic acid (other polyphenols) and ethyl gallate (phenolic acid).

3.2 ADME analysis

The eighteen abundant RRPM were subjected to ADME analysis. RRPMs violating more than two parameters as per Lipinski's rule (hesperetin 3'-O-glucuronide) were ruled out from further analysis. Seventeen abundant RRPMs were found to obey Lipinski's rule, thereby exhibiting drug likeliness. The results obtained from ADME analysis are represented in Table 1, Figure 3 gives the bioavailability plots of the abundant RRPM identified in the experimental samples and those with high binding affinity. The compounds satisfying majority of the criteria, *i.e.*, lipophilicity (LogP)< 5, size < 500 g/mol, polarity (total polar surface area/TPSA) < 140 Å, flexibility (number of rotatable bonds) < 9, insolubility < 6, and insaturation (Csp3) < 1, shows good oral bioavailability (Krüger *et al.*, 2020; Mahanthesh *et al.*, 2020).

Table 1: ADME properties of the abundant red rice polyphenols

S.No.	Molecules	Lipinski rule (Drug likeliness)				TPSA* (Å)	Water Solubility LogS (ESOL)		GI Absorption	Bioavailability score
		Mol weight (<500Da)	Lipophilicity (Log P <5)	H bond donor (<5)	H-bond Acceptor (<10)					
1	3'-Hydroxydaidzein	270.24	0.52	3	5	90.9	-3.37	Soluble	High	0.55
2	3'-Hydroxyequol	258.27	1.62	3	4	69.92	-3.49	Soluble	High	0.55
3	3'-Hydroxy-O-desmethylangolensin	274.27	1.1	4	5	97.99	-3.61	Soluble	High	0.55
4	3'-O-Methylcatechin	304.29	0.49	4	6	99.38	-2.57	Soluble	High	0.55
5	4-Hydroxybenzaldehyde	122.12	0.79	1	2	37.3	-1.87	Very soluble	High	0.55
6	7,4'-Dihydroxyflavone	254.24	1.08	2	4	70.67	-4.03	Moderately soluble	High	0.55
7	Caffeic acid 4-O-glucoside	342.3	-1.63	6	9	156.91	-1.22	Very soluble	Low	0.11
8	Coumarin	146.14	1.65	0	2	30.21	-2.29	Soluble	High	0.55
9	Coumaroyl glucose	472.44	0	5	10	170.82	-2.92	Soluble	Low	0.55
10	Dihydroxybenzoic acid	154.12	0.4	3	4	77.76	-1.89	Very soluble	High	0.56
11	Eriodictyol	288.25	0.16	4	6	107.22	-3.26	Soluble	High	0.55
12	Esculetin	178.14	0.45	2	4	70.67	-2.28	Soluble	High	0.55
13	Ethyl Gallate	198.17	0.49	3	5	86.99	-2.01	Soluble	High	0.55
14	Gallic acid	170.12	-0.16	4	5	97.99	-1.64	Very soluble	High	0.56
15	Hesperetin 3'-O-glucuronide	478.4	-1.72	6	12	192.44	-3.39	Soluble	Low	0.11
16	Malic acid	134.09	-1.37	3	5	94.83	0.32	Highly soluble	High	0.56
17	p-Hydroxybenzoic acid	138.12	0.99	2	3	57.53	-2.07	Soluble	High	0.85
18	Pinocembrin	256.25	1.27	2	4	66.76	-3.64	Soluble	High	0.55

*According to Veber*et al.*, (2002), Topological Polar Surface Area (TPSA)< 140 Å indicates good oral bioavailability.

Fifteen RRPM was found to show good oral bioavailability and high gastrointestinal absorption (as given by Boiled-Egg model). Caffeic acid 4-O-glucoside and coumaroyl glucose were excluded from molecular docking studies due to their low oral bioavailability and gastrointestinal absorption. Bioavailability score was found to be highest for *p*-hydroxybenzoic acid (0.85), followed by 2,4 dihydroxybenzoic acid, gallic acid and malic acid, with a score of 0.56. Twelve other compounds exhibited a bioavailability score of 0.55 and least value was recorded by caffeic acid 4-O-glucoside (0.11).

3.3 Molecular docking analysis

The fifteen potent compounds obeying Lipinski's rule with good oral bioavailability and high GI absorption along with the control drug, nelfinavir, were further subjected to molecular docking analysis to understand their inhibitory action against SARS-CoV-2 M^{pro}. The binding affinity of these compounds with the protein were determined based on the values of binding energy (kcal/mol) obtained from Autodock software. The lesser the value of binding energy more is the binding affinity of the ligand with the target protein.

Docking analysis (Table 2) revealed that 7,4'-dihydroxyflavone (-11.03 kcal/mol) and eriodictyol (-10.75 kcal/mol) had stronger binding affinity with SARS-COV-2 M^{pro}compared to the control drug nelfinavir (-8.53 kcal/mol). Five other phenolic compounds (3'-hydroxyequol, 3'-O-methylcatechin, pinocembrin, 3'-hydroxy-daidzein and 3'-hydroxy-O-desmethylangolensin) also showed a good binding affinity with the target protein with binding energy values ranging between -6.57 kcal/mol to -8.04 kcal/mol.

The docking poses and 2-D interactions of SARS-CoV-2 M^{pro} with 7,4'-dihydroxyflavone and eriodictyol are presented in Figure 4.

Among all the docked complexes, 7,4'-dihydroxyflavone exhibited highest binding affinity with the least binding energy of -11.03 kcal/mol. It was observed that five different bonds were involved in the interaction of protein and ligand complex. It formed hydrogen bonds with the active sites THR190, ASP187 and non-active site TYR54. It also formed a carbon hydrogen bond and a pication bond at the active sites GLU166 and HIS41, respectively. The phenolic metabolite formed an alkyl bond at the active site residue MET165 and weak Van der Waals interactions with seven other amino acids. Overall, it formed bonds with 8 active sites of SARS-CoV-2 M^{pro}.

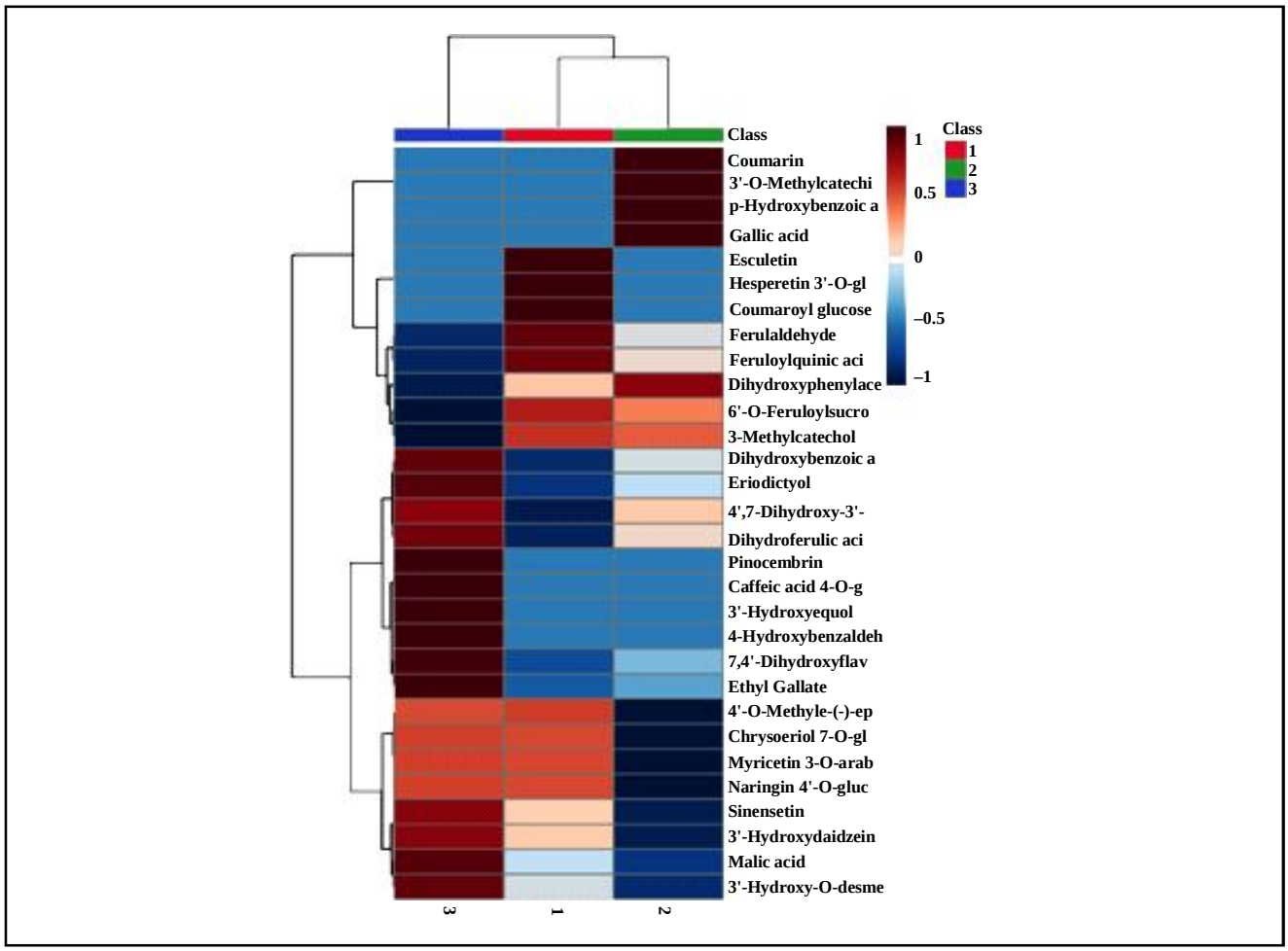

Figure 2: Heat map of top thirty phenolic compounds in red varieties (Class1: Veethivadankan, Class2: Samba Mosanam, Class 3: Kuzhiadichan).

Eriodictyol was also found to have a higher binding affinity (-10.75 kcal/mol), compared to the control drug. The structure of eriodictyol SARS-CoV-2M^{pro} complex was stabilized by three different interactions, *i.e.*, Van der Waals force, hydrogen bond and pication bonds. Hydrogen bonds were formed at the active site comprising the amino acid residues, THR190, ARG188, MET165, HIS41 and ASP187 of the protein. It also exhibited Van der Waals bonds with three active site residues MET49, GLU166 and HIS164. The ligand formed similar bonds with the amino acid residues TYR54, LEU167, PRO168, ALA191, VAL186 and GLN189. Eriodictyol was, thus found to bind with 11 active sites of SARS-CoV-2 M^{Pro}.

Additionally, eight more RRPM, *i.e.*, 3'-hydroxyequol, 3'-O-methylcatechin, pinocembrin, 3'-hydroxydaidzein, 3'-hydroxy-O-desmethylangolensin, esculetin, 4-hydroxybenzaldehyde and ethyl gallate also showed good binding potential with the target protein. The poses and interactions of the first five metabolites are represented in Figure 5 and described here.

3'-Hydroxyequol showed a good binding affinity (-8.04 kcal/mol) with the active sites of target protein by interacting with MET49, LEU141, GLY143, SER144, CYS145, HIS163, through hydrogen bonds. MET49 was found to interact with ligand through hydrogen, alkyl and pi-alkyl bonds. Additionally, pi-pi stacked bond along with pi-alkyl were observed between the ligand and HIS41 residue. Eight other amino acids can be observed forming weak Van der Waals interactions. An unfavorable donor-donor interaction was observed by GLN189 with the ligand molecule. 3'-Hydroxyequol formed bonds with a total of 15 active sites of SARS-CoV-2 M^{pro}.

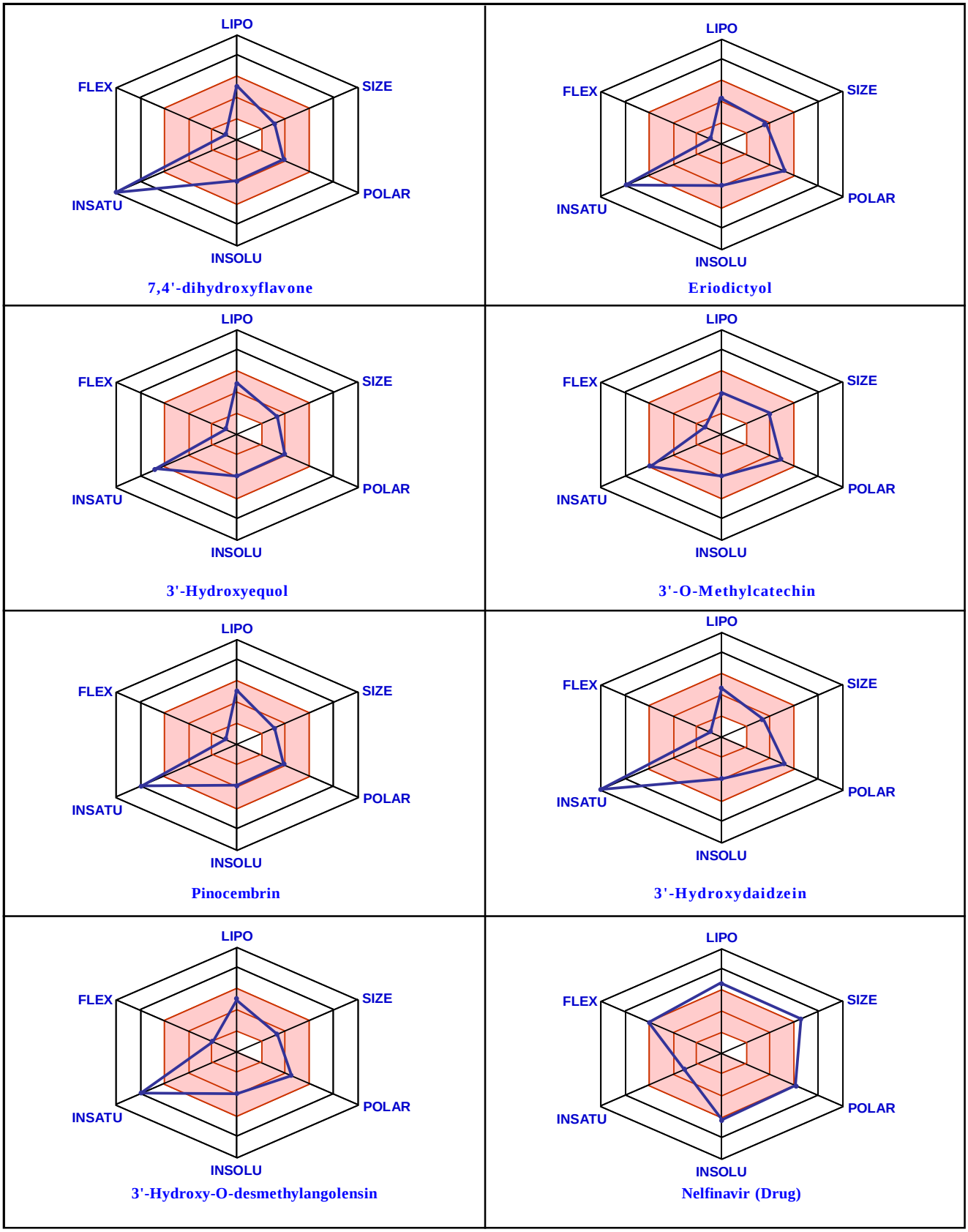

Figure 3: Bioavailability radar plots of nelfinavir (drug) and compounds exhibiting good inhibitory action against SARS-CoV-2 M^{pro}.

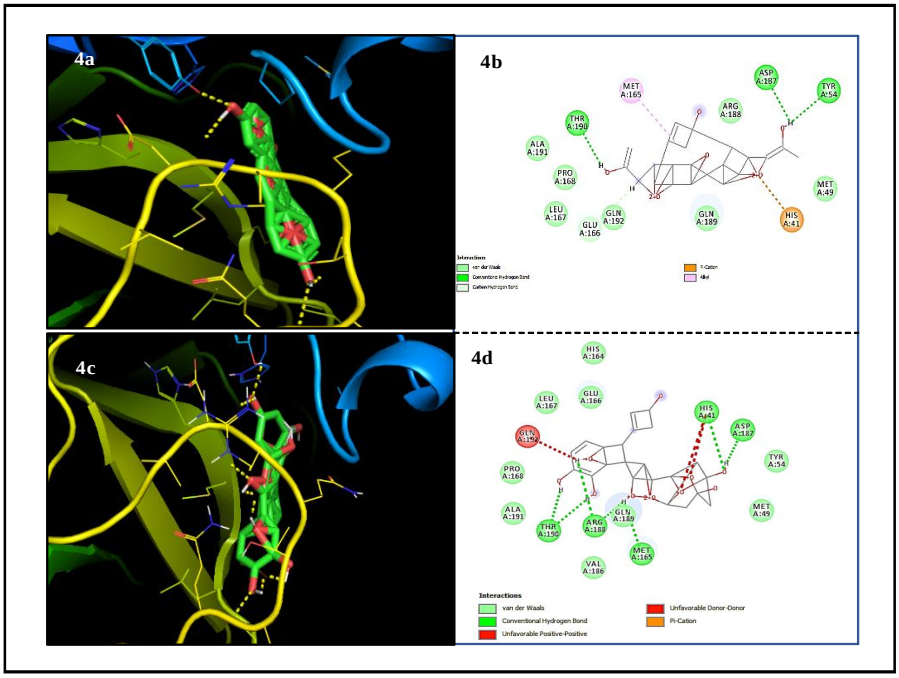

Figure 4: Molecular docking pose and 2-D view of interaction with SARS-CoV-M^{pro} of 7,4'-Dihydroxyflavone (Figures 4a and b) and Eriodictyol (Figures 4c and d).

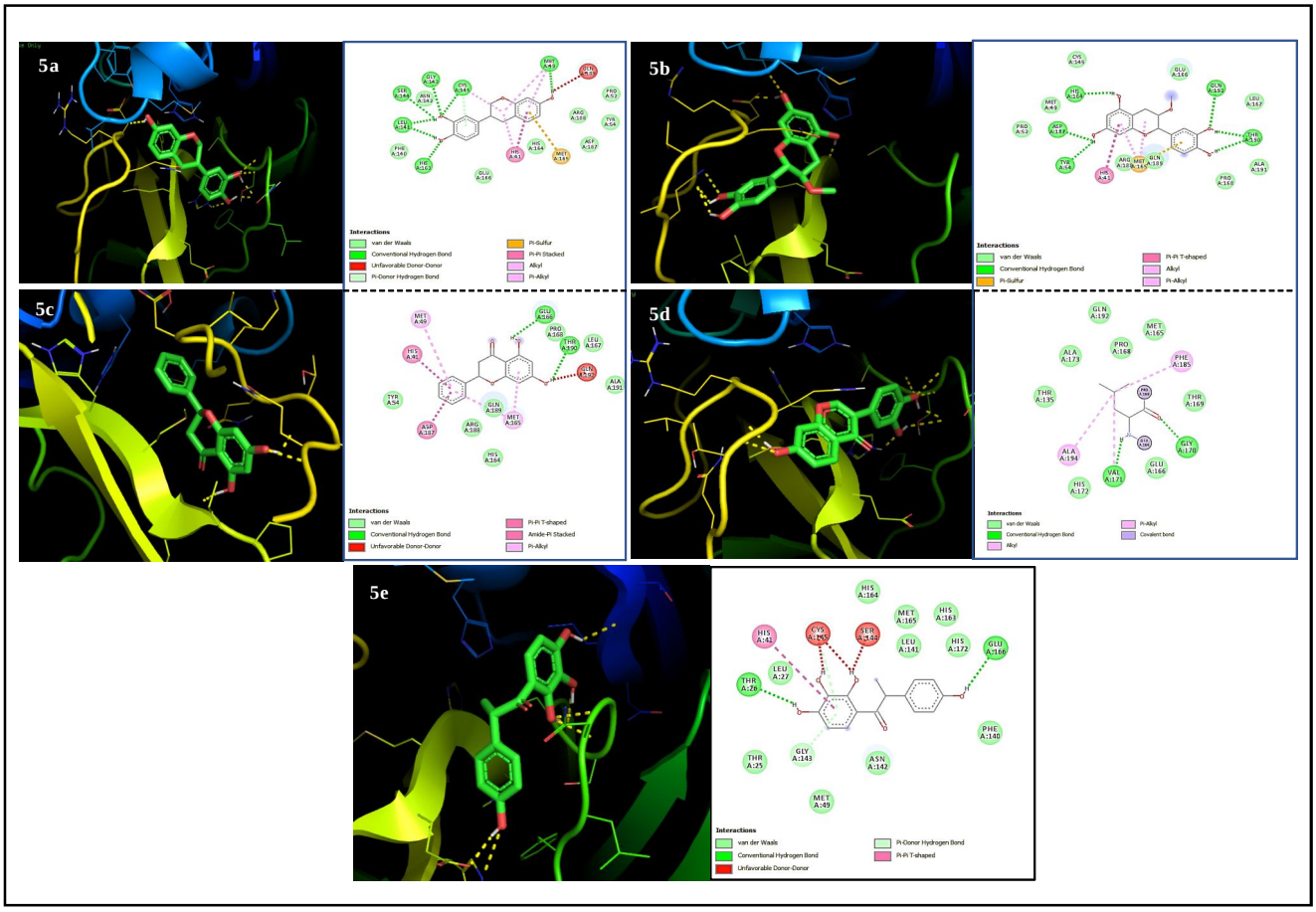

Figure 5: Molecular docking pose and 2-D view interaction of SARS-CoV-2 M^{pro} with 5a, 3'-Hydroxyequol, 5b, 3'-O-Methylcatechin, 5c, Pinocembrin, 5d, 3'-Hydroxydaidzein and 5e, 3'-Hydroxy-O-desmethylangolensin.

Table 2: Molecular docking results of fifteen bioavailable abundant red rice polyphenols

S. No.	Ligands	Binding Energy (kcal/mol)	Interaction with the active sites of SARS-CoV-2 M^{Pro}
1.	Nelfinavir (Control)	-8.53	MET49, ASN142, CYS145, MET165, GLU166, ASP187, ARG188, GLN189, THR190
2.	7,4'-Dihydroxyflavone (KC)#	-11.03	HIS41, MET49, MET165, GLU166, THR190, ASP187, ARG188, GLN189
3.	Eriodictyol (KC)	-10.75	MET49, THR190, ARG188, HIS164, GLU166, MET165, HIS41, ASP187, ARG188, GLN189, THR190
4.	3'-Hydroxyequol (KC)	-8.04	HIS41, PHE140, CYS145, ASN142, GLY143, SER144, CYS145, LEU141, GLY143, HIS163, MET49, HIS164, MET165, GLU166, ASP187
5.	3'-O-Methylcatechin (SM)	-7.96	HIS41, MET49, CYS145, HIS164, MET165, GLU166, ASP187, ARG188, GLN189, THR190
6.	Pinocembrin (KC)	-7.86	MET49, HIS41, HIS164 GLU166, THR190, ASP187, ARG188, GLN189, THR190
7.	3'-Hydroxydaidzein (KC)	-7.29	HIS172, GLU166, MET165
8.	3'-Hydroxy-O-desmethylangolensin (KC)	-6.57	HIS41, MET49, THR25, THR26, PHE140, LEU141, GLY143, MET165, GLU166, HIS163, HIS164, HIS172
9.	Esculetin (VV)	-5.81	ASN142, CYS145, GLY143, SER144, PHE140, LEU141, HIS163, HIS164, MET165, GLU166, HIS172, GLN189
10.	Dihydroxybenzoic acid (KC)	-5.39	-
11.	Coumarin (SM)	-5.36	-
12.	4-Hydroxybenzaldehyde (KC)	-4.93	HIS41, MET49, HIS164, MET165, ASP187, ARG188
13.	Ethyl Gallate (KC)	-4.91	MET165, GLU166, ASP187, ARG188, GLN189, THR190
14.	p-Hydroxybenzoic acid (SM)	-4.89	-
15.	Gallic acid (SM)	-4.75	-
16.	Malic acid (KC)	-2.4	-

The name of the red rice variety abundant in the respective polyphenol is mentioned in the parenthesis; KC-Kuzhiadichan; SM -Samba mosanam; VV -VethiVadakan.

3'-O-methylcatechin is another ligand with good binding energy (-7.96 kcal/mol). Six different interactions were involved in stabilizing the docked complex through Van der Waals force, hydrogen bond, pi-sulfur, pi-pi T-shaped bond, alkyl, and pi-alkyl bond. The ligand formed hydrogen bonds at the active sites HIS164, ASP187,THR190 and non- active sites TYR54 and GLN192, respectively. The metabolite showed pi-sulfur, alkyl and pi-alkyl interactions at the active site MET165. The ligand was found to interact with nine other amino acids through Van der Waals force. Thus, 3'-O-methylcatechin formed bonds with a total of 15 active sites of SARS-CoV-2 M^{pro}.

Pinocembrin M^{pro} complex had a binding energy of -7.86 kcal/mol and exhibited five different interactions. The active sites, HIS41 and ASP187 coupled with the ligand through an amide-pi stacked bond and pi-pi T-shaped bond, respectively. Hydrogen bonds were formed with the active sites MET165,GLU166 and THR190. The ligand showed a pi-alkyl interaction with the active sites MET165 and MET49. Seven other amino acid residues were involved in the formation of Van der Waals bond with the ligand molecule. The complex also showed a donor-donor interaction with the GLN192 residue.

3'-hydroxydaidzein was observed to have a binding energy of -7.29 kcal/mol. A covalent bond interaction was observed with the active site GLU166 and another amino acid residue PRO168. The ligand shared two hydrogen bonds with GLY170 and VAL171. The structure was stabilized by alkyl and pi-alkyl bond through interaction with PHE185 and ALA194 residues, respectively. VAL171 was found to have shared both hydrogen bond and pi-alkyl bond with 3'-hydroxydaidzein. It also formed bonds through Van der Waals force with eight other amino acid residues.

3'-hydroxy-O-desmethylangolensin was observed to have a binding energy of -6.57 kcal/mol. Altogether four different bonds were involved in the interactions -Van der Waals force, hydrogen bond, pi-donor hydrogen bond and pi-pi t-shaped bond. Hydrogen bonds were formed with the active sites THR26 and GLU166 and a pi-donor hydrogen with another active site GLY143. Bonds due to weak Van der Waals were formed with ten other amino acid residues.The phenolic compound also formed a pi-pi T-shaped bond at the active site of HIS41.

4. Discussion

The present study demonstrated a great diversity in the composition of polyphenolic metabolites among the three Indian red rice varieties, VV, SM and KC. Flavonoids were the largest group of polyphenols present across all three red rice varieties, followed by phenolic acids and other polyphenols. A recent study conducted by Haldipur and Srividya (2020) also reported flavonoids to be the most abundant polyphenols in two other pigmented rice varieties (Bamboo rice and Garudan Samba). Similar polyphenols reported in the study were coumarin, dihydroquercetin, dihydroquercetin 3-O rhamnoside, pinocembrin, procyanidin dimer B, sinapic acid-hexoside, naringin 4' -glucoside, 3-methoxynobiletin,esculetin, and isorhamnetin. Polyphenols like *p*-coumaric acid, ferulic acid, gallic acid, *p*-hydroxybenzaldehyde, and isorhamnetin identified in the experimental samples were also reported in pigmented rice varieties from Italy (Zaupa *et al.*, 2015) and Thailand (Seekhaw *et al.*, 2018). In a study by Kotamreddy *et al.* (2020), vanillic acid was reported to be absent in red rice varieties, similar to the observation in the current study.

Through, molecular docking analysis, it was found that several of the RRPM exhibited good binding potential at the active sites of the target protein M^{Pro} indicating them to be potential antiviral agents against SARS-CoV-2.

Eriodyctiol showed a high binding potential with SARS-CoV-2 M^{pro} at eleven active sites. Eriodyctiol has been reported for the first time in the present study in pigmented rice metabolome. A glucoside of the compound, *i.e.*, eriodyctiol-7-glucoside has been reported previously in Chinese brown rice genotypes (Dong *et al.*, 2014). A recent study by Deshpande *et al.* (2020) also reported eriodyctiol with a good binding potential (-6.7 kcal/mol) against SARS-CoV-2 M^{Pro}.

7,4'-dihydroxyflavone exhibited the best binding affinity among all other RRPMs with interactions at eight active sites of SARS-CoV-2 M^{pro}. No study till date has reported the antiviral activity of this metabolite.This is probably the first study to identify the antiviral potential of 7, 4'-dihydroxyflavone against COVID-19 virus. A structurally similar phenolic metabolite -3, 4'-dihydroxyflavone has been reported to show antiviral activity against influenza virus (Hossain *et al.*, 2014).

Among eight other RRPM which exhibited antiviral activity against SARS-CoV-2, only one of them, *i.e.*, esculetin have been reported earlier. Vijayaraj *et al.* (2020) reported the potential antiviral activity of an ester of esculetin identified in Marine Sponges against COVID-19 virus based on its binding potential with SARS-CoV-2 M^{Pro}. Few of these metabolites have been reported previously for activity against different viruses. Le *et al.* (2019) reported the antiviral activity of pinocembrin against Zika virus. 3'-hydroxydaidzein has been reported to show antiviral activity against influenza virus (Özçelik *et al.*, 2006).

The main antiviral RRPMs - 7,4'-dihydroxyflavone and eriodyctiol and other RRPMs with activity against SARS-CoV-2 M^{Pro} were found to be abundant in Kuzhiadichan rice variety. Interestingly, a recent study has shown strong negative correlation between rice consumption and the number of COVID-19 patients based on data obtained from nineteen countries from five continents (Watanabe and Inuma, 2020). A recent *in silico* study (Fujii, 2020) postulated that the micro RNA of rice (MIR2097-5p) can prevent COVID-19 by suppressing SARS-CoV-2 viral micro RNAs and HIPK2 target proteins.

Hence, this rice can be considered as a potential functional food in COVID-19 management.

5. Conclusion

The study showed that the three Indian red rice cultivars are rich sources of different bioactive phenolic metabolites. 7,4'-dihydroxyflavone and eriodyctiol with high binding capacity on multiple active sites of SARS-CoV-2 target protein could be considered as potential candidates for antiviral drug development. The red rice variety-Kuzhiadichan, abundant in eight potential antiviral metabolites could be recommended as functional foods for COVID 19. Alternatively, the concentrate form of these phenolics could be used in the development of antiviral nutraceuticals which could act synergistically in the prevention and management of SARS-CoV-2 infection. Identification of the antiviral pharmacological action of

these RRPM is an important milestone for scientists, health practitioners, and pharmacologists for future drug and functional food product development. Further, *in vitro* and *in vivo* studies are warranted for the validation of the antiviral activity of these red rice phenolic metabolites.

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Conflict of interest

The authors declare that there are no conflicts of interest relevant to this article.

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