Research Square

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Computational screening of phytocompounds from Moringa oleifera leaf as potential inhibitors of SARS-CoV-2 Mpro

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Research Article

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Abstract

Background: Coronavirus Disease (COVID-19), caused by novel SARS CoV-2 is rapidly spreading all over the World creating a global public health emergency at unprecedented levels. Till today, no effective treatments or vaccines against this global pandemic is reported and hence to identify lead compounds having potential action in controlling the spread the pandemic is a global concern. This study aimed at *in silico* screening of phytocompounds from *M.oleiera* leaf against novel SARS CoV-2 main protease (M^{pro}) through molecular docking. *M.oleiera* is an Indian medicinal plant as well as a vegetable, all parts of the plant is medicinally useful and is being used in many of the traditional and Ayurvedic medicinal preparations.

Result: When the 19 compounds identified from *M.oleifera* leaf methanolic extract by Liquid Chromatography Mass Spectrometry (LCMS/MS) analysis and 5 FDA approved anti-viral drugs were screened *in silico* with SARS CoV-2 main protease (M^{pro}), the following compounds showed top interaction; apigenin-7-O-rutinoside (-8.8 kcal/mol), Mudanpioside (-8.3 kcal/mol), isoquercetin (-8 kcal/mol), isoquercitrin (-8 kcal/mol), quercetin (-7.8 kcal/mol) and dihydroquercetin (-7.8 kcal/mol). Anti-viral drugs: Raltegravir (-7.2 kcal/mol), Lopinavir-Ritonavir (-7.7 kcal/mol), maraviroc (-8.2 kcal/mol), Nelfinavir (-8.3 kcal/mol) and Tipranavir (-9.2 kcal/mol) also showed active interaction with M^{pro}. Preliminary phytochemical screening of methanol extract showed the presence of flavonoids, cardiac glycosides, phenols, coumarins, saponins, steroids and phytosteroids. *In vitro* antioxidant activity of methanolic extract of *M.oleifera* also showed greater activity, which would ameliorate the post-COVID secondary infection.

Conclusion: Hence these compounds from *M.oleifera*, which are our diet based components, which can interact with the M^{pro} and curtail COVID-19 virus multiplication in the host cell.

Background

Corona viruses (SARS-CoV-2) are enveloped viruses belonging to the family *Coronaviridae,* and subfamily *Coronavirinae.* The virion of SARS-CoV-2 consists of crown shaped peplomers and possess positive stranded genome of ~30kb size consisting of a 5' cap and 3' poly A tail. A helical capsid is also present within the viral membrane consisting of genomic RNA complexed with nucleocapsid. There are four genera of corona viruses; *alpha, beta, gamma* and *delta*. Alpha and beta causes infections in mammals while the other two affects birds and mammals commonly [1]. HCoV-229E, HKU1, HCoV-NL63 and HCoV-OC43 are the human corona viruses which cause mild upper respiratory infection to highly contagious Severe Acute Respiratory Syndrome coronavirus (SARS-CoV) and Middle East Respiratory Syndrome coronavirus (MERS CoV) [2].

As per the recent report on 29th July 2020 of COVID-19 by WHO total 16 558 289 were infected people and a total of 656 093 deaths globally (https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200729-covid-19). The causative agent of ongoing COVID-19 pandemic is highly contagious novel Severe Acute Respiratory Syndrome- Corona Virus-2 (SARS-CoV-2). No vaccines or effective treatments are available till today against this globally affected pandemic and scientists all over the World are investigating the possibility to develop vaccines and/or medicines. All the prevailing prophylaxis against SARS-CoV-2 is symptomatic to handle mild to severe forms of symptoms associated with the infection [3]. Hence the situation warrants discovery of potential medications to control SARS-CoV-2 pandemic from spreading.

The corona virus uses the heteromeric spike protein present on its surface to interact with the ACE2 (angiotensin-converting enzyme-2) abundantly present in many human cell types [4]. After entering the cell, two polyproteins encoding several crucial non-structural proteins from their genomic RNA is translated including Chymotrypsin-like protease (3CLpro) or main protease (M^{pro}) and papain like protease (P^{pro}) [5,6]. Open reading frames, ORFs, ORF1a and ORF1b of SARS CoV-2 and MERS CoV-2 encodes two cysteine proteases, viz; a papain-like protease (PL^{pro}) and a 3C like protease (main protease (M^{pro}) are encoded by ORF1a. PL^{pro} is involved in the cleavage of first three cutting sites of its polyprotein and M^{pro} is involved in cleaving other eleven positions leads to the release of sixteen non-structured proteins (nsp). Since this

autocleavage process is necessary for viral maturation and replication, M^{pro} remains as the important drug target against COVID-19 infection [7]. In this study, we aimed to identify bioactive compounds from *Moringa oleifera* leaves, which bind with M^{pro} of SARS CoV-2.

M.oleifera is most widely distributed and cultivated species across India. It is a medium sized tree, about 5 to 10 m in height and found in wide array of climatic conditions [8]. The plant is used to cure more than 300 ailments. Its medicinal properties include; antidiabetic [9], anticancer [10], [11], Neuroprotectant [12], [13], antiulcer [14], antipyretic [15], antihelminthic [16], antiretroviral [17], antiarthritic [18], antimicrobial [19], antioxidant [20], antifertility [21], hepatoprotective activity [22], hypotensive [23], analgesic [24], wound healing [25], anticonvulsant [26] and reported to have inhibitory actions against several viruses including HIV [27], HSV [28], HBV [29], EBV [30], FMDV [31] and NDV [32]. In the present study, a total of 19 leaf compounds were screened and those having higher binding affinity with M^{pro} can be a good target for further anti-viral drug research.

Methods

1. **Plant material collection, Identification and preparation**

The plant material was identified by DNA barcoding analysis and the voucher specimen and herbarium (No. 486) was deposited at St. Albert's College Herbarium (SAC), Ernakulam, Kerala, India. *M.oleifera* leaves were collected, shade dried and powdered. Soxhlet extraction was carried out by taking 20 g of the leaf powder in 1000 ml of methanol. The extract thus obtained was concentrated using rotary evaporator and lyophilized.

2. **Phytochemical Screening**

Preliminary phytochemical screening was done to detect the presence of Carbohydrates, Flavonoids, Quinones, Glycosides, Cardiac glycosides, Terpenoids, Phenols, Coumarins, Phlobatannins, Anthraquinones, Tannins, Saponins, steroids and phytosteroids using standard methods.

3. ***In vitro* Antioxidant Activity**

Antioxidant activity of *M.oleifera* leaf methanolic extract was done by DPPH (2,2-diphenyl-1-picrylhydrazyl) method [33]. Ascorbic acid was taken as the standard.

4. **Identification of compounds from*oleifera* methanolic extract by LCMS/MS Analysis**

Bioactive compounds present in the extract was tentatively identified by LCMS/MS (Schimadzu) analysis. The column used was C18 and a gradient elution of 0.1% formic Acid in water and acetonitrile was employed. The flow rate was 0.3ml/min and the duration of the analysis was 30 minutes.

5. **ADME Prediction**

All the secondary metabolites detected from *M.oleifera* were subjected to ADME (Absorption Digestion Metabolism Excretion) prediction using SWISS ADME[34]. Following parameters were assessed; Lipophilicity (LogP), water solubility, gastrointestinal absorption (GI), Blood Brain Barrier (BBB) permeability, p-gp substrate, Drug likeliness (Lipinski's Rule) and Bioavailability score.

6. **Molecular Docking Analysis**

High-resolution three-dimensional X-ray crystal structure of SARS CoV M^{pro} (PDB ID: 6Y2F) was downloaded from Protein Data Bank. Water molecules were removed and hydrogen atoms were added using PyMol software. The SDF structure of the selected ligands were downloaded from PubChem and they were converted to PDB format using Open babel software and then to PDBQT using AutoDock tools. The following compounds were identified tentatively by LCMS/MS analysis of *M.oleifera* methanolic extract, which were used for docking studies; Apigenin-7-O-rutinoside, Mudanpioside J, Isoquercetin, Isoquercitrin, Quercetin, Dihydroquercetin, Luteolin, Neochlorogenic acid, Vicenin 2, Catechin, Epicatechin, Rutin , Marumoside B, Scutellarin, Rhamnetin, Astragalin, Apiin, Marumoside A and Niazirin. The FDA approved drugs Nelfinavir, Raltegravir and Lopinavir-Ritonavir, maraviroc, Tipranavir were also docked against SARS CoV2 M^{Pro}. Molecular docking analysis was done using AutoDock Vina [35].

Results

Phytochemical Screening

Presence of flavonoids, cardiac glycosides, phenols, coumarins, saponins, steroids and phytosteroids were detected from the methanolic extract of *M.oleifera* leaf.

In vitro antioxidant activity

Table:1 showing the result of *in vitro* antioxidant assay

Concentration (µg/ml)	Percentage Inhibition of DPPH radical	
	M.oleifera Extract	Ascorbic Acid
20	90.00%	48.00%
40	96.20%	67.80%
60	97.40%	92.87%
80	98.10%	94.08%
100	98. 9%	94.54%

Identification of compounds from *M.oleifera* methanolic extract by LCMS Analysis

Compounds such as Apigenin-7-O-rutinoside, Mudanpioside J, Isoquercetin, Isoquercitrin, Quercetin, Dihydroquercetin, Luteolin, Neochlorogenic acid, Vicenin 2, Catechin, Epicatechin, Rutin , Marumoside B, Scutellarin, Rhamnetin, Astragalin, Apiin, Marumoside A and Niazirin were identified tentatively from *M.oleifera* leaf methanolic extract. Among these 19 compounds, 14 were flavonoids (Apigenin-7-O-rutinoside, isoquercetin, isoquercitrin, quercetin, dihydroquercetin, luteolin, vicenin2, Catechin Epicatechin, Rutin, Scutellarin, Rhamnetin, Astragalin and Apiin), monoterpene glycoside (Mudanpioside J), phenolic acid (Neochlorogenic acid), phenol (Marumoside A, Marumoside B) and nitrile glycoside (niazirin).

ADME Prediction

Table:2 showing the results of ADME prediction of secondary metabolites detected from *M.oleifera* methanolic extract

Sl. no.	Compound	Molecular mass	lipophilicity	water solubility	GI absorption	BBB permeability	p-gp substrate	Drug likeliness	Bioavailability score
1.	Apigenin-7-O-rutinoside	578.5	-6.4	moderately soluble	Low	No	Yes	No	0.17
2.	Apiin	564.49	-0.68	Soluble	Low	No	Yes	No	0.17
3.	Astragalin	448.38	-0.09	Soluble	Low	No	No	Yes	0.17
4.	Catechin	290.27	0.83	Soluble	High	No	Yes	Yes	0.55
5..	Dihydroquercetin	304.25	0.51	Soluble	High	No	No	Yes	0.55
6.	Epicatechin	290.27	0.85	Soluble	High	No	Yes	Yes	0.55
7.	Hyperoside	464.38	-0.38	Soluble	Low	No	No	No	0.17
8.	Isoquercetin	464.38	-0.48	Soluble	Low	No	No	Yes	0.17
9.	Luteolin	286.24	1.73	Soluble	High	No	No	Yes	0.55
10.	Marumoside A	297.3	-0.53	Soluble	High	No	No	Yes	0.55
11.	Marumoside B	459.44	-1.68	Soluble	Low	No	No	Yes	0.17
12.	Mudanpioside J	630.59	0.8	Soluble	Low	No	Yes	Yes	0.17
13.	Neochlorogenic acid	354.31	-0.46	Soluble	Low	No	No	Yes	0.11
14.	Niazirin	279.29	0.25	Soluble	High	No	No	Yes	0.55
15.	Quercetin	302.24	1.23	Soluble	High	No	No	Yes	0.55
16.	Rhamnetin	316.26	1.63	Moderately soluble	High	No	No	Yes	0.55
17.	Rutin	610.52	-1.51	Soluble	Low	No	Yes	No	0.17
18.	Scutellarin	286.24	1.81	Soluble	High	No	No	Yes	0.55
19.	Vicenin 2	594.52	-1.98	Soluble	Low	No	Yes	No	0.17
	Anti-viral drugs								
	Raltegravir	444.42	1.46	Moderately soluble	Low	No	Yes	Yes	0.55
	Lopinavir-Ritonavir	1349.75	8.21	Insoluble	Low	No	Yes	No	0.17
	Maraviroc	513.67	4.75	Poorly soluble	High	No	Yes	Yes	0.55
	Nelfinavir	567.78	4.41	Poorly soluble	Low	No	Yes	Yes	0.55
	Tipranavir	602.66	6.06	Insoluble	Low	No	Yes	Yes	0.56

Molecular docking Analysis

See Figure 2.

Discussion

Preliminary Phytochemical Screening

Potterat, 1997 [36] reported the presence of alkaloids, triterpenoids, flavonoids, tannins, saponins, and glycosides in methanolic extract of *M.oleifera*. These are essential phytochemicals in the leaf that represent total phenolics, different enzymes (ascorbic acid oxidase, polyphenol oxidase, and catalase) and vitamins [37];[38]. Phenolic compounds are also reported from moringa leaf extracts which act as natural antioxidants and serve as first line defense against free radical induced cellular damage [39]. Flavonoids are the bioactive phytocomponents which are used for treatment of allergies,

inflammation, ulcers, tumors and viral infections while saponins are reported to have antihypertensive and hypocholesteremic effects [10],[40].

In vitro antioxidant activity

Most of the medicinal plants are a major source of phytochemicals with proven antioxidant property [41]. Moringa especially treated as a nutritive as well as a medicinal plant in the Indian traditional medicine. Results of the present study reveal that the antioxidant and anti-viral property of *Moringa oleifera* from a natural source. There was a 90% and 98.9% inhibition of DDPH free radical at a concentration of 20 µg/ml and 100µg/ml respectively, showing that even at a low dose of 20 µg/ml the moringa extract proved to be an effective antioxidant (table:2). Similar results were also reported by Fitriana et al., 2016 [42] revealing that the methanolic extract shows highest antioxidant activity. It is proven that flavonoids and other phenolic compounds present in the plant have potent antioxidant and chelating property. According to Suphachai, 2014, [43] there exists a correlation between antioxidant activities with total phenolic compound. Methanolic extracts will have a total phenolic compound higher than the other solvents because methanol is the highest polar solvent which can pull out more polyphenol compounds. The presence of polyphenol compounds such as quercetin and kaempferol in the leaves may also account for greater antioxidant activity. The observed antioxidant property of the extract would ameliorate the post-COVID secondary infection.

ADME Prediction and Molecular docking Analysis

Compounds from *M.oleifera* leaf were docked against M^{pro} of SARS CoV2 for the identification of potential inhibitors of the same. Few compounds that showed top interactions were Apigenin-7-O-rutinoside (-8.8 kcal/mol), Mudanpioside (-8.3 kcal/mol), isoquercetin (-8 kcal/mol), hyperoside (-7.8 kcal/mol), quercetin (-7.8 kcal/mol) and dihydroquercetin (-7.8 kcal/mol). All the compounds used in the present study showed a similar interaction with M^{pro} as in the case of the FDA approved standard anti-viral drugs like Nelfinavir (-8.3 kcal/mol), Raltegravir (-7.2 kcal/mol), Lopinavir-Ritonavir (-7.7 kcal/mol), maraviroc (-8.2 kcal/mol) and Tipranavir (-9.2 kcal/mol) studied here.

Apigenin-7-O-rutinoside (-8.8 kcal/mol), is a flavonoid, showed higher binding affinity with M^{pro} than the FDA approved antiviral drugs Raltegravir (-7.2 kcal/mol), Lopinavir-Ritonavir (-7.7 kcal/mol), Maraviroc (-8.2 kcal/mol) and nelfinavir (-8.3 kcal/mol) but it was found to be a P-gp substrate, having low GI absorption and low bioavailability score. Whereas Mudanpioside J (-8.3 kcal/mol) is a monoterpene glycoside which also showed higher affinity with M^{pro} than Raltegravir (-7.2 kcal/mol), Lopinavir-Ritonavir (-7.7 kcal/mol), Maraviroc (-8.2 kcal/mol) and showed same affinity as of nelfinavir (-8.3 kcal/mol). it was also found to have low GI absorption. Isoquercetin, hyperoside, quercetin and dihydroquercetin showed much higher binding affinity with M^{pro} than the anti-viral drugs Raltegravir and Lopinavir-Ritonavir. Luteolin also exhibited binding affinity similar to the anti-viral drug Lopinavir-Ritonavir. Isoquercetin and hyperoside, showed low GI absorption where quercetin and dihydroquercetin had high GI absorption rate and none of them were P-gp substrate. All the anti-viral drugs except maraviroc were having low GI absorption. Also, all of them were P-gp substrate.

All the other phytocompounds showed binding affinity greater than -7 kcal/mol, but with lesser affinity with M^{pro} than the anti-viral drugs used here. Neochlorogenic acid (-7.6kcal/mol) is a phenolic acid which showed low GI absorption and low bioavailability score. Vicenin 2 (-7.6 kcal/mol), catechin (-7.5 kcal/mol), Epicatechin (-7.4 kcal/mol) and Rutin (-7.4 kcal/mol) are flavonoids showed potential interaction by forming more hydrogen bonds with M^{pro}. Catechin and epicatechin had high GI absorption and high bioavailability score unlike vicenin2.

Maramoside B is a phenol, showed a binding affinity of -7.3kcal/mol but GI absorption was low. Scutellarin (-7.3 kcal/mol), is a flavonoid exhibited strong interaction with M^{pro} but without forming any conventional hydrogen bonds. It also showed high GI absorption and bioavailability score. Rhamnetin (-7.2 kcal/mol), astragalin (-7.2 kcal/mol) and apiin are flavonoids and Marumoside A and niazirin (-7 kcal/mol) both are phenolic compounds also showed good binding

affinity with $M^{pro.}$ Rhamnetin, marumoside A and niazirin had high GI absorption rate and bioavailability score whereas astragalin and apiin were having low GI absorption and bioavailability score.

Anti-viral drugs such as Raltegravir, Lopinavir-Ritonavir, Maraviroc, Nelfinavir and Tipranavir were also screened for tracing possible interaction with M^{pro}. Raltegravir (-7.2 kcal/mol) is an anti-retroviral drug, used along with other drugs to relieve the HIV infection [44]. Lopinavir-Ritonavir (-7.7 kcal/mol) is a combination of two medications; lopinavir and ritonavir, used to control HIV/AIDS infection [45]. Maraviroc (-8.2 kcal/mol) is a FDA approved chemokine receptor type (CCR5) antagonist, which blocks the entry of HIV to cells [46]. Nelfinavir (-8.3 kcal/mol), is anti-retroviral drug and a protease inhibitor, used for treating Human Immunodeficiency Virus (HIV) [47].Tipranavir (-9.2 kcal/mol) is also an anti-viral medication used to treat HIV infection in combination with ritonavir.

After the screening and molecular docking of bioactive components from *M.oleifera* leaf, it was found exhibiting strong binding affinity with the novel corona virus main protease (M^{pro}). The results were comparable to the interactions of the FDA approved antiviral drugs studied here. Further cell line studies and biochemical assays would establish its activity. Since natural products are always serve as an excellent source of lead molecules for many diseases without much side effects unlike in the case of many synthetic drugs in use today. *M. oleifera* is a universal tropical plant easily available, easily extractable and economically viable source of these compounds.

Conclusion

The present study in an *in silico*-based approach which reveals the possibility of identification of potent SARS CoV2 M^{pro} inhibitors from plant sources. Our study gives an idea of the action of phytoconstituents from *M.oleifera* leaf against the main protease of Corona Virus. Among the 19 compounds screened, apigenin-7-O-rutinoside showed highest activity against SARS CoV-2 M^{pro}. Moreover, these compounds are also found to have antioxidant property which would ameliorate the post-COVID secondary infection.

Abbreviations

COVID - Coronavirus Disease

SARS CoV2 -Severe Acute Respiratory Syndrome Coronavirus 2

FDA - Food and Drug Administration

SARS - Severe Acute Respiratory Syndrome

MERS - Middle East Respiratory Syndrome

HCoV - Human Coronavirus

M^{pro} - Main protease

ACE2 - angiotensin-converting enzyme-2

LCMS - Liquid Chromatography Mass Spectrometry – LCMS

HIV - Human Immunodeficiency Virus

HSV - Herpes simplex Virus

HBV - Hepatitis B Virus

EBV - Epstein-Barr Virus

FMDV - Foot-and-Mouth Disease Virus

NDV - Newcastle Disease Virus

ADME - Absorption Distribution Metabolism Excretion

PDB - Protein Data Bank

SDF - Structure Data File

GI - Gastro Intestinal

BBB - Blood Brain Barrier

P-gp - P-glycoprotein

DPPH - 2,2-diphenyl-1-picrylhydrazyl

Declarations

Ethics approval and consent to participate

Not Applicable

Consent for publication

Not Applicable

Availability of data and material

All data and materials are available upon request.

Competing interests

The authors do not have conflicts of interest.

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Authors' contributions

AND carried out the analysis and drafted the manuscript. TJJ supervised the work and finalized the manuscript.

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Figures

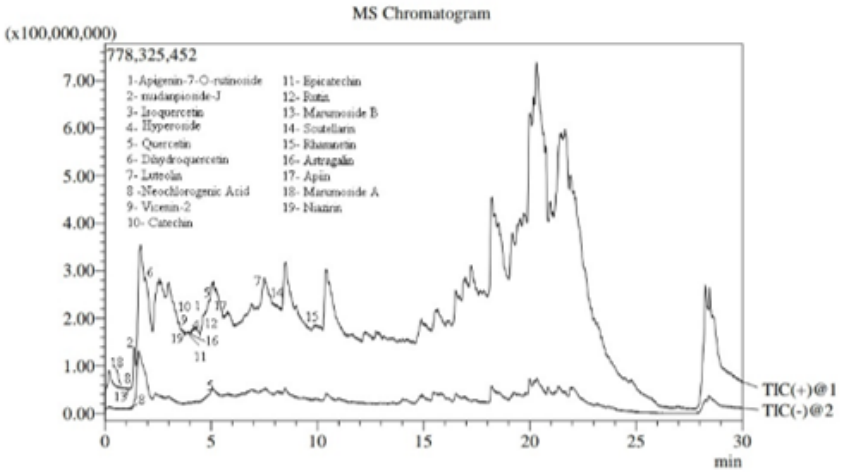

Figure 1

chromatogram

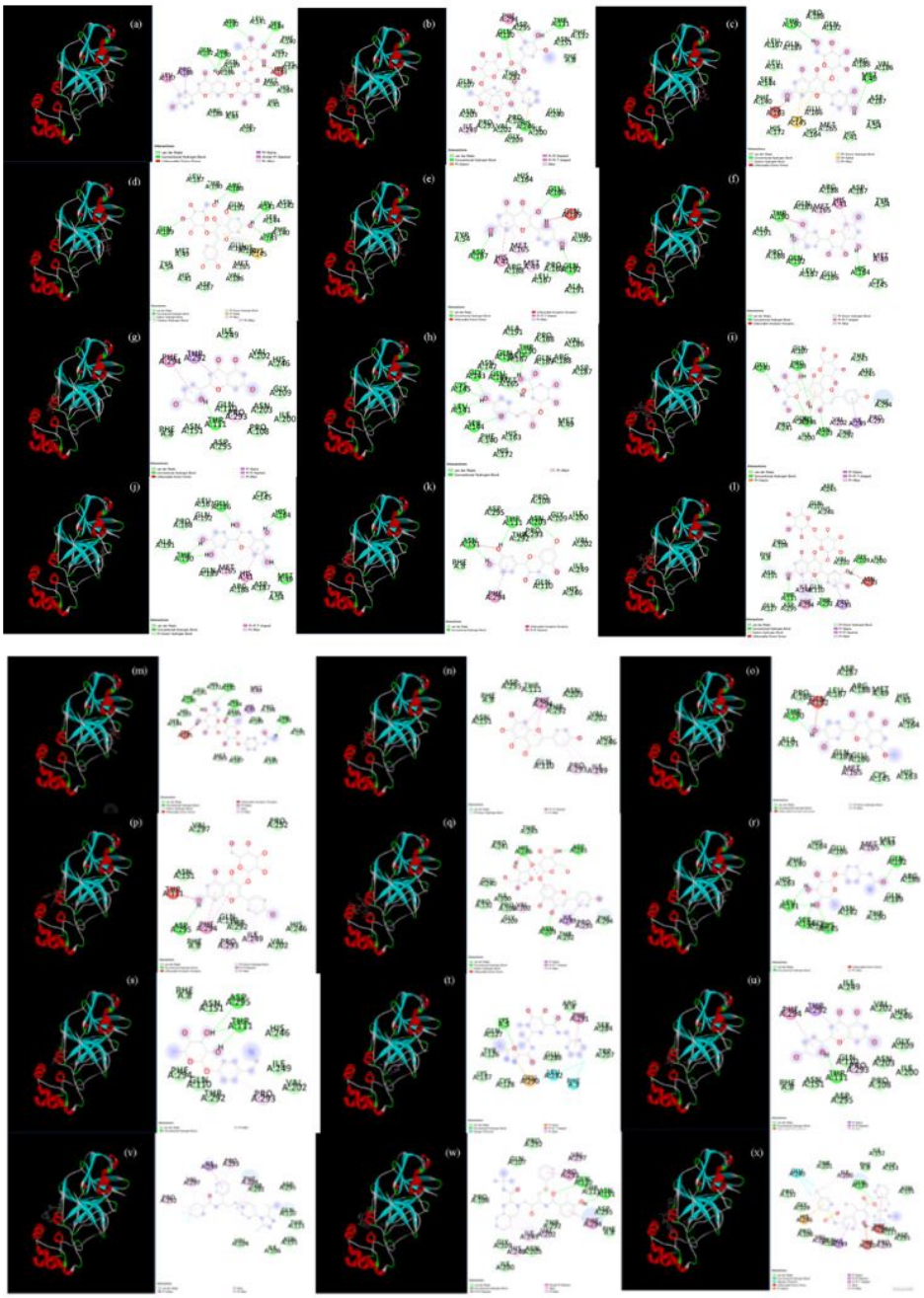

Figure 2

showing the 2-dimensional interactions of Apigenin-7-O-rutinoside (a), Mudanpioside J (b), Isoquercetin (c), Isoquercitrin (d), Quercetin (e), Dihydroquercetin (f), Luteolin (g), Neochlorogenic acid (h), Vicenin 2 (i), Catechin (j), Epicatechin (k), Rutin (l), Marumoside B(m), Scutellarin (n), Rhamnetin (o), Astragalin (p), Apiin (q), Marumoside A(r), Niazirin (s), Raltegravir (t), Lopinavir-Ritonavir (u), Maraviroc (v), Nelfinavir (w) and Tipranavir (x) with SARS-CoV-2 Mpro.

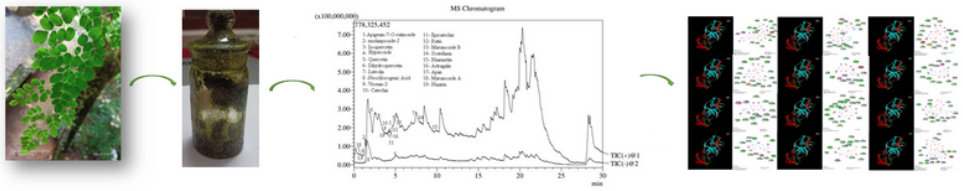

Figure 3

Graphical abstract