TO STUDY THE EFFECT OF DRUG, PHYTOCHEMICAL & VITAMIN AGAINST COVID-19 MAIN PROTEASE USING IN SILICO STUDIES

A THESIS REPORT

Submitted in partial fulfillment of the requirements for the award of the degree

Of

Bachelor of Technology

In

Biotechnology

Under the supervision

Of

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JUNE 2022

STUDENT'S DECLARATION

I hereby declare that the work presented within the Project report entitled

"TO STUDY THE EFFECT OF DRUG, PHYTOCHEMICAL & VITAMIN AGAINST COVID-19 MAIN PROTEASE USING IN SILICO STUDIES"

submitted for partial fulfillment of the wants for the degree of Bachelor's of Technology in Biotechnology Engineering at Jaypee University of data Technology, Waknaghat

is an authentic record of my work dispensed under the supervision of

Dr. Jata Shankar

Associate Professor

This work has not been submitted elsewhere for the reward of the other degree/diploma. I'm fully liable for the contents of my project report.

Signature of Student

Priyanka Shukla

181831

Department of Biotechnology & Bioinformatics

Jaypee University of Information Technology, Wakhnaghat, India

June 2022

CERTIFICATE

This is to certify that the work reported in the project report named is accurate. "TO STUDY THE EFFECT OF DRUG, PHYTOCHEMICAL & VITAMIN AGAINST COVID-19 MAIN PROTEASE USING IN SILICO STUDIES." in partial fulfillment of the requirements for a Bachelor of Technology in Biotechnology with a specialty in medical biotechnology and submitted to the Biotechnology Department at the Jaypee University of Information Technology, Waknaghat. It is genuine documentation as a part of B. Tech. Biotechnology project report by Priyanka Shukla (181831) from July 20, 2021, until May 20, 2022, under the leadership of, Dr. Jata Shankar, Associate Professor, Jaypee University of Information Technology, Waknaghat, Department of Biotechnology. To the best of my knowledge, the assertion mentioned above is correct.

Signature of supervisor

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JUIT, Wakhnaghat

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Thank you

Priyanka Shukla (181831)

Department of Biotechnology.

INTRODUCTION

Outline

The recent outbreak of a unique coronavirus disease (COVID-19), which resulted from severe acute respiratory syndrome (SARS), has now been declared a world pandemic. The WHO designated this epidemic as a Public Health Emergency of International Concern on January 30, 2020 [1], and named it COVID-19 in 2020. There are no specific drugs discovered so far, and given the danger factors linked with this condition, a therapy technique to treat this disease to limit transmission is urgently needed [1].

Coronaviruses (CoVs) are an enormous viral family that causes diseases starting from colds to severe respiratory infections. Furthermore, some COV strains are zoonotic, meaning they will be passed from animals to people. SARS-CoV may be a coronavirus genus that originated within the civet cat and horseshoe and first appeared in southern China in 2003. This disease afflicted 8096 people, leading to deaths [2].

Another is that the geographic area Respiratory Syndrome-CoV, which was named after the Dromedary Chamber on the peninsula, contained 2494 virus cases and 858 deaths.

The Novel Coronavirus (SARS-CoV-2) was discovered within the Chinese city of Wuhan, which incorporates a population of 11 million people, leading to the coronavirus 2019. (COVID-19). By Groundhog Day, 2020, the number of cases had risen to 17,400, with 362 fatalities and cases. The number of infections skyrocketed, increasing the timeframe to 1.8 days.

We produced a database illustrating how COVID-19 has spread to over 12 million cases worldwide and killed 560,000 people by July 10, by gathering 180 reports from the planet Health Organization (WHO).

Within four months, SARS-CoV-2 had contacted the planet and was killing many people every day [3].

Corona has produced one outbreak within the last several decades.

With the newest COVID-19 outbreak and also the World Health Organization's declaration of a plague on March 11, 2020, scientists throughout the planet are concerned. The pandemic had resulted in 26,504,030 illnesses and 873,821 deaths as of September 4, 2020, with the numbers continuing to rise fast. Corona are positive-sense single-stranded polymer (RNA) coated viruses with a genomic size of 26–32 kb that belong to the Coronaviridae family and Coronavirus subfamily [4]. It's capable to infect both humans and animals. Coronaviruses are classified into four categories reckoning on their serotypes and kinds. The four varieties of corona alpha corona, beta corona, gamma corona, and delta corona . Corona with CoV and CoV infections include HKU1, HCoV-NL63, HCoV-229E, HCoV-OC43, MERS-CoV, SARS-CoV, and SARS-CoV-2.

SARS, MERS, and COVID-19, respectively, have caused two epidemics within the last 20 years, furthermore mutually epidemic caused by corona.

Coronavirus have produced one outbreak within the last several decades.

In 2002, SARS-CoV became a worldwide threat in Southern China, infecting 8,098 individuals and killing people [5]. Merscorona infection also resulted in a very regional pandemic within the Middle East in 2012, with 2,494 cases and 858 fatalities. On December 31, 2019, a replacement virus that causes COVID-19 was discovered in Wuhan, China, and was called SARS-CoV-2 because it shares around 70% of its genes with SARS. SARS-CoV-2 medications primarily target RNA-based RNA polymerase, papain-like protease,

3-chymotrypsin-like protease, and spike glycoprotein protein. [6] In 2002, SARS-CoV became a worldwide threat in Southern China, infecting individuals and killing people. [6]

SARS-CoV-2 enters human cells by a right away interaction between the S protein and therefore the human angiotensin-converting enzyme. Chloroquine phosphate and hydroxychloroquine are highly recommended for treating severe cases because of their restricted modes of action. One example is the alkalization of cell phagocytozomes. Drugs that work as antiviral such remdesivir, ivermectin, and lopinavir, moreover as peptide EK1, neuraminidase inhibitors, and nucleoside analogues, are recommended as stimulant agents for SARS-CoV-2 [7, 14]

Why it is called $3CL^{pro}$ or M^{pro}

The functional polypeptides are released from the polyproteins after extensive proteolytic processing. [3] The 33.1-kD HCoV 229E major proteinase (Mpro), also known as 3C-like proteinase (3CLpro) because its cleavage-site selectivity is comparable to that of picoronavirus 3C proteinases [15, 30] , is primarily responsible for this.

Structure

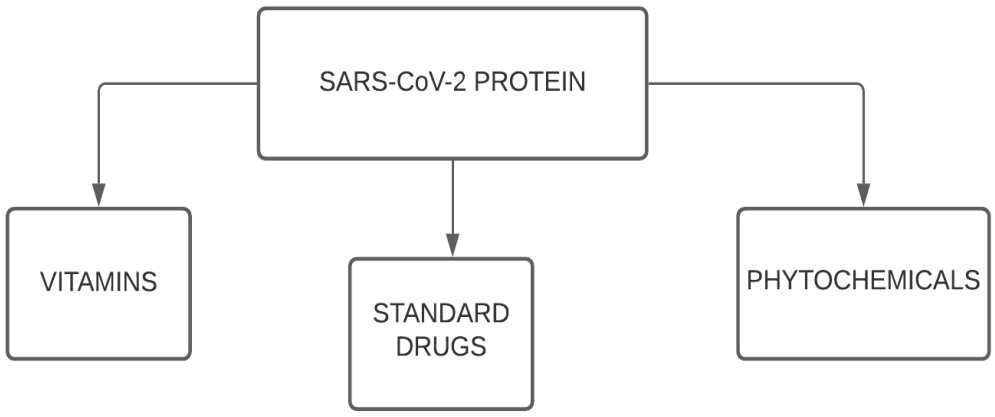
SARS-CoV-2 may be a single-stranded positive-sense RNA virus that belongs to the Betacoronavirus genus. SARS-CoV-2 is closely linked to 2 bat-derived SARS-like corona viruses [8, 19] , but it's further distant similarity from SARS-CoV and geographic region respiratory syndrome corona virus. RNA-based meta genomic next-generation sequencing was utilised to detect somebody's coronavirus from two pneumonia patients during the Wuhan epidemic in 2019. It measured many bp long. . Sequence and nucleotide similarity to the corona virus circulating in Rhinolophus , SARS-CoV-2 is consistent with evolutionary

study supported S, and N genes, SARS-CoV-2 is possibly a new strain of corona virus that was individually transferred from animals to humans [16, 18, 20, 21].

COVID-19 has no particular vaccinations or therapies available or efficient diagnosis at this point. However, numerous clinical trials testing putative therapies are currently underway [9, 10, 12, 14].

AIM

To Examine the effects of phytochemical and vitamins on the SARS Covid Main Protease, as well as their interactions with other drugs



★ ANALYSIS OF:

1. SPIKE PROTEIN VS PHYTOCHEMICALS
2. SPIKE PROTEIN VS VITAMINS
3. SPIKE PROTEIN VS STANDARD DRUGS LIKE IVERMECTIN AND AMPHOTERICIN B

★ MOLECULAR DOCKING OF THE ABOVE-MENTIONED ENTITIES

METHODOLOGY

1. Ligand Preparation

For 2D Structures

1. Launch UCSF Chimera.

2. Go to PubChem and type in the name of the inhibitor.

3. Get the string SMILES

4. Navigate to UCSF Chimera Tools -> Structure Editing -> Build Structure -> SMILES string (paste the string from Pub Chem) -> Apply -> Close.

5. We've completed our ligand molecule. [8]

6. Select Tools -> Structure Editing -> lower the structure once again (here set steepest descent steps: 1000 and Conjugate gradient steps:1000) -> Minimize

7. Next, click OK to add hydrogens, then Assign Charges to reduce. Enter OK after selecting Gasteiger. This will provide the molecule's net charge.

8. Click OK. Save the ligand to the working directory as follows: save the file as.mol2

For 3D Structures

1. Chimera at UCSF

2. Go to PubChem and type in the name of the inhibitor.

3. Save the 3D structure as SDF file and open it in UCSF Chimera.

4. Select Tools -> Structure Editing -> Minimize Structure once again (here set steepest descent steps: 1000 and Conjugate gradient steps:1000) -> Minimize

5. Now click OK to add hydrogens, then Assign Charges to reduce. Enter OK after selecting Gasteiger. This will provide the molecule's net charge. Click OK.

6. Save the ligand to the working directory as follows: save the file as.mol2

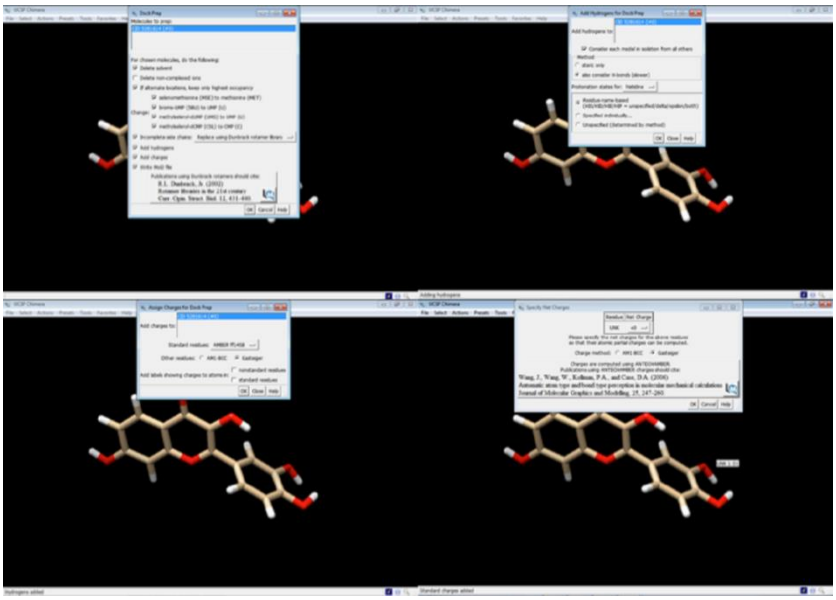


Fig 1 : Ligand Prepration

2. Protein Preparation

1.Start the Discovery studio.

2.Open the RSCB PDB in your web browser and look for the PDB ID.

3.Save the structure as a PDB file [22, 27]

4.In Discovery Studio, open the pdb structure.

5.Go to macromolecule -> clean protein -> show clean report.

6.Now go to Protein Minimization -> Protein Selection -> Algorithm Selection -> Yes.

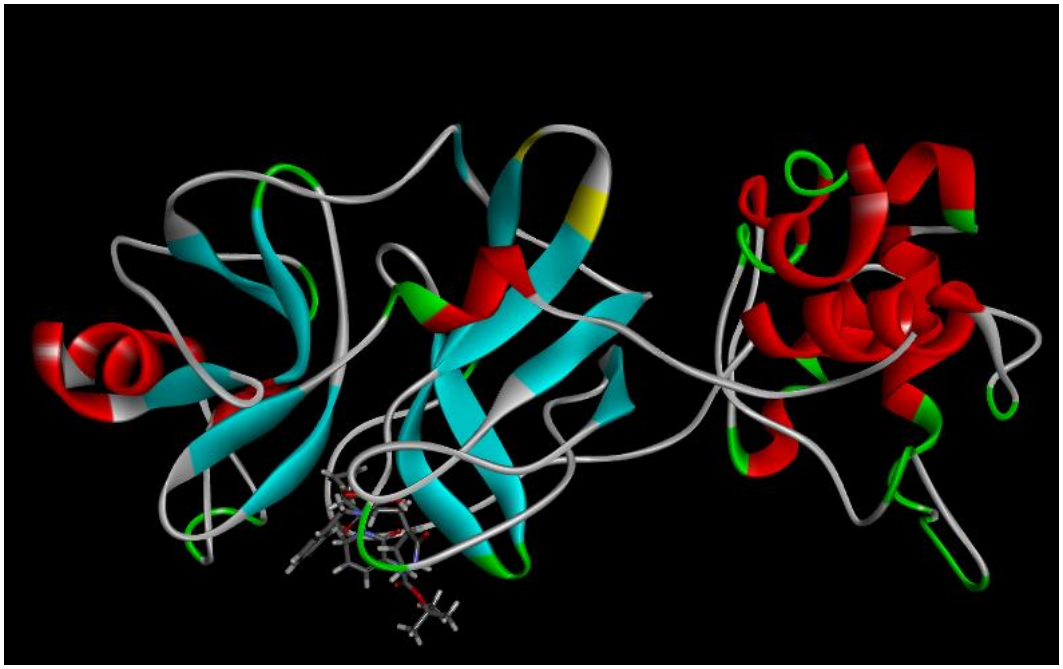


Fig . 2 : Protien Structure

3. Grid Generation

1. Go to File->Read Molecule->5R84.pdb

2. Water -> Edit -> Delete

3. Add H -> All polar -> Edit -> Hydrogens Ok

4. Charges -> Add Kollman charges -> Edit

5. Charges -> Compute Gastegier charges -> Edit

6. Atoms -> Assign type AD4

7. Select File -> Save -> PDBQT [24, 27]

8. Select -> Choose from the following options: string -> Residue sets -> Ligands -> Dismiss

.mol2 files -> Ligand -> Input -> Open -> Ok

10. Ligand -> Output -> PDBQT Write

11. Select ->

Choose from the following options: string -> Residue sets -> Ligands -> Add-> Remove

12. Grid -> Macromolecules -> 5R84 -> Save as.pdbqt

13. Grid -> Map types -> Ligand selection

14. Grid -> Grid Box -> Center -> Center on Lignand

15. File -> Save Current -> Close

16. Grids -> Output -> gpf -> grid.gpf -> save

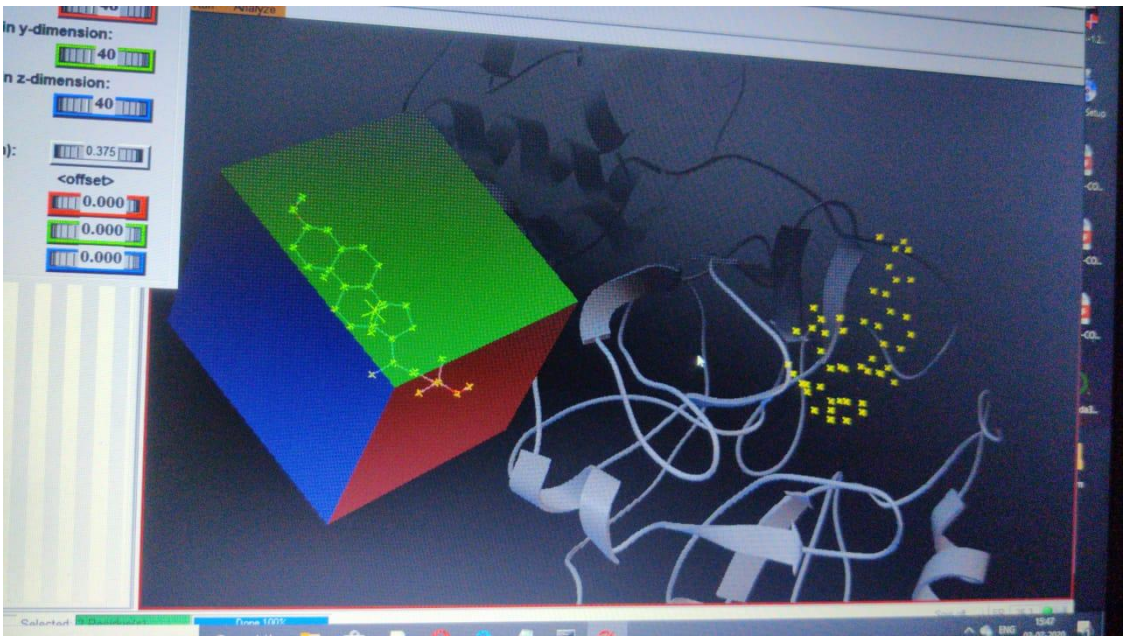
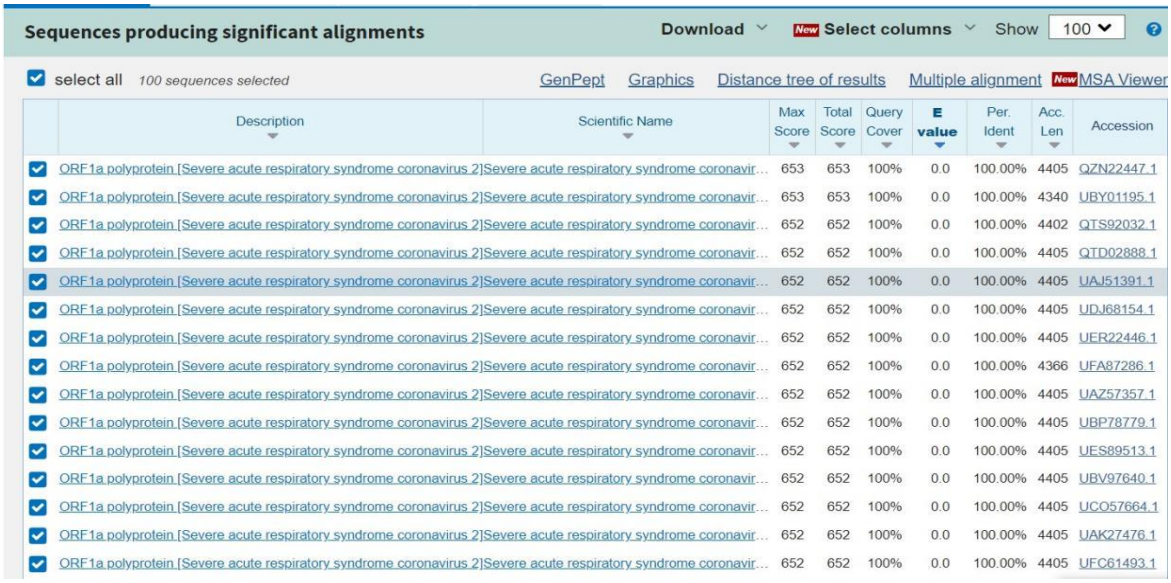


Fig . 3 : Grid Prepration

4. Protein Blast analysis

As illustrated in fig. 4, we utilised the protein's PDB ID to locate it in the protein data bank at this point. We used the protein's FASTA format. The sequences were then found with greater than 99 percent accuracy using protein BLAST. PDB [10, 23] was used to download the top 10–15 structures and their aligned sequences.



Sequences producing significant alignments Download New Select columns Show 100

select all *100 sequences selected* GenPept Graphics Distance tree of results Multiple alignment New MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
ORF1a polyprotein [Severe acute respiratory syndrome coronavirus 2]	Severe acute respiratory syndrome coronavir...	653	653	100%	0.0	100.00%	4405	QZN22447.1
ORF1a polyprotein [Severe acute respiratory syndrome coronavirus 2]	Severe acute respiratory syndrome coronavir...	653	653	100%	0.0	100.00%	4340	UBY01195.1
ORF1a polyprotein [Severe acute respiratory syndrome coronavirus 2]	Severe acute respiratory syndrome coronavir...	652	652	100%	0.0	100.00%	4402	QTS92032.1
ORF1a polyprotein [Severe acute respiratory syndrome coronavirus 2]	Severe acute respiratory syndrome coronavir...	652	652	100%	0.0	100.00%	4405	QTD02888.1
ORF1a polyprotein [Severe acute respiratory syndrome coronavirus 2]	Severe acute respiratory syndrome coronavir...	652	652	100%	0.0	100.00%	4405	UAJ51391.1
ORF1a polyprotein [Severe acute respiratory syndrome coronavirus 2]	Severe acute respiratory syndrome coronavir...	652	652	100%	0.0	100.00%	4405	UDJ68154.1
ORF1a polyprotein [Severe acute respiratory syndrome coronavirus 2]	Severe acute respiratory syndrome coronavir...	652	652	100%	0.0	100.00%	4405	UER22446.1
ORF1a polyprotein [Severe acute respiratory syndrome coronavirus 2]	Severe acute respiratory syndrome coronavir...	652	652	100%	0.0	100.00%	4366	UFA87286.1
ORF1a polyprotein [Severe acute respiratory syndrome coronavirus 2]	Severe acute respiratory syndrome coronavir...	652	652	100%	0.0	100.00%	4405	UAZ57357.1
ORF1a polyprotein [Severe acute respiratory syndrome coronavirus 2]	Severe acute respiratory syndrome coronavir...	652	652	100%	0.0	100.00%	4405	UBP78779.1
ORF1a polyprotein [Severe acute respiratory syndrome coronavirus 2]	Severe acute respiratory syndrome coronavir...	652	652	100%	0.0	100.00%	4405	UES89513.1
ORF1a polyprotein [Severe acute respiratory syndrome coronavirus 2]	Severe acute respiratory syndrome coronavir...	652	652	100%	0.0	100.00%	4405	UBV97640.1
ORF1a polyprotein [Severe acute respiratory syndrome coronavirus 2]	Severe acute respiratory syndrome coronavir...	652	652	100%	0.0	100.00%	4405	UCO57664.1
ORF1a polyprotein [Severe acute respiratory syndrome coronavirus 2]	Severe acute respiratory syndrome coronavir...	652	652	100%	0.0	100.00%	4405	UAK27476.1
ORF1a polyprotein [Severe acute respiratory syndrome coronavirus 2]	Severe acute respiratory syndrome coronavir...	652	652	100%	0.0	100.00%	4405	UFC61493.1

Fig . 4 : Protien Blast Analysis

5. Phylogenetic Analysis

A phylogenetic tree could be a branching diagram that depicts the evolutionary relationship between distinct biological species or other entities supported physical or genetic similarities and differences. Simple Phylogeny Masa tools (https://www.ebi.ac.uk/Tools/phylogeny/simple phylogeny/) were wont to create the phylogenetic tree [11]

The following steps were performed:

Step 1: identify and procure a group of homologous DNA or protein sequences.

Step 2: Put the sequences within the query box as shown in fig. 5.

Step 3 :Measure the tree from the target sequence

Step 4 : A tree was inbuilt the way that clearly conveys evolutionary relationship between different biological species of corona virus as shown in fig.6.

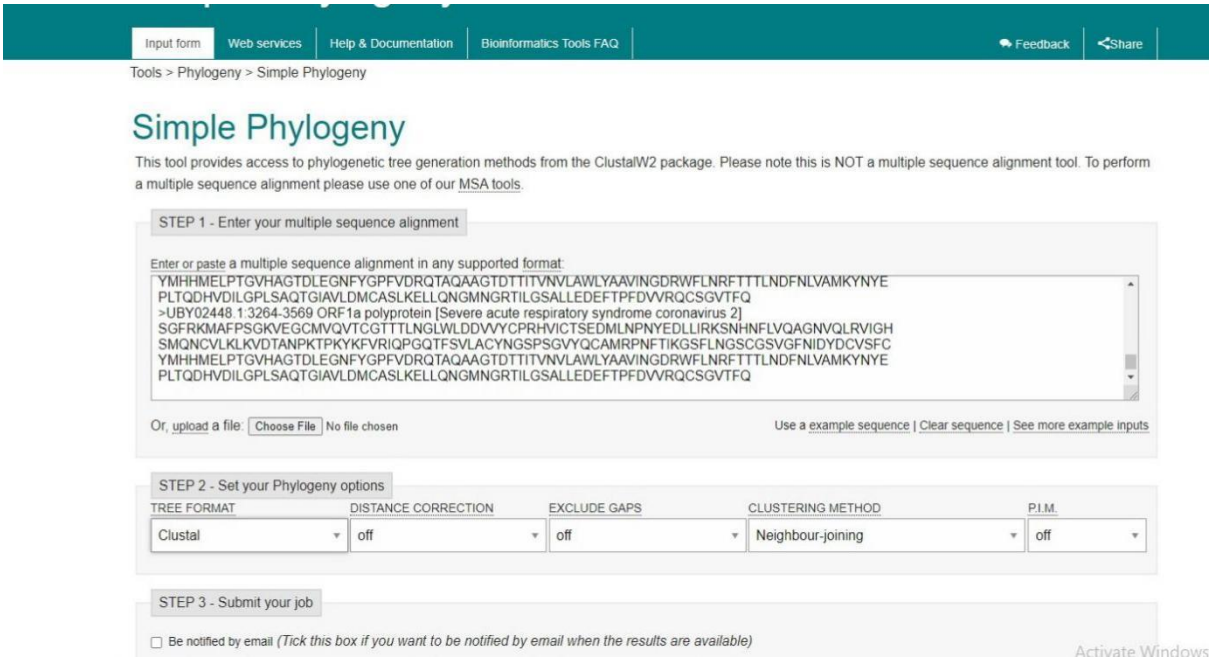


Fig . 5 .:Phylogeny

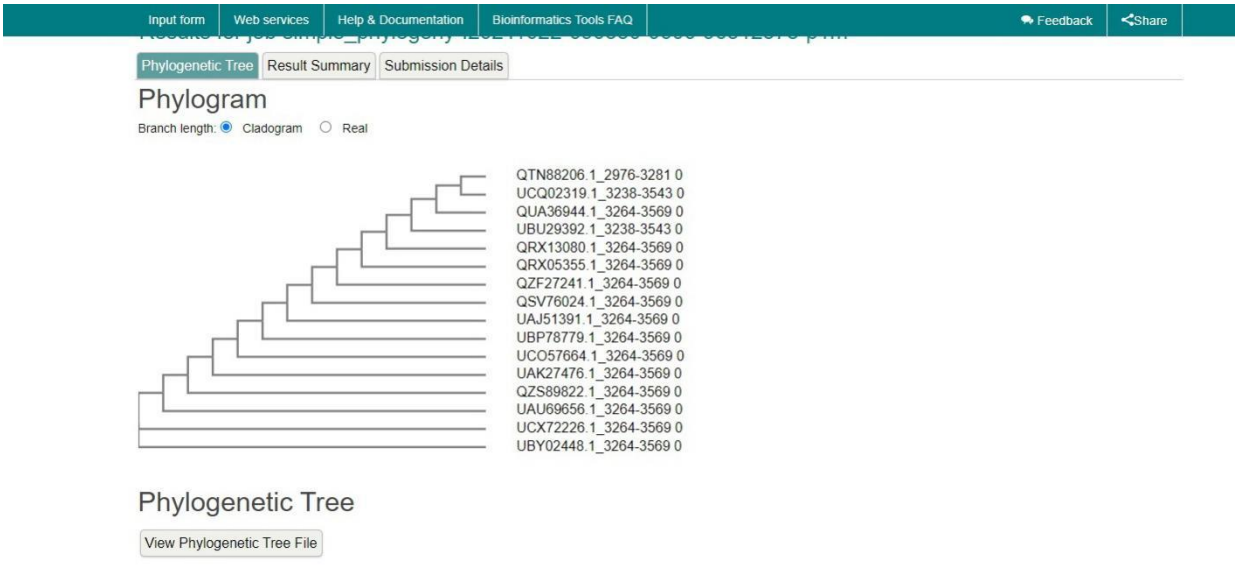


Fig.6.Phylogenetic Analysis

6. Molecular Docking

- Auto Grid
    1. Run -> Auto-Grid -> Program Pathname -> Browse -> C Drive -> (XProgram Files) -> The Scripps Research Institute -> Autodock -> 4.2.6 -> autogrid.
    2. For parameter filename -> Browse ->grid.gpf.
    3. Automatically a log filename file grid.glg will be created -> Launch. [29]
- Auto Dock
    1. Docking -> Macromolecule -> RigidFilename ->5R84.pdb
    2. Docking -> Ligand -> Choose -> .mol2 file -> Accept
    3. Docking -> Output -> Lamarkian -> Save as dock.dpf
    4. Run -> Auto-Dock -> Program Pathname -> Browse -> C Drive -> (XProgram Files) -> The Scripps Research Institute -> Autodock -> 4.2.6 -> autodock.
    5. For parameter filename -> Browse ->dock.dpf.
    6. Automatically a log filename file dock.dlg will be created -> Launch. [28]

Analyzing 2D and 3D Poses

- Through Auto dock
    - visualize -> Docking -> Open -> File Location -> dock.dlg.
    - visualize -> Conformations -> Play Rank by Energy(Best pose appears)

- Through Discovery Studio
    - File -> Open -> Protein

- Convert -> dockfile.dlg to dockfile.pdb
- Delete everything except coordinates. [25]
- Remove lower remaining part
- Drag pdb file from notepad

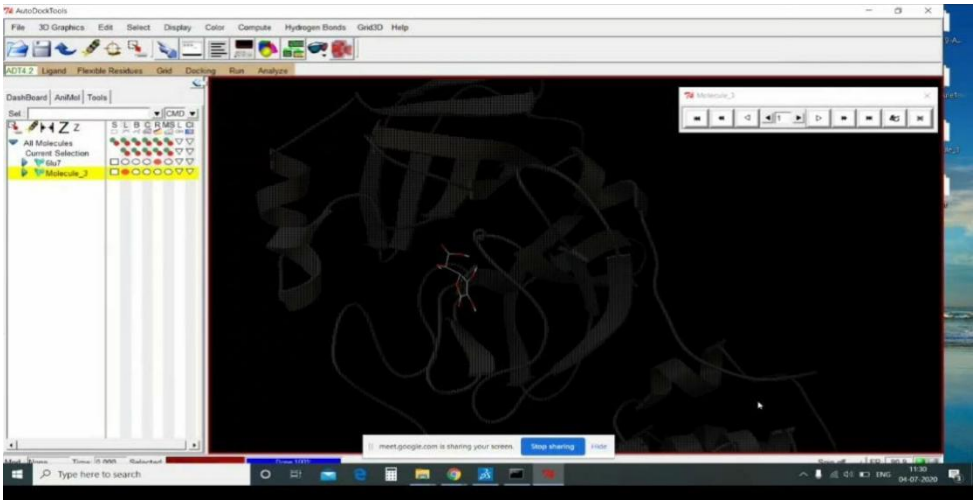


Fig .7 : 3D view of Protein

- Software's used

To visualise the results collected from the server, we used UCFS Chimera, a molecular visualisation programme. The docked positions and 2D interaction plots were also prepared using Discovery Studio Visualizer [8] Autodock 4.2.6 was used to predict how tiny

compounds attach to receptors with known 3D architectures using a suite of automated docking technologies. We used PubChem, Drugbank, and NCBI [13] while surfing the web.

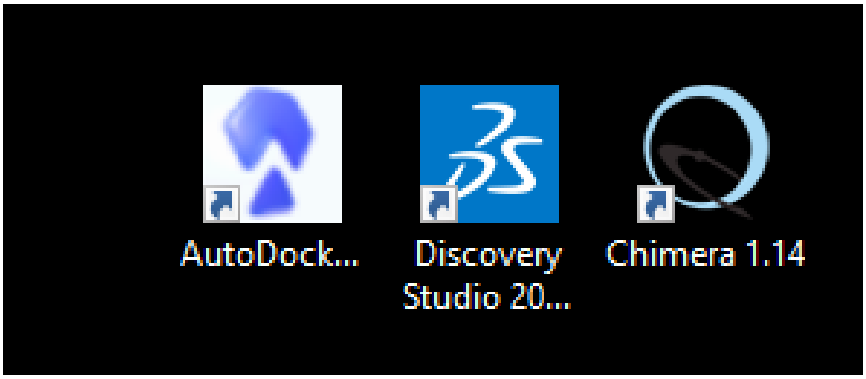


Fig. 8 : Tools used

RESULTS

1. SARS Covid Main Protease vs Phytochemicals Docking Results

Compound	BE(Kcal/Mol)	EE(Kcal/Mol)	IE(Kcal/Mol)
Gallic Acid [8]	-4.77	-0.48	-6.26
Quercetin [27, 25]	-5.73	-0.18	-7.52

Table 1 : Phytochemical

2. SARS Covid Main Protease VS Vitamin Docking Results

Compound	BE(Kcal/Mol)	EE(Kcal/Mol)	IE(Kcal/Mol)
Ascorbic Acid	-4.33	-0.22	-2.39
Vitamin D	-6.37	+0.01	-2.31

Table 2 : vitamins

3. SARS Covid Main Protease VS Standard drugs Docking Results

Compound	BE(Kcal/Mol)	EE(Kcal/Mol)	IE(Kcal/Mol)
Ivermectin	-6.39	-0.04	-9.68
Remdesivir	-7.194	-7.713	

Table 3 : Standard drug

Mucormycosis

• "Mucor" is a fungus that can be found in the environment and in soil. It only causes illness when immunity is extremely low.

• Mucormycosis is an old infection. It has been observed in people with weakened immune systems, such as those with uncontrolled diabetes following a transplant, as well as several cancer therapies [31]

• A rapid increase in Mucormycosis cases has been noted. Infection caused by Mucormycosis can result in vision loss and even menacing death.

• Mucor infection can happen during or after a Covid-19 infection weeks following Covid-19 recovery.

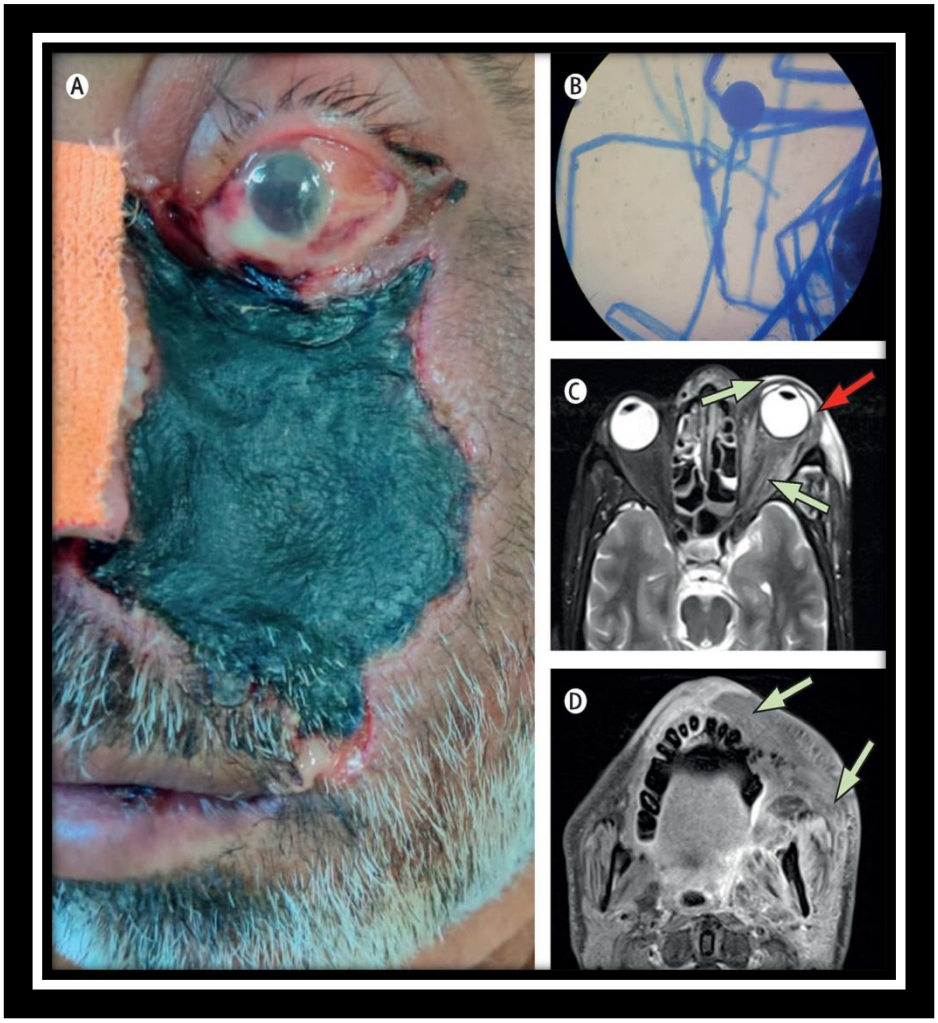


Fig 9 : Mucormycosis CT scan images

(Source:http://avignadiagnostics.com/creating-clarity/mucormycosis-affecting-covid-19-patients/)

What's creating this Mucor overabundance?

- COVID-19 has the potential to aggravate diabetes and perhaps create diabetes in previously healthy people.

- The Covid 19 infection reduces the number of some types of white blood cells and lowers immunity.

- Some medications used to treat Covid-19 infection, such as steroids and tocilizumab, can exacerbate immunological state.

Infection risk increases when Covid infection, high blood sugar levels, and immunosuppressive therapies are all present [32].

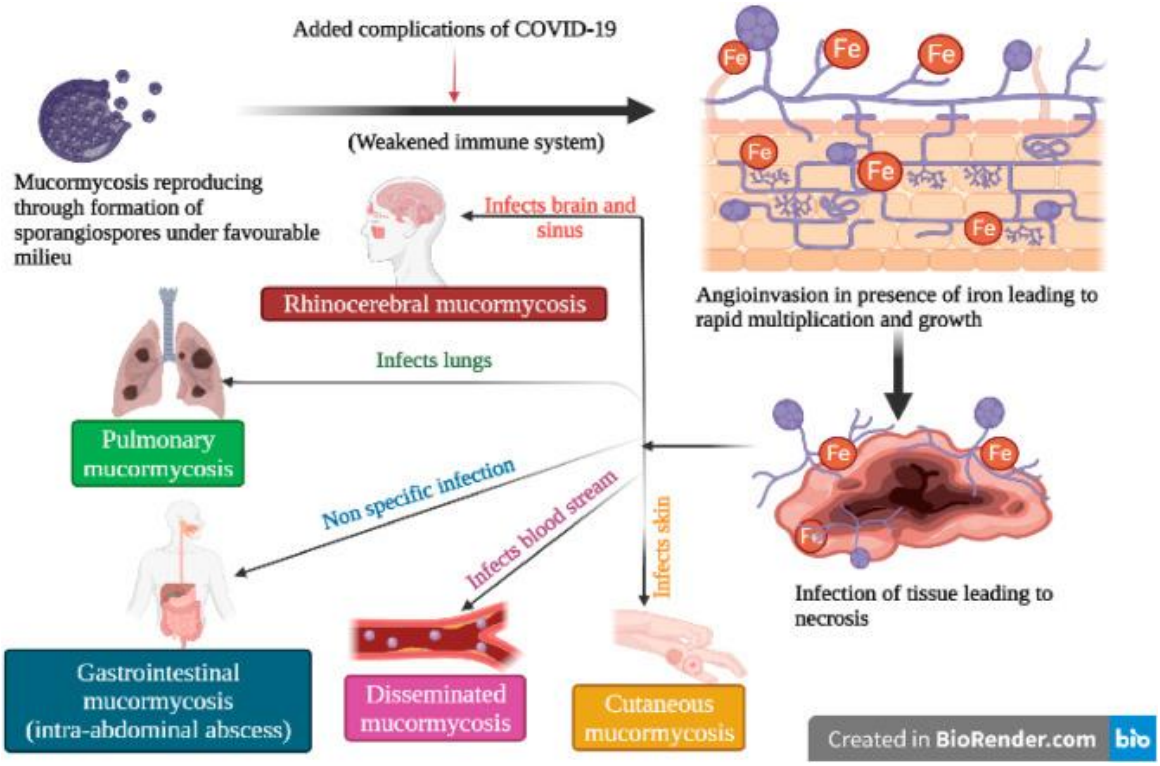


Fig 10: Complications in covid which cause mucormycosis

(Source:http://avignadiagnostics.com/creating-clarity/mucormycosis-affecting-covid-19-patients/)

Antifungal Drug in Mucormycosis

Mucormycosis is a dangerous infection that requires treatment with antifungal medications such as amphotericin B, or isavuconazole. Amphotericin B, posaconazole, and isavuconazole are administered intravenously. Fluconazole, voriconazole, and echino candins are not effective against the fungi that cause mucormycosis [28, 32]. Mucormycosis frequently necessitates surgery to remove diseased tissue

Treatment and Diagnosis

- To review Covid treatments to minimise immune compromised by treating co-morbid illness/blood sugar control and Covid illness including aspergillosis [11, 33].
- Daily checks for progression to orbital/intracranial involvement using the Mucor Checklist.
- KOH Smear/ Biopsy of affected lesion with adequate care to confirm diagnosis
- Radiology — CT/MR scans to determine the degree of the disease.

In the early stages, radiological indications may be mild and limited, with no evidence of florid sinusitis or bone erosion. The absence of these symptoms does not rule out the diagnosis [33].

- Ampho B/ Posaconazole antifungal .

Consider antifungal treatment before microbiological confirmation in cases of high clinical suspicion.

• Surgical debridement as soon as the systemic illness has stabilised, with facilities in place for post-operative care and ventilation.

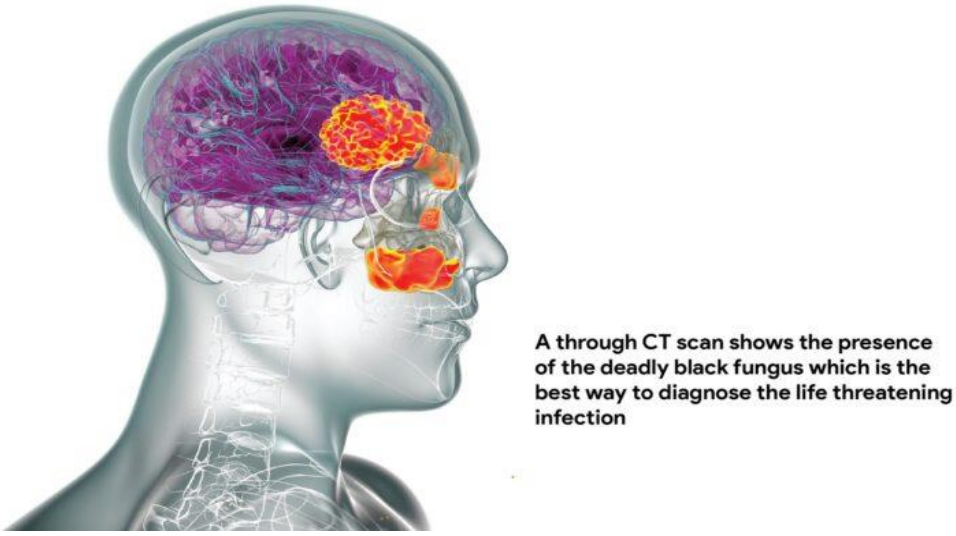


Fig 11: Diagnosis of mucormycosis

(Source:http://avignadiagnostics.com/creating-clarity/mucormycosis-affecting-covid-19-patients/)

Fig 12 : Symptoms of mucormycosis (

(Source:http://avignadiagnostics.com/creating-clarity/mucormycosis-affecting-covid-19-patients/)

Opportunistic Fungal Pathogens

Invasive mycoses caused by opportunistic fungal pathogens have increased dramatically within the last 20 years. Individuals undergoing solid-organ transplantation, blood and marrow transplantation (BMT), and surgical process, additionally as those with AIDS, ceroplastic disease, immunosuppressive therapy, advanced age, and premature birth, are all in danger for developing serious fungal infections. A growing number of pathogens, including well-known Candida alb, Cryptococcus, and Aspergillum fumigates, are linked to serious life-threatening infections. [34]

Other Candida and Aspergillum species, like Rhodo spp., and *Geotrichum capitatum*, zygomycetes, hyaline moulds, like Fusarium, Acremonium, Scedosporium, Paecilomyces, and Trichoderma species, and a good kind of dematiaceous fungi Medical mycology has evolved into a difficult study of infections caused by a good form of taxonomically diverse opportunistic fungi. The message to clinicians and clinical microbiologists is that no fungus is uniformly nonpathogenic: any fungus can cause a lethal infection in an immune compromised host and may never be dismissed as a contaminant out of hand.

Opportunistic mycoses pose significant diagnostic and therefore therapeutic challenges because of the complexity of the patients in danger of infection and the diverse and array of fungal pathogens. Clinical suspicion and therefore the retrieval of appropriate material for culture and histopathology are required for diagnosis.

We review selected and fewer common opportunistic mycoses during this article, that specialize in what's known about their susceptibility and resistance to new and established antifungal agents. Although several exciting new antifungal agents with improved activity and potencies are now available, it's important to recollect that the widespread and injudicious use

of any anti-infective agent in a very severely immunocompromised host may result in superinfections caused by organisms that are both unusual and drug resistant. [35]

SARS-CoV-2 in COVID-19 patients are observed in worrying numbers round the world White fungi implicated in such infections. During this review, we focused on global epidemics of fungal with SARS-CoV-2, the function of the human system, and a thorough understanding of these fungi in order to delineate the significance of such in the deterioration of COVID-19 patients' health [16, 17].

SARS-CoV-2 infection impairs CD4 + lymphocyte response, allowing fungi to require over host cells and cause severe fungal like candidiasis, mucormycosis, invasive pulmonary aspergillosis (IPA), and others.

Pulmonary aspergillosis caused by COVID-19 (CAPA). Mucormycosis and CAPA, has an a deathrate of 66% in India and 60% in Colombia. Furthermore, morbidity rates of are observed in Belgium, European country, France, and Germany, respectively. Voriconazole, Isavuconazole, and Echinocandins are among the antifungal medicines wont to treat fungal coinfection in COVID-19 patients.

SARS-CoV-2 weakens the system, allowing numerous fungus to use true and produce life-threatening health problems. Immunity boosting, good cleanliness and sanitation, and appropriate medicine supported the diagnosis are all necessary to stop the mortality and morbidity of fungal infections. [36]

PHYTOCHEMICALS AS ANTIOXIDANT AGENT

Antioxidants are chemicals that protect cells from damage caused by extremely reactive, unstable molecules called "free radicals." it's critical for our health to keep up a balance between antioxidants and free radicals in our bodies. Free radicals cause cell damage, which has been associated with variety of chronic disorders. Plant foods like fruits, vegetables, whole grains, beans, nuts, and seeds include phytochemicals, which are present substances. Many phytochemicals are shown in lab experiments to function antioxidants, neutralising free radicals and reducing their ability to cause damage. Vitamins C and E, additionally because the mineral selenium, appear to directly prevent free radicals not just within the laboratory, but also within the build [32].

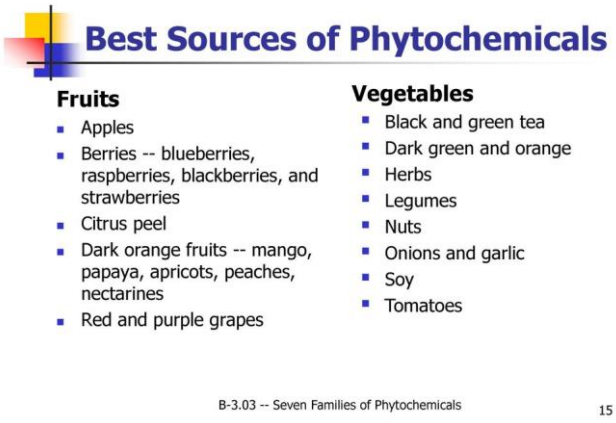
However, when it involves phytochemicals, lab test findings don't always reflect the results within the body. Many phytochemicals with high antioxidant ratings in lab testing cannot even be absorbed by the gut. Many of them, however, could also be counteracted by beneficial bacteria within the colon, leading to other molecules which will be absorbed.

Phytochemicals and therefore the molecules that generate from them appear to safeguard health during a kind of ways. Some can make cancer cells more likely to self-destruct, while others can halt carcinogens from starting the cancer formation process. They'll also prevent cancers from growing new blood vessels and cells. Some are anti-inflammatory.

Many phytochemicals also appear to help our bodies' ability to take care of a healthy balance of antioxidants and free radicals. The human antioxidant weapons system is formed of a posh network of enzymes and other chemicals that function together similarly like antioxidant elements from meals. [36]

A variety of metabolites derived from plants and other natural sources are shown to inhibit pathogenic fungi. Terpenes, saponins, alkaloids, coumarins, peptides, and proteins are just a few of the structural classes represented by these compounds. The growing number of multidrug-resistant fungus strains necessitates the event of newest antifungal compounds to combat fungal resistance mechanisms. This has prompted a hunt for alternative therapeutics, particularly medicinal plants and compounds isolated from them, with antifungal properties. Another issue is that the limited number of medication on the market as a results of the stringent regulations and sophisticated test process for potential candidate compounds. Various sorts of plant-based antifungal compounds against various fungi were identified and described during this review. [37]

As discussed, similarly, some studies have demonstrated a link between these natural compounds and their antifungal mechanisms of action. The cytomembrane disruption mode and also the interaction with intracellular molecules model are the 2 main mechanisms of action that result in programmed death. Because many compounds exhibit their potency through multiple mechanisms, it may be difficult to simplify the mechanisms of action in plant secondary metabolites. As a result, instead of grouping metabolites into the biosynthetic group, it's critical to conduct an in-depth examination of the compounds subgroups. Interfering with supermolecule and protein synthesis in cells may be a brand new drug target if there aren't any negative side effects and/or interactions with the human system. Additionally, the efflux pump within the future, inhibition is predicted to be significant in antifungal resistant strains.



Fi g . 13 : Phytochemicals

(Source: https://slideplayer.com/slide/12430)

Antimicrobial resistance may evolve due to COVID drug practises

One of the foremost pressing issues in modern medicine is antimicrobial resistance. Because the treatment of COVID-19 is increasingly reliant on pharmacological interventions, there's a greater risk of antimicrobial resistance evolving and spreading faster. A study conducted in a very tertiary hospital environment revealed concerning microbe colonisation patterns over long periods of your time. It also highlighted the wide selection of antimicrobial resistance gene reservoirs found in hospitals, which could aid within the emergence and spread of newest antibiotic resistance modes . [38]

Due to the COVID-19 pandemic, admissions and discharges from tertiary hospitals have increased dramatically within the last year, with many hospitals reaching capacity. Co-infection risks have significantly increased as a results of hospitalizations exceeding normal capacity because of COVID-19 combined with impaired immune function in patients. [39]

With drugs frequently replaced by new therapeutic options, the fear of increased antimicrobial resistance evolution and spread are a reality. There was an increasing demand for and misuse of varied drugs within the treatment of COVID-19 no matter paucity of scientific evidence.

. Social media has also played an alarming role in increasing the recognition (both positive and negative) of some drugs, including variation of unproven pharmacological substances. Furthermore, poor drug penetration in COVID-19 patients may lead to the rapid evolution of multidrug resistance. [40]

Furthermore, antibiotic medication was delivered to all or any instances in an

exceedingly single-center trial in Wuhan including 36 non-survivors with COVID-19, with 61.1 percent using combination therapy and 38.9 percent on one antibiotic intake . It should be emphasised that neither of the previous investigations included any information on microbial detection.

Although antibiotic therapy for bacterial pneumonia is universally established in clinical practise for H1N1 influenza, the case in SARS-CoV-2-related pneumonia is substantially different and ambiguous. Antibiotic or antiviral medication to treat co-infections in COVID-19 individuals, on the opposite hand, might be considered a good method, howsoever these patients are within the minority. An example, teicoplan, a narrow-spectrum antibiotic that's mostly active against Gram-positive organisms like staphylococcal infections, has previously been shown to inhibit the initial stage of the center East respiratory syndrome coronavirus life cycle in host cells.

Antibiotic prophylaxis appears to be used mostly to avoid bacterial co-infections among hospitalised patients, as observed within the COVID-19 instances. It's worth noting that, during this worldwide outbreak, incorrect antibiotic usage could contribute significantly and silently to the event of AMR . Unfortunately, recent investigations have revealed that antibiotic treatment for COVID-19 hospitalised patients is routinely utilized in numerous countries .

To minimise antibiotic consumption and stop the emergence and spread of antimicrobial resistant bacteria during the COVID-19 pandemic, coordinated interventions and antibiotic stewardship programmes are strongly suggested . Furthermore, proper data and records on Amr prevalence before and after the pandemic are going to be beneficial in evaluating the efficiency of those interventions and preventive measures. Furthermore, a microbial genomic comparative analysis of the DNA sequences involved in drug resistance before, during, and

after the COVID-19 pandemic should give significant information on the speed of genetic modifications also as clarify the potential mechanisms driving AMR development.

FELUDA: A Test for SARS-CoV-2 detection (Made in India)

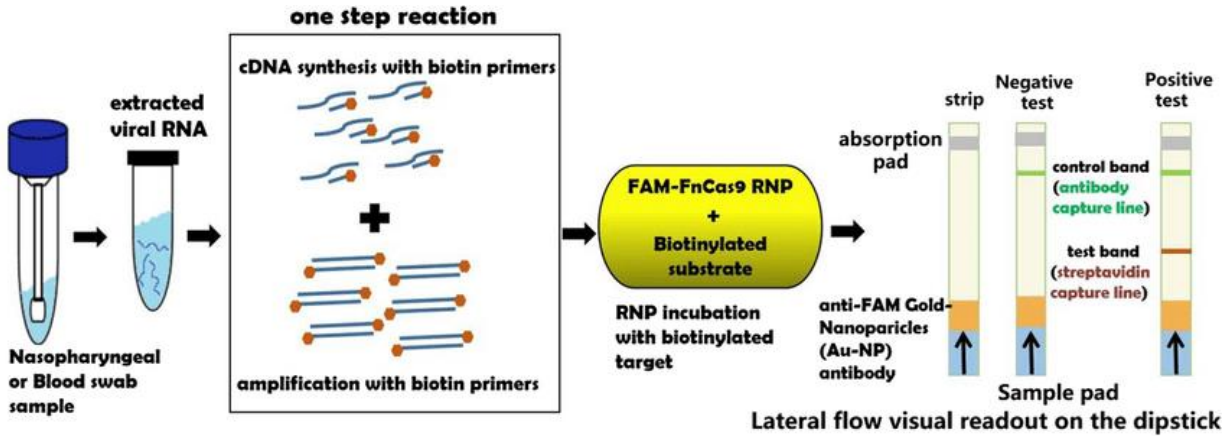


Fig : 14 : Feluda test working

1. *Available for much lesser cost than RT-PCR*
2. *96% sensitivity and 98% specificity, much higher than 70% for Real-time PCR*

(Source:http://moodle.juit.ac.in:81/moodle/pluginfile.php/23899/mod_resource/content/1/CRISPR-BASED-DIAGNOSTICS.pptx)

CONCLUSION

Binding and other interactions between SARS Covid Main Protease and phytochemicals (gallic acid and Quercetin), vitamins (vitamin D and ascorbic acid), and few medicines was carried out. Free energy, intermolecular energy, and electrostatic energy were computed (Ivermectin and Amphotericin B). The highest levels of binding with SARS-CoV-2 main proteins were found in quercetin and vitamin D. As a result, computational methods offer a viable alternative to many fundamental studies, and they can be used to anticipate virus particle interctions and to develop improvised therapeutic interventions.

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