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# Luteolin: A Dietary Molecule as Potential Anti-COVID-19 Agent

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# Abstract

Luteolin (Lut) is an important plant-derived flavonoid that is widely distributed in edible herbs and vegetables. Studies on animal and human models have shown that Lut exhibits various pharmacological properties, viz. anti-inflammatory, anti-cancer, anti-oxidant, anti-apoptotic, and neurotrophic actions. The ongoing pandemic coronavirus disease-2019 (COVID-19), is a disease of the respiratory tract that consists of mild to severe symptoms of pneumonia including fever, muscle aches, sore throat, coughing, and shortness of breath. It is of particular concern in older people and patients with chronic diseases having cardiovascular and blood clotting issues or who have compromised immune. This situation prompted us to evaluate the bioactive compounds which are being used to prevent respiratory-related illness. Lut is one such compound which is used as an anti-inflammatory agent. Several studies have explained the protective nature of Lut by inhibiting virus entry and fusion with human receptors in old SARS-CoV that had emerged in 2003. Thus, regular consumption of foods having adequate amount of Lut in our diet may be helpful in inhibiting the SARS-CoV-2 infection as well and may prevent the consequent symptoms in COVID-19 patients. In present work, we have carried out the molecular docking studies of Lut with six different SARS-CoV-2 encoded key proteins. The FDA-approved drug remdesivir was also evaluated as control to compare the results. Lut showed excellent inhibitory action against papain-like proteinase, a main protease of SARS-CoV-2. Lut was also many times more active than remdesivir. Therefore, the foods which have Lut in adequate amount might be explored further for potential use against COVID-19

# 1. Introduction

Luteolin (Lut) is a naturally occurring plant-derived flavonoid that is widely distributed in edible herbs, vegetable and fruits such as bird chilli, oregano, juniper berries, celery seeds and parsley in significant amount [1]. Besides the high abundance of Lut in dietary substances (figure 1), its bioavailability is very low that is approximately 4.10% at a dose of 50 mg/kg because of significant first pass effect [2]. After dietary intake, Lut is converted into its glucuronide/sulphate conjugates as in rat model study or remains in free form as in human plasma. In the same study, the maximum plasma concentration ($C_{max}$) and half-life ($t_{1/2}$) of Lut were found as 1.97 ± 0.15g/ml and 4.94 ± 1.2 h after oral administration of 14.3 mg/kg dose in rat model [3]. Evidence-based studies on cell culture, animal and human model have shown that Lut is well known for exert its pharmacological properties, namely anti-inflammatory, anti-cancer, anti-oxidant, anti-apoptotic, and neurotrophic actions [4]. Lut also affects the osteoclast differentiation, myeloid differentiation and oligodendrocyte maturation though activating or inhibiting metabolic pathways [5, 6]. Moreover, some studies have reported the cardio protective properties of a high intake of dietary Lut during ischemia-reperfusion injury in various rat models [7, 8]. In one study, Si et al. had evaluated the vasorelaxation effect of Lut on rat aortic rings model by using Western blot and fluorometric assays. In this experiment, the effect of Lut was found directly on vascular endothelial cells (ECs) which led to nitric oxide (NO) production followed by vascular relaxation [9]

The recent outbreak, coronavirus disease−2019 (COVID−19) which is caused by severe acute respiratory syndrome coronavirus−2 (SARS-CoV−2), has become a pandemic disease of the respiratory tract having mild to severe symptoms of pneumonia [10]. Globally, more than 0.3 million peoples have been died so far because of the fast-spreading nature of SARS-CoV−2 from human to human. Most of the people have common signs and symptoms of respiratory illness including fever, muscle aches, sore throat, coughing, and difficulty breathing [11]. Most of the infected population has very few symptoms or no symptoms at all [12]. On the other hand, older people and chronic patients having cardiovascular and blood clotting issues or diabetes or who have compromised immune systems are at risk of developing severe symptoms of COVID−19 upon infection [13]. Due to the severity of symptoms andthe mortality rate of this pandemic, we have evaluated the bioactive compounds have been used to prevent respiratory illnesses. Based on the literature survey, we found that Lut may be a bioactive compound of interest. Literatures have claimed that Lut showed protective effects against acute lung injuries via inhibition of PI3K/Akt and MEK/ERK pathways in neutrophils [14]. In a study, Lut inhibited HIV−1 infection in reporter cells as well as primary lymphocytes and thus showed anti-HIV activity [15]. Furthermore, few studies have explained the protective nature of Lut by inhibiting the virus entry and fusion with human receptors in old SARS-CoV that had emerged in 2003 in China [16, 17]. Thus, consumption of foods having adequate amounts of Lut in our daily diet may play a crucial role inhibiting the SARS-CoV−2 infection and prevent the consequent symptoms in COVID−19 patients. Inspired from these previous works, in present work, we have carried out molecular docking studies of Lut with six differentSARS-CoV−2 encoded key proteins. The docking results of Lut were also compared with FDA-approved drug remdesivir as control.

## 2. Method And Materials

2.2 Selection and preparation of ligand and protein receptors

To study the interactions between protein-ligand complex, the 3D structures of Lut and FDA-approved drug remdisivir were retrieved from the PubChem database as PubChem IDs 5280445 and 492405 in '.sdf' format, followed by preparation of '.pdb' format using the Gaussian tool. The crystallographic 3D structures of SARS-CoV−2 encoded proteins were downloaded from RSCB protein data bank (http://www.rscb.org). The PDB IDs of proteins were 6W02 (ADP ribose phosphatase of NSP3), 6Y2E (Free enzyme of the SARS-CoV−2 main protease), 6M03 (COVID−19 main protease in apo form), 6VSB (Prefusion 2019-nCoV spike glycoprotein with a single receptor-binding domain up), 6VYO (RNA binding domain of nucleocapsid phosphoprotein from SARS coronavirus 2), and 6VXX (SARS-CoV−2 spike glycoprotein close state).

*2.2 Procedure of Molecular Docking*

Molecular docking was done by using a high configuration computer system of Gigabyte Technology co. Ltd. to predict active binding sites in protein-ligand complexes. To get best results in term of binding energy, protein-ligand preparation was performed using AutoDock4.2.6 tool. Water molecules were

removed from the receptor file and Hetatm, hydrogen atoms, Kollman charges were added to the receptors. After this, the structure of ligand was cleaned up and theMMFF94 force field was applied before saving it in PDB format. In the PDB format of ligand detected root, number of torsions and aromaticity criterion were repositioned through the AutoDock 4.2.6 tool and then saved as PDBQT format. A grid map of dimensions 50Å x 50Å x 50Å with spacing of 0.375Å was generated and saved in GPF format. At the end, Lamarckian genetics algorithm was used to set the parameters in the calculation of the Vander Waals forces and the electrostatic terms. Observation and visualization of the results were carried out using Discovery Studio 3.0 Visualizer. Finally, analysis of docking results was done by calculation of binding energy (BE) with the help of the AD4 analysis option.

**Due to technical limitations, Table 1 is provided in the Supplementary Files section.**

# 3. Results

### 3.1 Analysis and prediction of binding affinity of Lut against 6W02

Molecular docking study was performed of Lut against the crystal structure of ADP ribose phosphatase of NSP3 from SARS CoV−2 in the complex with ADP ribose (6WO2) using AutoDock4 software. This study revealed the molecular interaction of Lut with Ala38, Ala39, Gly48, Val49, Ala50, Pro125, Leu126, Ser128, Ala129, Gly130, Ile131, Phe132, Ala154, Val155, Phe156 amino acids of 6WO2 protein as shown in Table 1. The negative value of the binding free energy (−9.37 kcal/mole) as shown in Table 2 indicates strong interactions of Lut with the 6WO2 protein.

### 3.2 Analysis and prediction of binding affinity of Lut against 6Y2E

Molecular docking study was performed of Lut against the structure of free enzyme of the SARS-CoV−2 main protease (6Y2E) using AutoDock4 software. This study revealed the molecular interaction of Lut with Thr24, Thr25, Thr26, Phe140, Leu141, Asn142, Gly143, Ser144, Cys145, His163, Met165, and Glu166 amino acids of 6Y2E protein as shown in Table 1. The negative value of the binding free energy (−7.22kcal/mole) as shown in Table 2 indicates strong interactions of Lut with the 6Y2E protein

### 3.3 Analysis and prediction of binding affinity of Lut against 6MO3

Molecular docking study was performed of Lut against the crystal structure of COVID−19 main protease in apo form (6MO3) using AutoDock4 software. This study revealed the molecular interaction of Lut with Thr24, Thr25, Thr26, Phe140, Leu141, Asn142, Gly143, Ser144, Cys145, His163, Met165, and Glu166 amino acids of 6MO3 protein as shown in Table 1. The negative value of the binding free energy (−7.08 kcal/mole) as shown in the Table 2 indicates strong interactions of Lut with the 6MO3 protein

### 3.4 Analysis and prediction of binding affinity of Lut against 6VSB

Molecular docking study was performed of Lut against the crystal structure of prefusion 2019-nCoV spike glycoprotein with a single receptor-binding domain up (6VSB) using AutoDock4 software. This study

revealed the molecular interaction of Lut with Glu773, lle1013, Ala1016, Glu1017, Arg1019, and Ala1020 amino acids of 6VSB protein as shown in Table 1. The negative value of the binding free energy (−6.75 kcal/mole) as shown in Table 2indicatesinteractions of Lut with the 6VSB protein

### 3.5 Analysis and prediction of binding affinity of Lut against 6VYO

Molecular docking study was performed of Lut against the crystal structure of RNA binding domain of nucleocapsid phosphoprotein from SARS coronavirus 2 (6VYO) using AutoDock4 software. This study revealed the molecular interaction of Lut with Trp52, Thr57, His59, Asn77, Arg92, lle94, Leu104, Ser105, His145, lle14, Gly147, Thr148, and Asn150 amino acids of 6VYO protein as shown in Table 1. The negative value of the binding free energy (−6.74 kcal/mole) as shown in Table 2 indicates interactions of Lut with the 6VYO protein

### 3.6 Analysis and prediction of binding affinity of Lut against 6VXX

Molecular docking study was performed of Lut against the crystal structure of RNA binding domain of nucleocapsid phosphoprotein from SARS coronavirus 2 (6VXX) using AutoDock4 software. This study revealed the molecular interaction of Lut with Thr998, Gln1002, Gln1005, Thr1006, and Thr1009 amino acids of 6VXX protein as shown in Table 1. The negative value of the binding free energy (−6.54 kcal/mole) as shown in the table 2 indicates the interactions of Lut with the 6VXX protein

### 3.7 Analysis and prediction of binding affinity of Remdesivir against SARS-CoV−2 encoded proteins

Molecular docking study was also performed of the standard drug remdesivir against all the proteins discussed above using Auto dock4 software. The negative values of the binding free energy such as −6.06, −5.44, −4.96, and −1.46 kcal/mole, as shown in the table 2, indicated significant molecular interactions against 6VYO, 6M03, 6W02, and 6Y2E proteins respectively. On the other hand, positive value of binding energy like +0.27, +0.62 kcal/mole as shown in the table 2, showed no interactions with 6VSB, 6VXX proteins respectively.

## 4. Discussion

The spike protein is responsible for the entry of virus into the host cell via interactions of S1 domain with host receptor (ACE2) followed by S2 segment mediated fusion of the host and viral membranes. This fusion allows the viral RNA to enter inside the host cells and thus spike proteins may act as therapeutic targets in drug designing studies. Main protease (M$^{pro}$, also called 3CL$^{pro}$) is the attractive drug target site in the treatment of COVID−19. These proteins play a central role in gene expression and replication of virus by using proteolytic process of replicase polyproteins. In this process, C-terminal end is cleaved by chymotrypsin-like cysteine protease (3C-like protease) whereas N-terminal end is processed by the papain-like protease (M$^{pro}$or PL$^{pro}$). Thus, both 3CL$^{pro}$ and PL$^{pro}$ provide new insights for the designing of protease inhibitors against SARS-CoV−2. Nucleocapsid protein of SARS-CoV−2 is a type of phosphoprotein that surrounds the viral genome in a helical ribonucleocapsid cavity. It plays an

important role in viral self-assembly. Thus, spike protein, main proteases and nucleocapsid proteins may be prime target sites for discovery of antiviral agents against COVID−19.

In the present study, molecular docking study reveals that Lut interacted in the active sites of target protein through both hydrogen as well as hydrophobic bonds. Lut displayed the highest binding affinity (−9.37 kcal/mole) towards papain-like protease (6wo2), a main protease through formation of strong hydrogen bonds with Val49, Gly130, Ile131, Pro125, and Ala154 amino acids residues in the active sites required for enzyme inhibition. Moreover, Lut showed significant binding energies (−7.22 kcal/mole for 6y2e, and −7.08 kcal/mole for 6mo3) through formation of hydrogen bonds with Met165, Ser144 and Gly143 amino acids residues of 6vyo enzyme as well as Thr25, Gln143, Cys145 and Met165 amino acids residues for 6mo3 enzyme and thus found to be significantly active against 3C-like proteinase enzymes of main protease. In addition, Lut exhibited weak binding affinities against spike proteins (−6.75 kcal/mol for 6vsb and −6.54 kcal/mol for 6vxx) and nucleocapsid protein (−6.75 kcal/mole for 6vyo) enzymes via formation of some hydrogen bonds with interacting amino acid residues. On the other hand, remdisivir, which was chosen as a control to compare our results was found to be moderately active in the active sites of nucleocapsid protein (6vyo) through requisite hydrogen bonds. It did not show promising results with main protease and spike proteins in our docking methods. The docking scores (values of binding energies) of the Lut and ragainst spike, main protease and nucleocapsid proteins using AutoDock4 are listed on Table 2.

In this study, Lut shows most stable docked structure in binding pocket of papain-like proteinase and found to be more active as compared to FDA-approved remdesivir drug. Although these results need to be further studied through *in-vitro* and *in-vivo* studies, this molecular analysis suggests that Lut could be used as an inhibitor of the main protease for the treatment of COVID−19.

**Table 2:** Binding energy (Kcal/Mole) and inhibition constant (µM) of Luteolin and control Remdisivir

| Experiments | | Luteolin | Remdesivir |
|---|---|---|---|
| | | BE (ΔG) in Kcal/mole | BE (ΔG) in Kcal/mole |
| SARS-CoV-2 encoded Proteins Targets | 6wo2 (Papain-like proteinase) | -9.37 | -4.96 |
| | 6y2e (3C-like proteinase) | -7.22 | -1.46 |
| | 6m03 (3C-like proteinase) | -7.08 | -5.44 |
| | 6vsb (Spike glycoprotein) | -6.75 | +0.27 |
| | 6vyo (Nucleocapsid protein) | -6.74 | -6.06 |
| | 6vxx (Spike glycoprotein) | -6.54 | +0.62 |
| | | | |
| Lipinski's Rule of Five | Molecular Weight | 286.24 | 602.58 |
| | MlogP (lipophilicity) | -0.03 | 0.18 |
| | H-bond donors | 4 | 4 |
| | H-bond acceptor | 6 | 6 |
| | No. of violation | Zero | 2 |
| | Lipinski's follow | Yes | No |

Note = BE: Binding Energy

# Conclusions

In concluding remarks, we have carried out molecular docking studies of Lut with randomly chosen six important protein receptors (spike, main protease and nucleocapsid protein) which are encoded by SARS-CoV−2. The docking results (binding energy) were compared with remdesivir, an FDA-approved drug against COVID−19. Our results revealed that Lut exhibited higher dock score than remdesivir against the proteins of SARS-CoV−2. In addition, Lut is also known to have pharmacological properties which of particular relevance in respiratory illnesses. Therefore, based on the promising docking results and the

medicinal importance of Lut, we propose that the Lut should be further studied to determine its viability as pharmacological agent against COVID−19.

## Declarations

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# Figures
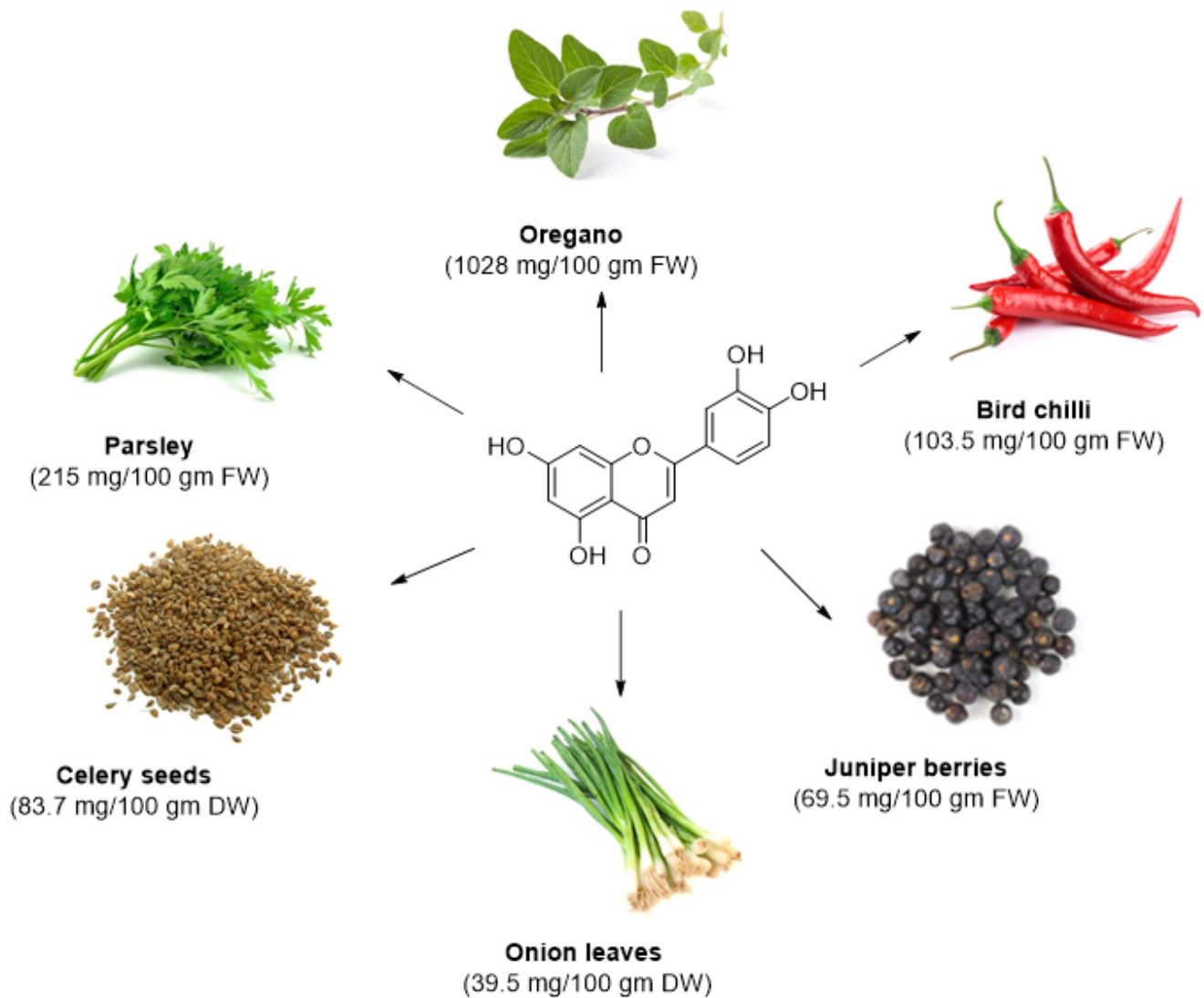
**Figure 1**

Chemical Structure of Luteolin and Dietary Sources with Amount (FW: Fresh Weight; DW: Dry Weight)

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.docx