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(REVIEW ARTICLE)

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Nutraceuticals in viral infections: A review

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Abstract

Nutraceutical is anything i.e. food or a component of food having health or medical advantages, including the treatment and prevention of disease. These products have been used to improve health, delay the onset of chronic conditions, postpone ageing, prolong mortality rates, defend against disease or improve the structure or functioning of the body. Nutraceuticals have been found beneficial in various disease conditions such as diabetes, cardiovascular diseases, gastrointestinal diseases, neurological disorders and viral infections. The present review was carried out to congregate the benefits of polyphenols such as rutin, hesperidin lactoferrin against SARs-Co-V-2, Enterovirus, Influenza and Human papillomavirus (HPV). The data manifest that polyphenols through various mechanisms such as direct binding to virus, binding with heparan sulfate proteoglycans, intracellular inhibition of viral replication or by enhancing effects of T and

NK 20 lymphocytes inhibits virus growth**.** The review was carried out using scientific search engines such as Google scholar, PubMed, Science direct, Medline and Science. Gov.

Keywords: Rutin; Hespiridin; SARS-CoV-2; Enterovirus; Lactoferrin; Hepatitis c virus

1. Introduction

Nutraceutical is anything i.e. food or a component of food having health or medical advantages, including the treatment and prevention of disease (Kalra, 2003). These are the products having both nutritious and therapeutic values. A nutraceutical food is a chemical that has physiological advantages or provide defense against chronic condition. It can be utilized to improve health, delay the onset of chronic conditions, postpone ageing, prolong mortality rates, defend against disease or improve the structure or functioning of the body. These drugs have received a lot of interest recently because of their ability to have beneficial effects on nutrition, safety, and treatment (Nasri et al., 2014). The drugs are classified as a dietary and herbal bioactive substances based on their origins. Herbal nutraceuticals help to improve the standard of life, increase longevity, and promote and maintain excellent health. Several conditions, such as malignancy, neurological conditions, and heart problems, viral infections, etc., have been successfully treated using nutraceuticals, according to studies (Sachdeva et al., 2020). The market for nutraceuticals worldwide, worth about USD 117 billion. A potential nutraceutical is one that offers the possibility of a certain health or medical benefit; it only becomes an established nutraceutical when there is enough clinical evidence to support it. It is disappointing to see that the vast majority of dietary supplements are in the "potential" stage of development. The nutraceuticals are categorized on basis of their chemical makeup, food sources and mechanisms of action. It involves natural food and fall into the categories such as fiber in food, probiotics, prebiotics, fatty acids, vitamins with antioxidants, polyphenols, omega 3 fatty acids, antioxidant and spices.

Plants have a wide range of bioactive substances that can be isolated and used medicinally. Bioactive substances can be used as an appropriate substitute for manufactured medications. According to the scientific community flavonoids is a unique class of therapeutic molecules due to their diverse therapeutic properties. Flavonoids are phytochemicals with

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polyphenolic secondary metabolites that work well as nutraceutical agents (Kaleem & Ahmad, 2018). Another major families of secondary metabolites are composed of phenolic chemicals. They exhibit a wide range of structural variations and are in charge of the main organoleptic qualities, such as color and taste, of meals and beverages made from plants. It has promising medicinal properties which is revealed by a deeper understanding of their structures and biological roles. Due to their extensive variety of pharmacological effects in the human body, flavonoids are more aptly referred to as nutraceuticals (Andrew & Izzo, 2017). Some of the polyphenolic compounds are mentioned below (Fig.1).

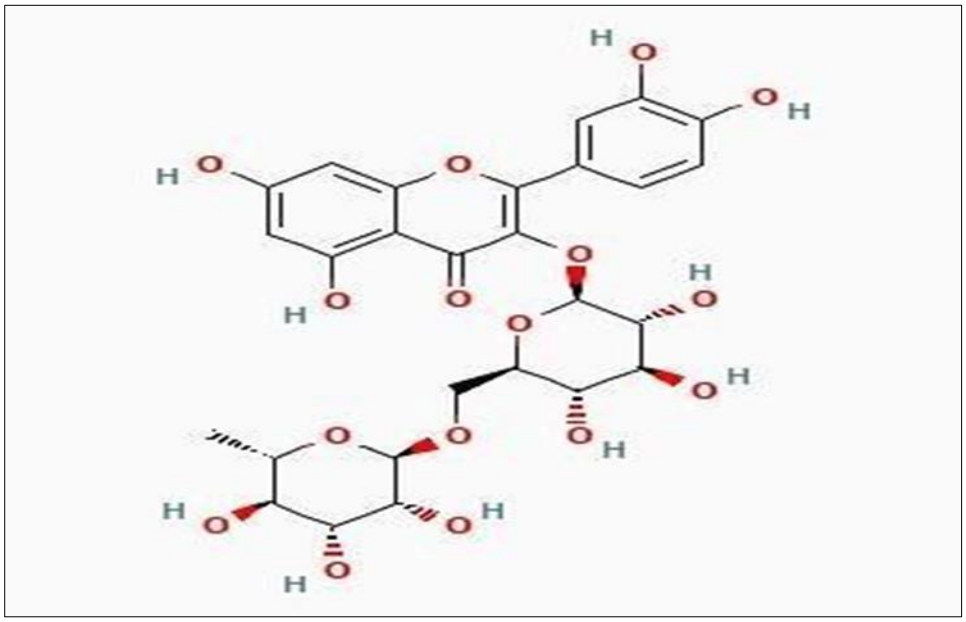

Figure 1 Structure of rutin; IUPAC3′,4′,5,7-Tetrahydroxy-3-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyloxy]flavone

2. Rutin

Rutin, also referred as vit- P, rutoside, quercetine 3 rutinoside or sophorin, is a citrus flavonoid found in buckwheat (Ganeshpurkar & Saluja, 2017). It has been studied for its potential to have a variety of pharmacological effects, including bactericidal, anticarcinogenic, antiparasitic, anti-inflammatory, antiviral, antiallergenic, cytoprotective, vasoactive, hypolipidemic, antispasmodic, antithrombotic and hypotensive. It is a low- molecular-weight polyphenolic molecule known as citrus flavonoid glycoside having a variety of physiological roles in human, animal and plant species. It is one of the better known natural antioxidants. It is metabolized to different compounds by the gut bacteria after consumption, therefore a small amount works efficiently. Higher plants show presence of rutin generally in fruits and fruit rinds; the examples are fruits and berries like Morusalba, Rutagraveolens, tea leaves, apples, and many more. Today, rutin has been observed for its wide range of nutraceutical effect in preventing various disease such as tumor, bacterial and viral infections, autism, airway infections, aging skin, and found effective as antiallergic, anti-inflammatory, vasoactive and antiprotozoal agent (Batiha et al., 2020).

2.1. Effect of Rutin in SARS-CoV-2

Numerous analytical investigations including in silico have discovered a number of currently available substances that may function as potent inhibitors of the SARS-CoV-2 a major protease, stopping the virus's ability to replicate(F. Rahman et al., 2021). Rutin has been selected as one of these because of its strong affinity for the virus. Also, its inclusion in a number of conventional antiviral medications that were given to infected patients in China who had mild to moderate COVID-19 symptoms support its potential as a repurposed bioactive secondary metabolite towards SARSCoV-2 (Agrawal et al., 2021). Rutin significantly bound to the Mpro, RdRp, PLpro, and S-proteins of SARSCoV-2, according to a docking studies analysis. Mpro, a member of these four proteins, had the best binding ability and the lowest binding energy (8.9 kcal/mol), and it was maintained by hydrogen bonds having lengths ranging between 1.18 to 3.17 and hydrophobic interactions. Rutin's prospective therapeutic candidate was indicated by values of the projected inhibitory constant with SARS-CoV-2 essential proteins that ranged from 5.66 mM to 6.54 mM (Aurich & Thiele, 2016). After thorough *in-vitro* and in vivo research, this study concluded that rutin, either alone or in combination, may be utilised as a dietary supplement to combat COVID-19 (Fig. 2).

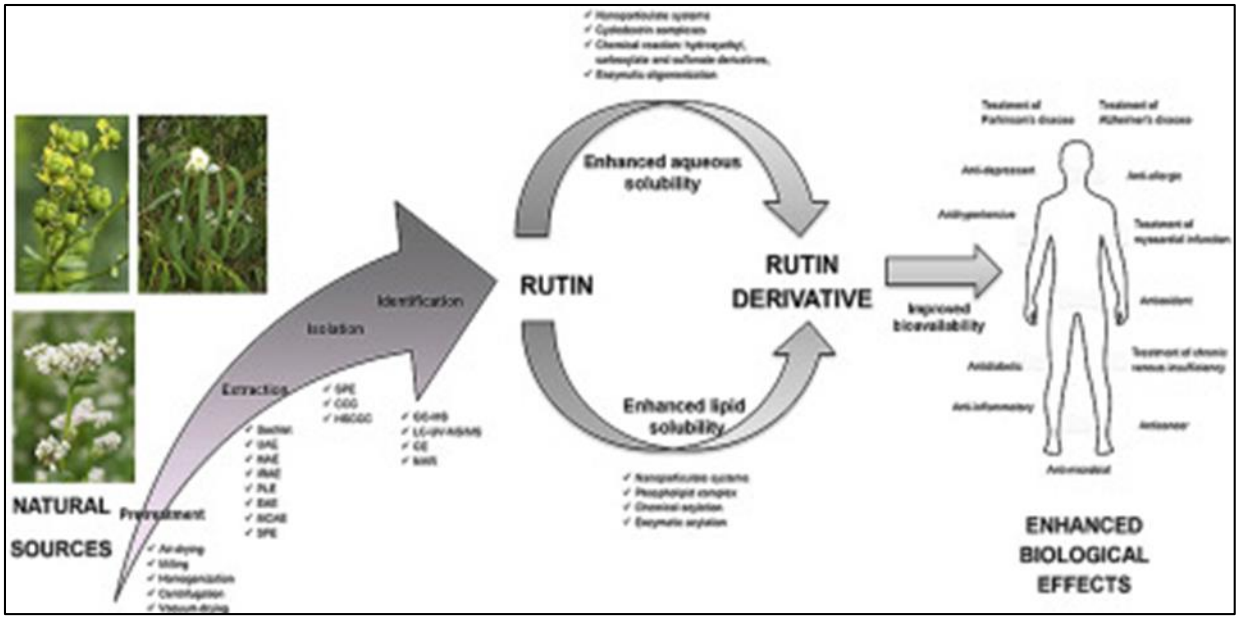

Figure 2 Various activities of rutin

2.2. Effect of Rutin on Enterovirus

Encephalitis, pulmonary edema, and mortality are all serious complications brought on by enterovirus A71 (EV-A71). In this work, the antiviral activity of flavonoids against EV-A71 was examined. Rutin were found to inhibit the enzymatic activity of recombinant EV-A71 3C protease (3Cpro) in a dose-dependent manner in an in-vitro inhibitor screening experiment. Rutin prevented the reproduction of EV-A71 in cells, according to results of a cell-based FRET test using an EV-A71 3Cpro cleavage motif probe(J.-Y. Lin et al., 2019). Rutin significantly decreased the EV-A71-induced cytopathic effect and viral plaque titers in RD cells culture, according to a virus replication assay. For rutin, the IC (50) values for plaque reduction against EV-A71 were 110 M, respectively. Rutin had therapeutic indices (CC50/IC50 of plaque reduction assays) above 10. According to the study, rutin prevent EV-A71 from replicating &exhibit a broad spectrum of anti-enterovirus activity. Vegetables, especially citrus herbs, contain flavonoids. Studies demonstrate their biological characteristics, which include anticancer, cardiovascular inhibitors, anti-inflammatory and antimicrobial effects. Their clinical usefulness is reduced by a lack of scientific evidence demonstrating the molecular mechanisms underlying their action.

Another study revealed the inhibition of 3Cpro activity and EV-A71 replication in RD cells by flavonoids. For in-vitro 3Cpro activity testing, EV-A71 3Cpro produced by E. coli was purified. A fluorescence resonance energy transfer (FRET) probe with a 3Cpro cleavage pattern was utilized to investigate the substrate selectivity of EV- A71 3Cpro in cells. Rutin was, significantly inhibited 3Cpro activity and EV-A71 replication (Liu et al., 2020).In 96-well plates, the study investigated the in-vitro anti-EV- A71 3Cpro activity of flavonoids such kaempferol, myricetin, rutin, chrysin, fisetin (Y.-J. Lin et al., 2012).

According to data, rutin reduce EV-A71 activity by more than 30% at a 200-M concentration. Rutin were chosen at concentrations of 0, 10, 100, 250, 750, and 1000 - M for a more thorough analysis of the 3Cpro inhibition investigation. Rutin demonstrated dose-dependent 3Cpro inhibiting activity. Based on HRP-based enzymatic tests, the results showed that rutin had dose-dependent inhibitory effects on the in-vitro cleavage activity of recombinant 3Cpro (Jash & Mondal, 2014) (Fig.3).

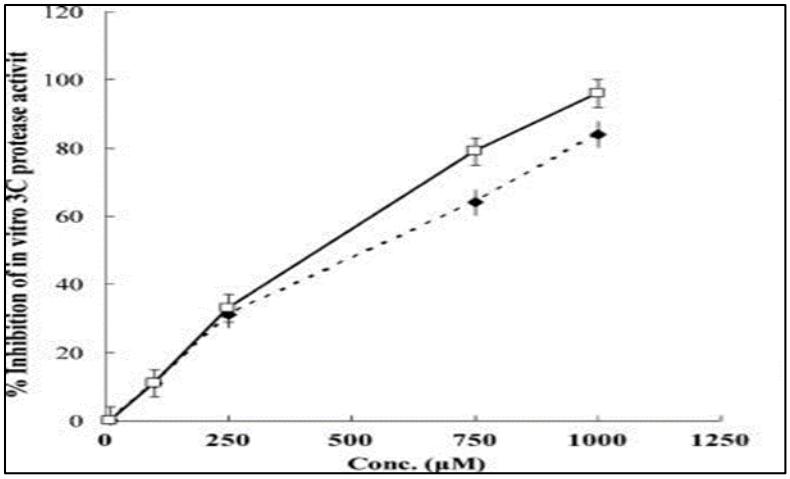

Figure 3 EV-A71 3Cpro activity is inhibited by rutin in a dose-dependent manner *in-vitro*.

3. Hesperidine

Citrus fruits include the flavanone glycoside hesperidin, anaglycone form, in high concentrations. The health advantages, including antioxidant, antimicrobial, antibacterial, anti-inflammatory and anticarcinogenic capabilities are associated with it (Gandhi et al., 2020). Such fruits are extensively utilized in the commercial sector, particularly for making juices. Huge quantities of byproducts, comprising peels, seeds, cell, and membrane remnant are also a good source of hesperidin. French chemist Lebreton originally discovered hesperidin in the white inner layer of citrus peels in 1828 (Julius et al., 2017). Hesperidin is hypothesised to play a role in plant defence mechanism. The sources are fruit of Citrus aurantium (bitter orange, petitgrain), orange juice (Citrus sinensis), Zanthoxylumgilletii in lemon of Rutaceae family, Agathosmaserratifolia (Lamiaceae) leaves in lime and also peppermint (Fig.4).

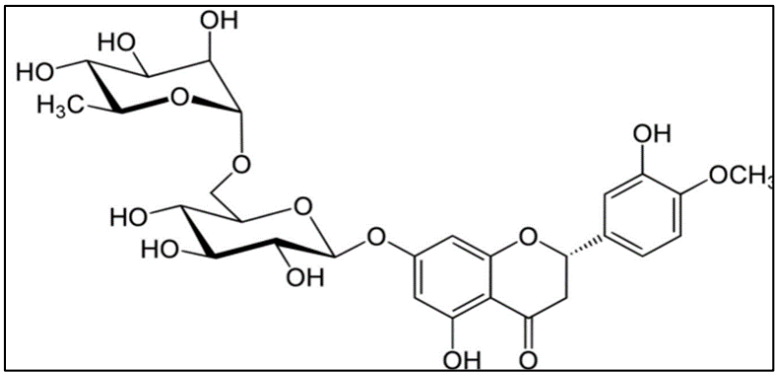

Figure 4 Structure of Hesperidine; IUPAC- (2S)-5-hydroxy-4′-methoxy-7-[α-l-rhamnopyranosyl-(1→6)-β-d-glucopyranosyloxy] flavan-4-one

A prominent flavonoid called hesperidin is present in citrus fruits like lemons and delicious oranges, as well as some other fruits, vegetables, and polyherbal preparations. Hesperidin has a efficiently bioavailability metabolite called hesperetin (Zanwar et al., 2014). Hesperidin possesses a number of pharmacological properties, including anti - hyperlipidemic, cardioprotective, antihypertensive, and antidiabetic effects. These properties are primarily related to an antioxidant defence mechanism and the inhibition of proinflammatory cytokine production (Kong et al., 2021). Hesperidin has demonstrated its positive effects in numerous randomised crossover experiments, which are mostly due to its anti-inflammatory and anti-atherogenic properties. Hesperidin's LD50 for acute oral toxicity studies was found to be greater than 2000 mg/kg, and its LD50 for subacute and sub - chronic toxicity studies was shown to be greater than 2000 mg/kg, demonstrating the sustainability of hesperidin in herbal preparations.

3.1. Effect of Hesperidin in Hepatitis-C

Globally, the viral hepatitis C infection is the major cause of liver diseases. There are numerous HCV genotypes that have been discovered all over the globe. Effective HCV medications are available, but they are expensive, especially for

infected patients in developing nations (Nouroz et al., 2015). Therefore, it is necessary to create affordable medications that target key HCV drug targets. This work was created to find antiviral antagonists using citrus fruit extracts against an important therapeutic target, NS3 protease, and HCV genotype 3a, which are primarily found in South Asian nations. Citrus fruits generally contain bioactive chemicals with antiviral properties (Mathew et al., 2015). Due to the 10 kb-long RNA genome that HCV possesses and the lack of a sequencing activity in the RNA-dependent RNA polymerases, different HCV genotypes and variants have emerged throughout the world (Venkataraman et al., 2018). The results of the current work demonstrate that the HCV genotype 3a NS3 protease was positive and functional in the NS4A fusing form, which can be exploited to create a FRET assay for the identification of anti-HCV inhibitors. Phytoconstituents out from Citrus family were tested for their ability to suppress NS3-NS4A activity using only a validated FRET assay. Hesperidin being abundant in grapefruit mesocarp, orange, bitter orange, and mandarin whole fruit extracts, could effectively inhibit the HCV NS3-NS4A protease, according to the results of the FRET assay, ESI- MS/MS, and molecular docking analyses. This suggests that citrus fruit extracts are really rich source of inexpensive organic ingredients with antiviral bioactive constituents (Khan et al., 2020). Hesperidin's capacity to suppress the activity of NS3-NS4A was confirmed by utilizing the FRET (Fluorescence Resonance Energy Transfer) assay during the evaluation of pure flavonoids. Hesperidin was confirmed to be a powerful inhibitor of the enzyme by the observation that it significantly decreased overall activity of NS3-NS4A.Ellagic acid, discovered in pomegranate pericarp extract was also effective against HCV NS3-NS4A protease(Khan et al., 2022) (Fig.5).

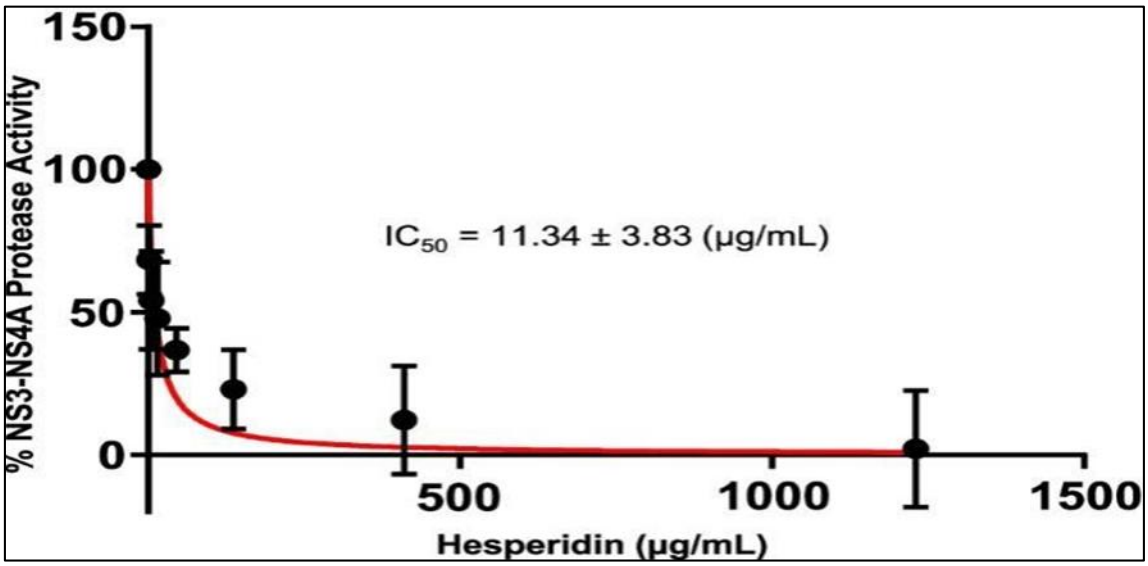

Figure 5 Hesperidin's ability to inhibit NS3-NS4A activity. Using the FRET test, hesperidin effectively reduced the activity of NS3-NS4A. [IC50 value of 11.34±3.83 µg/mL]

3.2. Effects of Hesperidin on Influenza virus

The influenza virus causes moderate to severe illnesses in seasonal epidemics and sporadic global pandemics in humans and animals. Every year, the influenza virus contaminated about 20% of the worldwide people, causing significant mortality and morbidity (Dong et al., 2014). Antiviral medication therapy is necessary to slow the progression of disease and stop the spread of viruses, but its availability and efficacy are constrained. One of the several cause was that the most recent antiviral medications target specific viral proteins (Halfon & Locarnini, 2011). Citrus fruit flavonoid hesperidin is recognized to have a variety of biological effects and antimicrobial effects against human viruses. However, hesperidin has a relatively poor solubility in water, and its target molecular structure for influenza virus is yet unclear (Meneguzzo et al., 2020b).

It has been observed that glucosyl hesperidin (GH), generated via regioselective transglycosylation using cyclodextringlucano-transferase, has biological activity that are comparable to or superior to those of hesperidin. Madin-Darby canine kidney (MDCK) cells were given GH following IAV inoculation to examine the inhibitory effect of GH on IAV infection. IAV replication was unaffected by GH treatment before to IAV inoculation, whereas IAV replication was significantly reduced by GH treatment concurrent with or following IAV inoculation. Analysis of GH's inhibition of two spikes in the surface of glycoproteins (Zu et al., 2012). IAV showed that, in contrast to receptor binding inhibition, GH suppresses viral sialidase activities, which is important in the entry and releasing stages of IAV infection. Between 0 and 25 mM of GH, MDCK cells showed no cytotoxic effects. The findings offered important knowledge for the creation

of new sialidase inhibitors for the treatment of influenza (Bouvier & Palese, 2008).Eleven different proteins (HA, NA, NP, NS1, NS2, PA, M1, M2, PB1-F2, PB1, and PB2) were encoded by the eight segmented RNA found in the influenza virus genome, although only NA and M2 have been shown to be effective targets for anti-influenza viral therapy. Recent research suggests two unique techniques to target viral genome for the development of antiviral drugs: altering cellular components necessary for the viral life cycle or inducing host antiviral responses.

A549 cell and MDCK cell, which were generated from the kidney epithelia of a dog and the lung epithelia of a cancer patient, respectively, are two host cells that influenza A/WSN/33 infects readily. Hesperidin is one of the flavonoid chemicals that has been discovered to have anti-influenza virus properties. In A549 cells and MDCK cells, hesperidin may have reduced the generation of influenza viruses. Shah Mahmud looked at the processes that underlie hesperidin's anti-influenza virus activity. By altering MAP kinase signaling pathways by upregulating p38 and JNK activation while downregulating ERK activation, our data showed that hesperidin improved cell-autonomous immunity (Shah Mahmud et al., 2017). With regard to both negative- & positive-sense a one-strand human respiratory viruses with RNA, bacillus ribonuclease functions as an antiviral medication.

3.3. Effect of Hesperidin in SARS-CoV-2

As per recent experimental and computational studies, hesperidin, a bioactive flavonoid present in large quantities within orange peel, stood for its strong affinity towards cellular receptors of SARS-CoV-2, surpassing drugs currently recommended for clinical trials (Jahangirifard et al., 2021) .So it was found highly promising for the prevention and treatment of COVID-19, together with other coexisting flavonoids like naringin, which may aid in limiting the immune system's pro-inflammatory reactivity. Hesperidin, a naturally occurring flavonoid compounds plenty in peels of citrus fruits, an intermediate product of the juice industry, and the primary flavonoid in sweet oranges and lemon, has been shown to possess a variety of advantageous biological properties, some of which it contributes with different citrus flavanols (Meneguzzo et al., 2020). Due to exceptional anti-inflammatory properties and exceptionally high binding affinity to the SARS-CoV-2 receptors, hesperidin and its aglycone hesperetin are desirable constituents for both preventive and therapeutic medications. Recent studies found that the citrus flavonoid hesperidin, which is abundant in citrus peel, exhibits considerable binding affinity to the SARS-CoV-2 protease domain, the receptor binding domain of the spike glycoprotein (RBD-S), and the receptor binding domain of the SARS-CoV-2 receptor (Sharma et al., 2017).

RBD-ACE2, the binding domain of the ACE2 at the protease domain, is in charge of viral replication and cell infection. Hesperidin's inhibitory actions against viral infection were thought to be best exemplified by the aforementioned extraordinary binding affinity to the three primary targets, which either prevented the virus from latching onto the ACE2 or prevented the virus from replicating in the cells. Hesperidin may therefore be a promising potent ingredient for medications that could be used to prevent or treat COVID-19, possibly in combination with other citrus flavonoids.

Researchers from Indonesia discovered through a molecular docking research that hesperidin had the least affinity to bind all three receptors (lowest docking score), blocking the viral infection and virus growth proteins. An antiviral medication called lopinavir was outperformed by hesperidin involved in the COVID-19 and nafamostat, a reference drug for RBD-S binding as per clinical studies. Hesperidin also outperformed a number of other organic substances. All of these citrus flavonoids may help to prevent viral infection and replication due to their affinity to the chosen receptors (Haggag et al., 2020). Another study examined all the proteins produced by the SARS-CoV-2 genes, compared them to those produced by other coronaviruses including SARS-CoV and MERS-CoV, and used those comparisons to simulate the protein structures.

The objectives of ACE2 from humans & type-2 transmembrane serine enzymes called protease were employed to search three different databases of licenced medications. These databases comprised the ZINC drug database developed by the FDA of the United States employing a virtual ligand screening technique, the database of frequently prescribed antiviral drugs (78 substances), along with the file of herbal products as well as conventional Chinese medicines (including reported frequently antiviral aspects from traditional Chinese medicine).The procedure revealed that the only substance capable of accurately targeting the Spike proteins and human ACE2 binding interface were hesperidin, and by overlaying the RBD-ACE2 complex on the hesperidin-RBD complex, a definite overlapping of all these interaction interfaces were seen. Hesperidin interacted with the ACE2 interface which implies that hesperidin may interfere with the way ACE2 and RBD interact and stop the virus from entering the cell. By employing a molecular docking method, hesperidin was found to have the maximum binding energy at the SARS-CoV-2 active site and was the best prospective inhibitor of Mpro/3CLpro. Rutin and diosmin were another potential inhibitor from citrus (Tallei et al., 2020).Furthermore, nelfinavir, a common HIV medication and one of the first choices for the treatment of COVID-19, was outperformed by both hesperidin and diosmin in their ability to bind to Mpro/3CLpro. The three docking targets were human ACE2, RNA-dependent RNA polymerase (RdRp), which synthesizes viral RNA from RNA templates and

participate in viral genome replication, transcription, and Mpro/3CLpro, which is involved in virus replication (Flores-Félix et al., 2021). Just eleven of the compounds brought into account were anticipated to be possibly effective towards COVID-19 according to the finding in a previous investigation that successful chemicals should target numerous critical proteins. Several flavonoids among these molecules demonstrated better binding interactions towards the targets than currently available synthetic anti-viral medications. Hesperidin showed the greatest average affinity number across each of the sites and by far the best binding number with human ACE2 among the predicted compounds (Wu et al., 2020). Consequently, it might be one of the compounds with the greatest potential for use at any stage of the illness as well as for prophylaxis (N. Rahman et al., 2020).

4. Lactoferrin

A substance called lactoferrin an 80-kDa iron-binding glycoprotein belonging to the transferrin family, is a component of exocrine secretions such as milk & saliva in addition to being present in neutrophil granules (Levay & Viljoen, 1995). Said to be involved in host defence, lactoferrin displays a wide variety of properties. Biological processes, such as those involved in antiviral, antimicrobial, antioxidant, immunomodulatory, cell-growth-regulating, and (Baveye and others, 1999) binding of several bioactive substances. In investigations carried out by Broxmeyer's group in the 1980s, lactoferrin's antiviral impact was first documented. They demonstrated how lactoferrin influences the myelopoiesis in mice exposed to a viral complex that has a friend's virus. Then, it was discovered that mice infected with a friend virus complex survived longer when lactoferrin was intraperitoneally administered (Lu et al., 1985). Target viruses that lactoferrin was demonstrated to have antiviral action against in the 1990s included hepatitis C virus (HCV), rotavirus, poliovirus (PV), cytomegalovirus (CMV), herpes simplex virus (HSV), human immunodeficiency virus (HIV), and respiratory syncytial virus (RSV). The researchers claim that lactoferrin has an early stages antiviral effect that prevents infection by viruses by either directly attaching to viral fragments or by inhibiting receptors within cells. Several studies have been conducted to examine the effectiveness of lactoferrin taken orally in preventing viral infections in both people and animals. Several studies claimed that consuming lactoferrin might have some protection against widespread viral infections (Fig.6).

Lactoferrin has been demonstrated to serve as an antiviral in both in vitro and in vivo experiments. Such antiviral capacity was initially discovered in mice infected with the friend's virus complex (FVC-P) variant that causes polycythaemia. (Lu et al., 1987). Lf is capable of inhibiting viruses, in part by iron chelation; nevertheless, subsequent administration of Lf to mice increases their survival rates and lowers the viral load of FVC-P. According to research, lactoferrin's antiviral mechanism is substantially more intricate (Redwan&Tabll, 2007). Lf interacts with a variety of surface molecules and ions, such as heparansulphate proteoglycans, cell receptors, and enveloped viral particles, disrupting viral maturation and allowing iron to bind at low pH levels due to its cationic properties. Inhibiting the activation of immunomodulators (Embleton et al., 2013). To investigate the mode of action of LF against various viruses, a collection of experimental bioassays has been prepared. Since LF's antiviral action may be initiated by interfering with a cellular target, tested by pre-incubating cells with the protein prior to their infection with viral particles, such as those from the hepatitis B virus (HBV), the HS-adapted Sindbis virus, the Semliki Forest virus, CMV, HSV-1, and HSV-2 (Hasegawa et al., 1994). Instead, LF can also disrupt with viral particles directly, such as HCV, adenovirus, feline herpes virus (FHV-1),and HIV, in order to exhibit its antiviral function. In several documented situations, LF appears to exert its antiviral effect in the first stages of process of infection. By pre-incubating HCV within human leukocytes, HepG2 cells, or Huh7.5 cells utilizing cLF before HCV infection, it had no influence upon the viral entrance, comparable findings with cLF have just been documented. Nevertheless, cLF completely inhibited viral entry. Camel LF may prevent the HCV virus's intracellular replication. (Berlutti et al., 2011, Andersen et al., 2004). Moreover, it has been noted that LF binds to heparansulphate, and several studies have shown the significance of heparansulphate at the cell surface for LF's antiviral effect. Surprisingly, a new study conducted by Calvarho et al. revealed the fact that the sulfate portion found in this glycosaminoglycan is accountable for the antiviral activity of LF since the efficiency of bLF adhering to cells was substantially dependent upon the level of sulfation of Glycosaminoglycan's (Hara et al., 2002).The capacity of this proteins and the hLF-derived Artificial Peptides (HLP) related to the n-terminal area of this protein (1-47 amino acids sequence), to avoid the spread of hepatitis B virus (HBV) along with reproduction, was evaluated in an attempt to comprehend the molecular processes that govern the antiviral hLF action. Experimental pigs were given a dietary supplement containing LF on a regular basis, and this increased their potential for immunological stimulation and enhanced their immune responses (Andersen et al., 2004) (Fig.6).

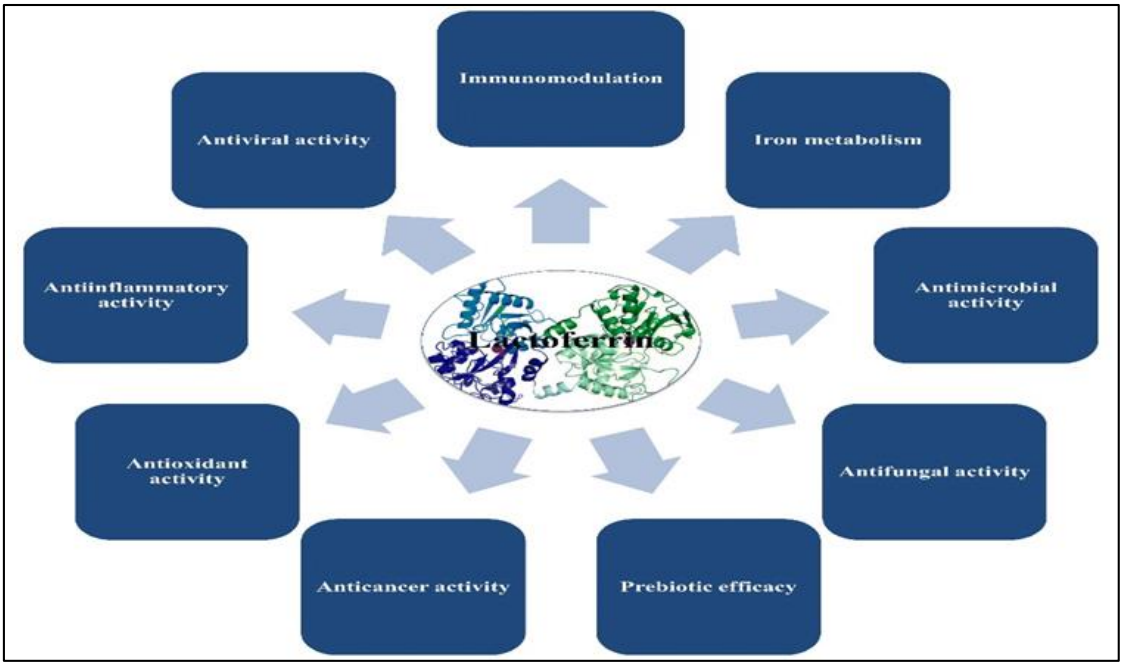

Figure 6 Multiple antimicrobial functions of lactoferrin. The antibacterial activities of lactoferrin against the Gram-positive and negative bacteria, and the antiviral activities of lactoferrin against the non-enveloped (naked) and enveloped viruses are shown

4.1. Effects of Lactoferrin on SARS-COV-2

The extremely hazardous coronavirus SARS-CoV-2 comprises a virus-sense RNA virus that is lipid-enveloped and belongs to the genus of coronavirus (Mirabelli et al., 2021).Mostly causes (Wang et al., 2020; Zamorano Cuervo & Grandvaux, 2020) infects the human respiratory tract, fever, dry cough, exhaustion, shortness of breath, muscle pain, and diarrhoea are some of the symptoms. A small percentage of patients may get acute respiratory disease syndrome, metabolic acidosis, septic shock, coagulation malfunction, or even mortality as a result of this condition. The entrance point for SARS-CoV-2 is the nasal cavity, and the main evacuation point is represented by the respiratory droplets. However, the faecal-oral propagation has to be taken into account, especially in the context having GI symptoms, as SARS-CoV-2 nuclear footprints have been discovered in the oesophagus, stomach, GI mucosa, duodenum, rectum, and faeces samples. It should be noted that SARSCoV-2 has been shown to persist longer in faeces samples than in respiratory samples. It has been proven that bLf has antiviral action in vitro against this Enveloped RNA virus. By competing with cell HSPGs, bLf has been demonstrated to block SARS-CoV-2 entrance similarly to other viruses. Furthermore, bLf binds to spike SARS-glycoproteins CoV-2's prevent infection and restrict viral entrance into host cells (Walls et al., 2020).As well as in the neurologic and hematologic symptoms (Campione et al., 2020, Sinopoli et al., 2022). A detailed in silico analysis of the functional structure between bLf& glycoproteins from spikes revealed the presence of 28 different interactions that endure for over 25 percent of the simulation period, which is consistent with its predicted substantial interaction energies. In depth, five hydrogen bonds, twenty residue pairs, and three salt bridges hydrophobic interactions have been discovered to be involved. To ascertain if some of the spike residues that bLf targeted were a part of the binding with ACE2(Hoffmann et al., 2020) has compared the average structure derived from the modelling of the interaction between ACE2 and the spike glycoprotein's C-terminal domain 1. Just two spike residues (Gly502 and Tyr505) were surprisingly shared by the intricate interfaces. Despite this, bLf occupies the same space above the up CTD1 domain as the ACE2 enzyme. Following the in silico results, the antiviral efficacy of bLf against SARS-CoV-2 was assessed in vitro. It has been proven that the anti-SARSCoV-2 action varies depending on the kind of experiment and bLf concentration. It is also widely known that bLf has a strong antiviral effect against a variety of RNA, DNA and enclosed or naked viruses. There has been mounting evidence from in-vitro research over the past year that bLf is effective against SARS-CoV-2. To exercise their antiviral effect, BLf either attaches straight to the SARS-CoV-2 spike proteins or disables host cell receptor. The in-vivo investigation carried out by Campione et al. supported these in-vitro findings. 30 patients suffering from COVID-19 who were treated with liposomal bLf experienced a rapid improvement in their clinical as compared to standard of care treatment, and there have been lesser symptoms and signs, a quick reversible transcriptase real-time (rRT)-PCR SARS-CoV-2 RNA negative transformation, and a decline in markers of inflammation such IL-6, d-dimer, and serum ferritin. In this section we provide the results of an investigation based on actual clinical practice, conducted by Italian physicians on their COVID-19 recipients who were receiving

therapy with bLf unloaded within liposome, in two ways by itself or in addition to different treatments, depending upon the subjects' symptoms. The individuals were in home-based separation while receiving bLf unloaded in liposome. These outcomes were contrasted with those seen in COVID-19 individuals who were not given bLf (Wang et al., 2020) (Fig. 7).

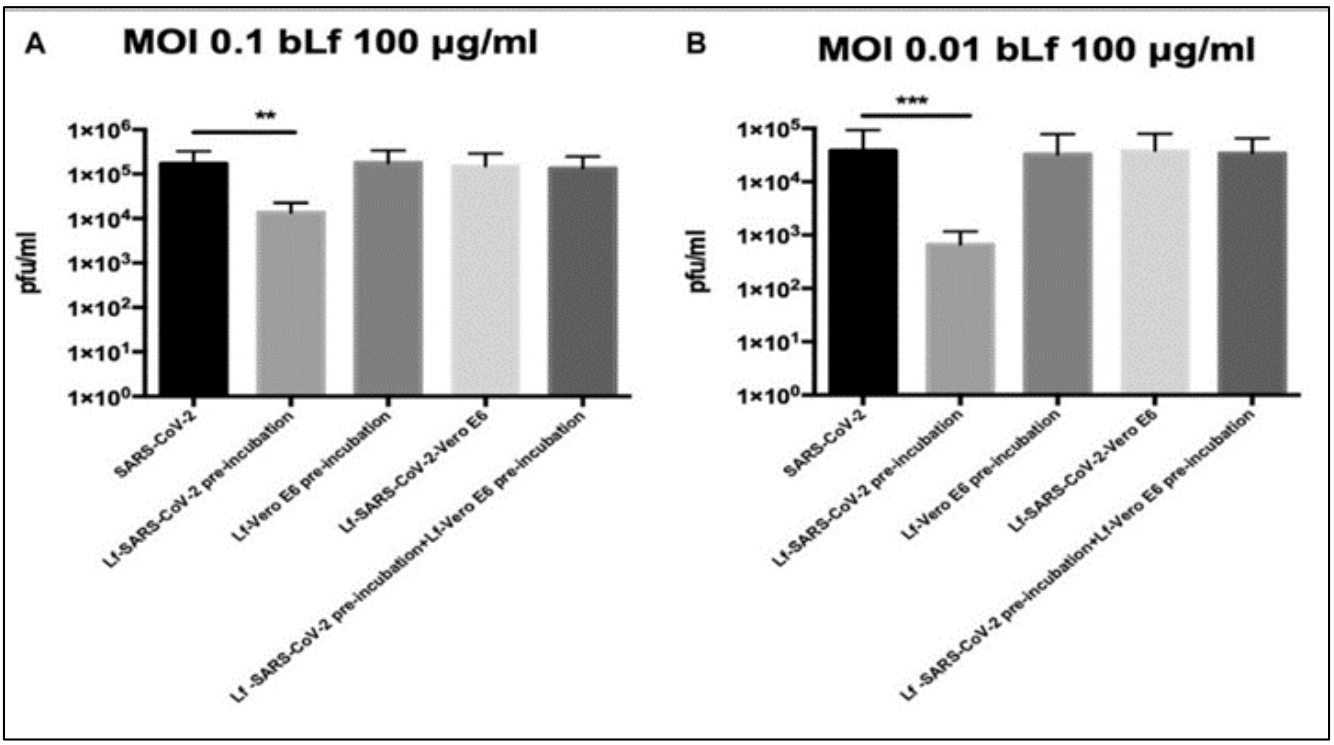

Figure 7 Plaque-forming units (pfu)/ml of SARS-CoV-2 observed in Vero E6 cells infected at multiplicity of infection (MOI) of 0.1 **(A)** and 0.01 **(B)** in the presence or absence of 100 µg/ml of bovine lactoferrin (bLf)

4.2. Effects of lactoferrin on H.P.V

Human papillomavirus (HPV) is a member of the Papillomaviridae family of naked DNA viruses. It affects skin or mucosal basal cells (de Villiers et al., 2004). This life cycle of virusis strongly influenced by the varying integrity of the epithelium. This could lead to the development of vaginal and respiratory system infections as well as cervical carcinoma and condyloma (Pinidis et al., 2016). According to estimates, the prevalence of HPV is11.7%. The largest incidence was seen in Sub-Saharan Africa (24.0%), Eastern Europe (21.4%), and Latin America (16.1%), (de Sanjosé et al.2007). According to the age-specific HPV distribution, the initial peak in Africa and America occurs at a younger age (25 Years), and it then rebounds at an older age (45 Years). The six most prevalent HPV genotypes worldwide are HPV-5, HPV-16, HPV-18, HPV-52, HPV-31, and HPV-58. Lactoferrin (LF), a multipurpose iron-binding glycoprotein essential for the innate immune response, is present in nearly every body fluids of humans. It is a low-cost protein made from milk that has no negative adverse reactions (Hadidi et al., 2018). Immunomodulatory, antiviral, antimicrobial, anticancer, antifungal, and anti-inflammatory effect are only a few of the numerous biological processes that LF participates in. (Kilic et al., 2017). There is proof that throughout the cell immunity process, LF is able to engage the CD14 receptor and the LPS binding protein. Moreover, proteolytic enzymes rapidly degrade it, which would prevent it from being used widely in application of antibiotics and antivirals. Although LF and transferrin are similar in many ways, they serve different purposes when used in living things. Transferrin appears to prevent cells from absorbing iron, whereas LF works to defend the mucosal membrane by forming bricks. With the potential to bind to negatively charged viral and microbial surfaces as well as DNA, heparin, and glycosaminoglycans, LF's cationic nature and ability to hold iron at acidic pH might be beneficial at infection and inflammation areas (Berlutti et al., 2011). Many in-vitro and in-vivo investigations have amply proven the protective LF's antiviral and antibacterial action. There are several hypothesised pathways for how LF works to stop infection by viruses (Wakabayashi et al., 2014). It includes binding to viral fragments (A), heparansulphate glycosaminoglycans (HSGA) (B), viral receptors (C), and intracellular localization (D) with a dose-related link to apoptotic or inflammatory pathways. According to reports, bovine LF (bLF) has a stronger inhibitory effect on HPV entry than human LF (hLF).

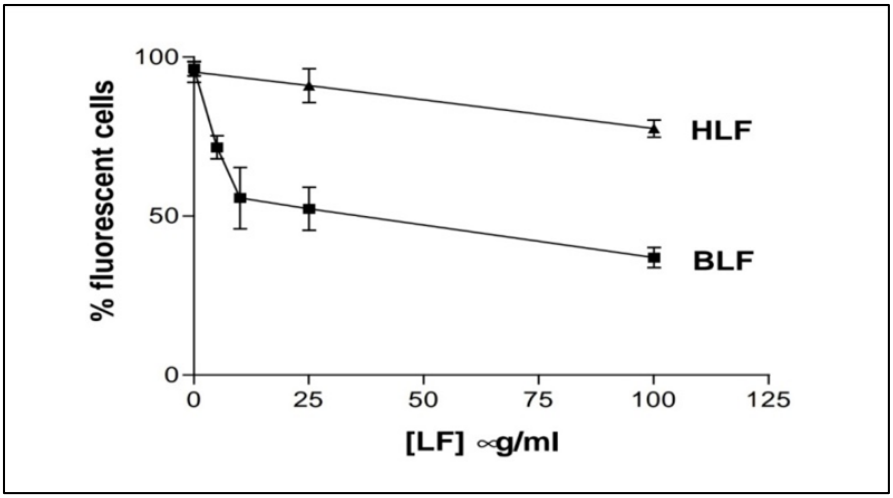

Figure 8 Lactoferrin inhibited HPV uptake. Dose response curve of inhibition of HPV-16 VLP uptake by bovine lactoferrin (BLF) and human lactoferrin (HLF)

4.3. Effect of lactoferrin against hepatitis c virus

The hepatitis C virus (HCV), which is thought to infect 59 million individuals globally, is a serious health issue and a major contributor to chronic liver disease (Williams, 2006). With a rate of as much as 20%, ten times more than any other country throughout the world, and the greatest incidence of HCV genotype 4, which is the cause of 90% of incidents, Egypt now has the most serious public health issue related to HCV a 55% prevalence of subtype 4a. According to studies, during the next 20 years, there will be a rise in HCV-related mortality. There is currently no HCV vaccine available, and interferon alone or in combination with ribavirin is the accepted therapy for chronic HCV infection (Nguyen & Keeffe, 2005). This HCV therapy is expensive, takes longer (12–72 weeks) to finish, and has significant side effects from the drug ribavirin, such as haemolytic anaemia that may necessitate dose decrease, limited efficacy, and ending the course of therapy. Lack of acceptable animal models and challenges in viral replication in cell culture have slowed the development of novel HCV treatments. LF has potent antiviral action against both naked and enclosed viruses throughout a broad spectrum. The description of LF action against the hepatitis C virus is the focus of this section (HCV). In cultured human hepatocytes (PH5CH8), HCV infection was successfully inhibited by both bLf and hLf, with bLf being the more active (Ikeda et al., 1998). A direct exchange between this investigation led to the identification of lactoferrin and the HCV envelope proteins E1 and E2 (Bartosch et al., 2003). In contrast to pre-treating cells with bLf, it has been shown that pre-incubating HCV with it prevents viral infection. Subsequent studies showed that immediately following bLf and HCV interaction, bLf prevented HCV entrance into cells by interacting with viral particles (Berlutti et al., 2011). The area of lactoferrin crucial for this function was found, and the binding activity of lactoferrin to the hepatitis C virus E2 envelope protein was enhanced. The results of this investigation were the first to identify a peptide produced from a natural protein. It stopped HCV infection by selectively interacting with the HCV E2 protein. Cs3-33, a synthetic peptide (amino acids 600–632) generated from the C-lobe of hLF, however it was less effective than intact LF, selectively prevented the entry of HCV into PH5CH8 cells by binding to HCV Envelope protein 2 (E2). Within the identical cell line, the Cs3-33 peptide pattern was tandem repeated and shown increased anti-HCV action; the triplicated peptide proved more powerful than the duplicate peptide, and each of them had been more effective than the one single peptide. The outcomes were comparable when a vesicular stomatitis virus pseudo type expressing native E1 and E2 genes and green fluorescent protein (GFP) was employed. Investigated was the ability of five synthesized peptides from the human LF C-lobe (17–33 residues from the Region defined by amino acids 600–632) to inhibit HCV cellular entrance. These peptides have different amounts of helices. The viral E2 that two of the five peptides attach to prevents HCV cells from entering. Remarkably, these two peptides possessed the highest helical shape, which shows that peptide helicity increased with peptide binding affinity for the viral envelope protein. It shows that as the peptide helicity rises, so does its affinity for the viral envelope protein, compared to the Nozaki peptide, which had a weaker affinity for HCV E2 (originating from the hLF C-lobe) (Abe et al., 2007). By blocking the interaction between the HCV E2-CD81 peptide and CD81, the cyanovirin Npeptide (101 residues) exclusively linked to the HCV E2 peptide and prevented viral cell entrance. A short peptide made from hLF is currently being tested in phase II clinical research as an antibacterial drug for allogenic bone marrow stem cell transplantation.

5. Discussion

A potential nutraceutical is one that offers the possibility of a certain health or medical benefit; it only becomes an established nutraceutical when there is enough clinical evidence to support it. The nutraceuticals are categorized on basis of their chemical makeup, food sources and mechanisms of action. Plants have a wide range of bioactive substances that can be isolated and used medicinally. According to the scientific community flavonoids is a unique class of therapeutic molecules due to their diverse therapeutic properties. Flavonoids are phytochemicals with polyphenolic secondary metabolites that work well as nutraceutical agents (Kaleem& Ahmad, 2018). The in-vitro and in vivo research for rutin has concluded that it, either alone or in combination, may be utilised as a dietary supplement to combat COVID-19.

The health advantages, including antioxidant, antimicrobial, antibacterial, anti-inflammatory and anticarcinogenic capabilities are associated these compounds (Gandhi et al., 2020). Fruits are extensively utilized in the commercial sector, particularly for making juices. Huge quantities of byproducts, comprising peels, seeds, cell, and membrane remnant are also a good source of hesperidin. Hesperidin's capacity to suppress the activity of NS3-NS4A in Hepatitis-C was confirmed by utilizing the FRET (Fluorescence Resonance Energy Transfer) assay during the evaluation of pure flavonoids.

By altering MAP kinase signaling pathways by upregulating p38 and JNK activation while downregulating ERK activation, the data showed that hesperidin improved cell-autonomous immunity (Shah Mahmud et al., 2017).

Hesperidin showed the greatest average affinity number across each of the sites and by far the best binding number with human ACE2 among the predicted compounds (Wu et al., 2020). Consequently, it might be one of the compounds with the greatest potential for use at any stage of the illness as well as for prophylaxis.

In addition to being a part of exocrine secretions like milk and saliva, lactoferrin, an 80-kDa iron-binding glycoprotein of the transferrin family, is also found in neutrophil granules (Levay & Viljoen, 1995). Both in vitro and in vivo studies have shown lactoferrin to be antiviral. There has been mounting evidence from in-vitro research over the past year that bLf is effective against SARS-CoV-2. Although LF and transferrin are similar in many ways, they serve different purposes when used in living things. Transferrin appears to prevent cells from absorbing iron, whereas LF works to defend the mucosal membrane by forming bricks. In contrast to pre-treating cells with bLf, it has been shown that pre-incubating HCV with it prevents viral infection. Subsequent studies showed that immediately following bLf and HCV interaction, bLf prevented HCV entrance into cells by interacting with viral particles.

6. Conclusion

The overall data has scientifically proved that these phytochemicals are potent inhibitor of viral infections.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors have no conflict of interest.

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