Journal Homepage: -www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/11777
DOI URL: http://dx.doi.org/10.21474/IJAR01/11777

RESEARCH ARTICLE

ASSESSMENT OF SOME NATURAL BIOACTIVE COMPOUNDS FOR INHIBITORY ACTIVITY AGAINST NOVEL COVID-19: A COMPUTATIONAL STUDY

Ashishkumar Swami[1], Sangita Sharma[2] and Liggy Andrews[3]

1. Research Scholar, Department of Chemistry, Hemchandracharya North Gujarat University, Patan-384265, Gujarat, India.
2. Professor, Department of Chemistry, Hemchandracharya North Gujarat University, Patan-384265, Gujarat, India.
3. Associate Professor, Department of Biochemistry, GMERS Dharpur College Patan-384265, Gujarat, India.

Manuscript Info

Manuscript History
Received: 25 July 2020
Final Accepted: 28 August 2020
Published: September 2020

Key words: -
Molecular Docking, COVID-19, Bioactive Compounds, Inhibition, Drug Likeness

Abstract

COVID-19 is a new coronavirus originated from Wuhan, China. In 2019. Twenty eight natural bioactive compounds (namely Amentoflavone, Apigenin, Bilobalide, Bilobetin, Catechin, Epigallocatechin, Fustin, Gallocatechin, Ginkgetin, Ginkgolide A, Ginkgolide B, Ginkgolide C, Glycitein, Isoginkgetin, Isorhamnetin, Kaempferol, Luteolin, Myricetin, Nobiletin, Procyanidin, Quercetin, Quercitrin, Rutin, Sciadopitysin, Tamarixetin, Ginkgolide J, Ginkgolide M, and Ginkgolide K) are selected for computational theoretical calculations of molecular docking with crestal structure of COVID-19 Main Protease 6LU7 and COVID-19 chymotrypsin-like protease Kinase- 2GTB. Lipinski's rule of five for drug likeness is applied to consider bioactive molecule as potential drug molecule. The interaction study is carried to assess to deactivate progression of COVID-19 using Auto Dock (4.2). Calculations are carried out on efficient shape-based search lemarckian genetic algorithm principle and a score base function. The binding energies are found between -5.59 to -1.75 in COVID-19 Main Protease 6LU7 and between -6.35 to -2.08 in chymotrypsin-like Protease 2GBT. Results from calculated data reveal that there is hydrogen bonding, electrostatic and vanderwaals are possible types of interactions. This data can help in identify best antiviral drug and consider some of the natural bioactive molecules as food supplements for development of inhibitor in the treatment of covid-19 stains.

Introduction:-

WHO has declared the COVID-19 a pandemic.1 after it was identified in Wuhan, a city in the Hubei province of china rapidly. Not only in china but in many countries of world including India it has spread. This is a new coronavirus because previously, it was never seen in humans. It coined as Coronavirus Disease 2019 (COVID-19) by the World Health Organization (WHO). In January 2020, it rapidly spread in China resulting in an epidemic throughout china, followed by an increasing number of cases in other countries throughout the world. For COVID-19 patients' common symptoms are Fever, Fatigue, Dry cough, Anorexia, Myalgias, Dyspnea and Sputum

Corresponding Author:- Prof. (Dr.) Sangita Sharma
Address:-I/c Head, Department of Chemistry, Hemchandracharya North Gujarat University, Patan-384265, Gujarat, India.

production. Other, less common symptoms are headache, sore throat, and rhinorrhea2. Mention the full form of abbreviations when they appear for the first time in the text.

Bats are considered as natural hosts for these types of viruses, yet several other species of animals are also known to be a source. Middle East Respiratory Syndrome Coronavirus (MERS-CoV) is transmitted to humans from camels and the Severe Acute Respiratory Syndrome Coronavirus-1 (SARS-CoV-1) is transmitted to humans from civet cats. The structure of the receptor-binding gene region is very similar to that of the SARS coronavirus, and to many bat coronaviruses but in a different clade3. The International Committee on Taxonomy of Viruses has proposed that COVID-19 is designated severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)4. In a phylogenetic analysis of 103 strains of SARS-CoV-2 from China, two different types of SARS-CoV-2 were identified, designated as Type L (accounting for 70 percent of the strains) and type S (accounting for 30 percent)5.

This infection of this virus spreads via Human-to-human transmission via respiratory route and it is quite contagious6. A person with infection coughs, sneezes, or talks can infect another person if it makes direct contact with the mucous membranes; infection can also occur if a person touches an infected surface and then touches his or her eyes, nose, or mouth. Droplets typically do not travel more than six feet (about two meters)7. So social distancing is suggested as only solution to avoid this virus because no medicine or vaccine is available for it. The incubation period for COVID-19 is thought to be within 14 days following exposure. Large-scale serologic screening may be able to provide a better sense of the scope of asymptomatic infections and inform epidemiologic analysis8. Severe illness can occur in otherwise healthy individuals of any age, but it predominantly occurs in adults with advanced age or underlying medical comorbidities. Common clinical features at the onset of illness are Cardiovascular disease, Diabetes mellitus, Hypertension, Chronic lung disease, Cancer, Chronic kidney disease6.

The diagnosis cannot be definitively made without microbiologic testing, but limited capacity may preclude testing all patients with suspected COVID-19. Local health departments may have specific criteria for testing. It will be very challenging to track and contain the spread of this novel virus due to its often ‘silent’ nature or clinical presentation reminiscent to other respiratory tract infections. At that time, there is no specific antiviral treatment for COVID-19. People with COVID-19 can seek medical care to help relieve symptoms 9.

Enzymes are essential for healthy digestion and are naturally produced by a healthy body. Various fruits, vegetables, and other foods supplements also are source of enzymes. Enzymes work best at your normal body temperature in range of 97°F to 99°F (36.1°C to 37.2°C). They work with other chemicals in the body and create chemical reactions in the body and its actually speed up the rate of a chemical reaction to help support life. Due to high fever and temperature increases in COVID-19 patients to 102°C and the structure of enzymes breaks down. They no longer function properly. Restoring your body temperature to its optimal range will help restore enzyme health. The pH level of your stomach or intestines and inhibitors affect enzyme activity. Enzymes functioning vary from one person to person and certainly has indirect effect on SARS-CoV-2 infected persons. So, taking of dietary enzymes will have a positive impact on health infected persons

Using X-ray crystallography to examine the structure of the proteases responsible for Covid 19, the researchers designed a series of α-ketoamide compounds that would bind and block the enzymes active site. The compounds they made were then tested in vitro in human cells and they have used their recombinant protein nanoparticle technology platform to generate antigens derived from the coronavirus spike protein. Scientists working towards a broad-spectrum antiviral and angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers able to combat all the coronaviruses identified a target – a ‘main protease’ that is essential for viral replication. Protease is produced in the stomach, pancreas, and small intestine. Protease breaks down proteins into amino acids. Molecular modeling based on sequencing data reveals diversity in a critical SARS-CoV-2 surface protein in comparison to close related bat. Coronaviruses (only 75 percent identical), potentially indicating mechanisms for adaptation to a life in the human host. While this is early in the outbreak, there are no specific drugs available to treat SARS-CoV-2. There is high sequence conservation between SARS-CoV-2 and related SARS-CoV in viral drug targets, such as in protease and polymerase enzymes10-13.

So here it is planned to find out bioactive compounds of natural herbal extracts already marketed as antioxidants, to treat some viral infections and used as food. Around 28 compounds are selected and these are subjected to Drug Likeness Assessment with Lipinski Rules of five and docked with crestal structure of COVID-19 main protease 6LU7 and COVID-19 Chymotrypsin-like protease kinase- 2GTB to find out interaction patterns.

Experimental Material and Methods:
Software used:
Gaussian 16W package14
Gauss view 6.015
Molecular graphics laboratory (MGL) tools Package
PMV- Python 2.7- language was downloaded from www.python.com16.
Msms- MSMS library is used by the Pmv module msmsCommands17
PCVolRen- The PCVolRen library is used in the PMV module18
ADT- AutoDock4.2. was downloaded from www.scripps.edu 19
Isocontour- The isocontour library is used by the Pmvmodule20
Vision- Vision Software is a visual-programming environment21
Cygwin (a data storage) c:\program and Python 2.7 were simultaneously downloaded from www.cygwin.com22

Preparation of natural Ligand compound:
Chemical 3D structures of the selected bioactive natural compound were retrieved from the Pub Chem database in the form of .sdf file format24 and optimized compound using Gaussain16W18 software. Then necessary conversation and visualization of these compound in .pdb format using Gauss view 6.0 software19. The compounds are subjected to arrange energy minimization and select torsion tree choose torsions and to calculate gasteiger charge using 'Prepare Ligand' in Auto dock.

Preparation of protein structures:
The crystal structure of COVID_19 main protease (PDB ID: 6LU7) and crystal structure of SARS protein chymotrypsin-like protease (PDB ID: 2GTB) was retrieved from the RCSB Protein Data Bank (PDB) in the form of .pdb file format23 and necessary changes like removal of water molecules, other atoms, extra chains and choose torsion charge, compute gasteiger charge were done using 'Prepare Protein' module of Auto Dock 4.219

Drug likeness assessment with Lipinski rules of five:
Insilco methods are computer-based methods widely used in the pharmacological field of science to help discover inhibitors with high binding capabilities with a protein target and drug-likeliness properties25 Lipinski's rule of five for drug likeness gives general for a bioactive molecule to be a potential drug and these are (i) molecular weight less than 500 gm/mol (ii) logP is not greater than 5 (iii) H-Bond donor not more than 5 (iv) H-Bond accepter not more than 10(v) polar surface area not greater than 140 A°2 (square angstrom) are studied here26. Drug-like rule is applied for selected 28 bioactive natural compounds. Drug-like properties of the selected compound were analyzed on the basis of physical properties of compounds, namely molecular weight (M.W.), partition coefficient (logP), number of hydrogen bond acceptors and number of hydrogen bond donor26. In table-1 reports the drug likeness of 28 selected bioactive compounds

Rutin (610.5), Procyanidin (594.5) Sciadopitysin (580.5) Myricetin (570.5), Ginkgetin (566.5), Isoginkgetin (566.5), Bilobetin (552.5) and Amentoflavone (538.5) violates rule 1 because molecular weight is greater than 500 gm/mol

As per rule 2, Sciadopitysin (6), Ginkgetin (5.7), Isoginkgetin (5.7) and Bilobetin (5.4) violates drug likeness because these compounds have logP value greater than 5.

For rule 3, Procyanidin (10), Rutin (10), Epigallocatechin gallate (8), Quercitrin (7), Amentoflavone (6), Gallocatechin (6) have H-Bond donor value more than 5 so violates this rule.

Rutin (16), Myricetin (14), Procyanidin (13), Epigallocatechin gallate (11), Ginkgolide C (11), Quercitrin (11) has H-Bond accepter value more than 10 so violates rule 4.

For rule 5 is violated by Rutin(266Å²), Procyanidin(230Å²), Epigallocatechin gallate (197Å²), Quercitrin (186Å²), Myricetin(184Å²), Amentoflavone (174Å²), Ginkgolide C (169Å²), Bilobetin (163Å²), Ginkgetin (152Å²), Isoginkgetin (152Å²), Ginkgolide B (149 Å²), Ginkgolide J (149Å²), Ginkgolide M (149Å²) and Sciadopitysin (141Å²) because these compounds have polar surface area values greater than 140 Å².

According to drug lead likeness rule of three, if violation of rules is more than 3 such bioactive molecules are not considered as drug suitable for treatment. But data reveals that there is some exception where compounds are taken

for interaction study even after violation of drug likeness rules27. Natural product compounds outliers under a fifth rule: compound classes that are substrates for biological transporters are exception to the rule 5. Here on the basis data available a new criteria of drug likeness available i.e. compounds with electron number more than 220 is considered. Drug likeness volition of any rule 1 or 2 are negligible for study28.

Table 1:- Physiochemical Properties of Bioactive Natural Compound.

No.	Name	Molecular Formula	M.W. g/mol [a]	Log P	H.B.D.C. [b]	H.B.A.C. [c]	R.B.C. [d]	T.P.S.A. [e]	H.A.C. [f]	T.A.C. [g]	N.E.C. [h]	D.L.V. [i]
1	Amentoflavone	$C_{30}H_{18}O_{10}$	538.5	5	6	10	3	174 Å²	40	58	278	3
2	Apigenin	$C_{15}H_{15}O_5$	270.24	1.7	3	5	1	87 Å²	20	35	137	0
3	Bilobalide	$C_{15}H_{18}O_8$	326.3	-0.3	2	8	1	119 Å²	23	41	172	0
4	Bilobetin	$C_{31}H_{20}O_{10}$	552.5	5.4	5	10	4	163 Å²	41	61	286	3
5	Catechin	$C_{15}H_{14}O_6$	290.27	0.4	5	6	1	110 Å²	21	35	152	0
6	Epigallocatechin gallate	$C_{22}H_{18}O_{11}$	458.4	1.2	8	11	4	197 Å²	33	51	238	3
7	Fustin	$C_{15}H_{12}O_6$	288.25	1.3	4	6	1	107 Å²	21	33	150	0
8	Gallocatechin	$C_{15}H_{14}O_7$	306.27	0	6	7	1	131 Å²	22	36	160	0
9	Ginkgetin	$C_{32}H_{22}O_{10}$	566.5	5.7	4	10	5	152 Å²	42	64	294	3
10	Ginkgolide A	$C_{20}H_{24}O_9$	408.4	0.6	2	9	1	129 Å²	29	53	216	0
11	Ginkgolide B	$C_{20}H_{24}O_{10}$	424.4	-0.4	3	10	1	149 Å²	30	54	224	1
12	Ginkgolide C	$C_{20}H_{24}O_{11}$	440.4	-1.4	4	11	1	169 Å²	31	55	232	2
13	Glycitein	$C_{16}H_{12}O_5$	284.26	2.4	2	5	2	76 Å²	21	33	148	0
14	Isoginkgetin	$C_{32}H_{22}O_{10}$	566.5	5.7	4	10	5	152 Å²	42	64	294	3
15	Isorhamnetin	$C_{16}H_{12}O_7$	316.26	1.9	4	7	2	116 Å²	23	35	164	0
16	Kaempferol	$C_{15}H_{10}O_6$	286.24	1.9	4	6	1	107 Å²	21	31	148	0
17	Luteolin	$C_{15}H_{10}O_6$	286.24	1.4	4	6	1	107 Å²	21	31	148	0
18	Myricetin	$C_{27}H_{22}O_{14}$	570.5	1.9	0	14	13	184 Å²	41	33	164	3
19	Nobiletin	$C_{21}H_{22}O_8$	402.4	3	0	8	7	81.7 Å²	29	51	212	0
20	Procyanidin	$C_{30}H_{26}O_{13}$	594.5	2	10	13	4	230 Å²	43	69	310	5
21	Quercetin	$C_{15}H_{10}O_7$	302.23	1.5	5	7	1	127 Å²	22	32	156	0
22	Quercitrin	$C_{21}H_{20}O_{11}$	448.4	0.9	7	11	3	186 Å²	32	52	234	3
23	Rutin	$C_{27}H_{30}O_{16}$	610.5	-1.3	10	16	6	266 Å²	43	73	320	5
24	Sciadopitysin	$C_{33}H_{24}O_{1}$	580.5	6	3	10	6	141 Å²	43	67	302	3

		0										
25	Tamarixetin	$C_{16}H_{12}O_7$	316.26	1.9	4	7	2	116 Å²	23	35	164	0
26	Ginkgolide J	$C_{20}H_{24}O_{10}$	424.4	-0.4	3	10	1	149 Å²	30	54	224	1
27	Ginkgolide M	$C_{20}H_{24}O_{10}$	424.4	-0.3	3	10	1	149 Å²	30	54	224	1
28	Ginkgolide K	$C_{20}H_{22}O_9$	406.38	0.1	2	9	1	129 Å²	29	51	214	0

[a]M.W. g/mol= Molecular weight in gram per mole [b] H.B.D.C.=Hydrogen Bond Donner atom Count
[c]H.B.A.C.= Hydrogen Bond Accepter Atom Count [d]R.B.C.= Rotational Bond Count
[e]T.P.S.A.= Total Polar Surface Area [f]H.A.C.= Heavy Atom Count
[g]T.A.C.=Total Atom Count [h]N.E.C.= Number of Electron Count
[i] D.L.V.=Drug Likeness Volition

Molecular docking:
Selected natural bioactive compounds were docked with COVID-19 main protease and protein chymotrypsin-like protease. Best binding conformations were generated using the Lamarckian genetic algorithm for each compound in Auto dock 4.2.29. The binding free energy was empirically calculated based on the energy terms and a set of coefficient factors. A three-dimensional grid of interaction energy was calculated using Auto Grid based on the macromolecular coordinates in docking simulations30. Various parameters like binding energy in kcal/mol, Ligand efficiency, inhibition constant (Ki)uM (micromolar), Intermolar energy in kcal/mol, vdW + Hbond + desolv energy in kcal/mol, Electrostatic energy in kcal/mol, Final total internal energy in kcal/mol, Torsional free energy in kcal/mol, Unbound system's energy in kcal/mol, RMSD (simply root-mean-square deviation) in angstrom value are calculated. Docking calculations occur using the molecular mechanics with generalized Born and surface area solvation (MM/GBSA) model, and solvated interaction energy (SIE) methods. The protein molecules were assumed in rigid form and bioactive compounds in flexible form during docking at 298.15 k temperature.

Results and Discussion:-

In this study, selected low risk 28 natural bioactive compounds are interacted with protease enzyme. Interaction results will help us to identify possible protease inhibitors and suitable antiviral drugs to inhibit activities against novel COVID-19. The 28 bioactive compounds selected are part of many herbal medicines that are used as antioxidants and food supplements. The 28 bioactive compounds belong to family of bioactive Biflavonoid, Bioflavonoid, Flavanol, Flavanonol, Flavone, Flavonol, Isoflavone, Proanthocyanidin and Terpene lactone. Group wise distribution already given in table-2and 3. Molecular Docking Analysis Score with crystal structure of COVID-19 main protease Kinase- 6LU7 is given in table-2. COVID_19 main protease (PDB ID: 6LU7) protein chain A contain 306 residues c=1499, N=402, O=444, S=22 total 2367 of molecules system with 11395 valance electrons31.

Amentoflavone is biflavonoid, found in fruits and it is formed by two molecules of Apigenin oxidative coupling. Amentoflavone is obtained from Viburnum prunifolium32. Ginkgetin is a biflavonoid, derived from an amentoflavone, and isolated from Ginkgo biloba and Dioon. It works as an anti-HSV-1 agent, an antineoplastic agent, inhibitory activity and a metabolite33. Isoginkgetin is a biflavonoid derived from leaves of Ginkgo biloba, it is a potent inhibitor of matrix metalloproteinase 9 and Pre-mRNA splicing34. Sciadopitysin is a biflavonoid obtained from an Amentoflavone. It is a bone density conservation agent and a platelet aggregation inhibitor35. Apigenin is a plant-derived flavonoid with significant promise as a skin cancer chemo preventive agent. Apigenin, a flavone abundantly found in fruits and vegetables, exhibits antiproliferative, anti-inflammatory, and antimetastatic activities through poorly defined mechanisms36. Luteolin is a natural polyphenolic flavonoid, with potential anti-oxidant, anti-inflammatory, apoptosis-inducing and chemo preventive activities. This inhibits tumor cell proliferation and suppresses metastasis37. Myricetin is a flavone isolated from the leaves of Myrica rubra plants. It acts as a cyclooxygenase 1 inhibitor, an antineoplastic agent, an antioxidant, a plant metabolite, a food component and a hypoglycemic agent38. Nobiletin is flavone with plant metabolite property and an antineoplastic agent39. Quercitrin is a flavone, used as an antioxidant, an antileishmanial agent, carbonyl reductase inhibitor, aldehyde reductase inhibitor, tyrosinase inhibitor and a plant metabolite40. Quercetin also produces anti-inflammatory and anti-allergy effects mediated through the inhibition of the lipoxygenase and cyclooxygenase pathways, thereby preventing the production of pro-inflammatory mediators. It has a role as an antibacterial agent, an antioxidant, a

protein kinase inhibitor, an antineoplastic agent, an EC 1.10.99.2 inhibitor, a plant metabolite, a phytoestrogen, a radical scavenger, a chelator and an Aurora kinase inhibitor41. Rutin is a rutinoside that is quercetin with the hydroxy group, acts as a metabolite and an antioxidant. Rutin have a variety of biological activities including antiallergic, anti-inflammatory, antiproliferative, and anticarcinogenic properties. It acts as potential protectors against human diseases such as coronary heart disease, cancers, and inflammatory bowel disease42. Tamarixetin is a flavone derived from a quercetin. It works as a metabolite and an antioxidant. Isorhamnetin is the methylated metabolite of quercetin43. Isorhamnetin used as a potent prevents drug of anti-cancer, inhibitor proliferation, endothelial dysfunction and superoxide production44. Bilobetin is found in fats and oils and it is isolated from Ginkgo biloba45. Catechin used as a metabolite it has a two enantiomer46. Epigallocatechin Gallate is a phenolic antioxidant compound which obtained in number of plants such as green and black tea under acts as chemo preventive agent for cancer47. Gallocatechin is a catechin, isolated from Acacia mearnsi, obtained from adzuki bean, green tea, redcurrants, gooseberries and marrowfat peas. It behaves as a metabolite and Potential nutraceutical. Procyanidins are members of the proanthocyanidin class of flavonoids. They are oligomeric compounds, formed from catechin and epicatechin molecules and in oxidative conditions they get depolymerized yield in cyanidin. But these compounds may help prevent cancer48. Bilobalide is a terpenoid ticlatone found in extracts of Ginkgo biloba49. Ginkgolide A is a diterpene lactone and found in Ginkgo biloba leaves. It is a highly active PAF antagonist cage and show variety of inflammatory and immunological disorders50. Ginkgolide B is a diterpenoid trilactone51. Ginkgolide C along with the other active constituents of Ginkgo biloba are responsible for the inhibition of PAF activity52. Ginkgolide M is isolated only from the root of the gingko biloba tree53. Ginkgolide J extracted from Ginkgo biloba, reduces apoptotic damage in cultured chick embryonic neurons as neuroprotective and is used as antioxidant and superoxide scavenging54. Ginkgolide K isolated from Ginkgo biloba, induces protective autophagy through the AMPK/mTOR/ULK1 signaling pathway with neuroprotective activity55.

Fustin is a flavanonol sometimes it known as "dihydrofisetin" obtained from young fustic and in the lacquer tree and it shows effective interaction with neuronal death cell56. Kaempferol is natural flavonols acts as an antioxidant by reducing oxidative stress, antibacterial agent, a human xenobiotic metabolite, a human urinary metabolite and a human blood serum metabolite and it is currently under consideration as a possible cancer treatment57. Glycitein is a soy isoflavone and acts as plant metabolite, a phytoestrogen and a fungal metabolite and it is also metabolized by human gut microorganisms and may follow metabolic pathways similar to other soy isoflavones. Glycitein is a biomarker for the consumption of soya beans and other soya products58.

Many components selected here are present in Ginkgo biloba plant as minor or major component. The Ginkgo biloba leaf extracts are used for improved blood flow, radical scavenging, vesoprotection, and anti-PAF (platelet aggregating factor) activity. Ginkgolides separately are used in treatment of lung weakness, asthma, coughing, cancer, ischemic cardiovascular and cerebrovascular diseases and used as protective against the immune system, central nervous system, and ischemic injury59. Extracts of Ginkgo biloba may be used covid-19 patients as food supplements or herbal medicine. Molecular docking pictorial presentation is given in Figure-1 for COVID-19 main protease protein (PDB ID: 6LU7) with Ginkgolide-J.

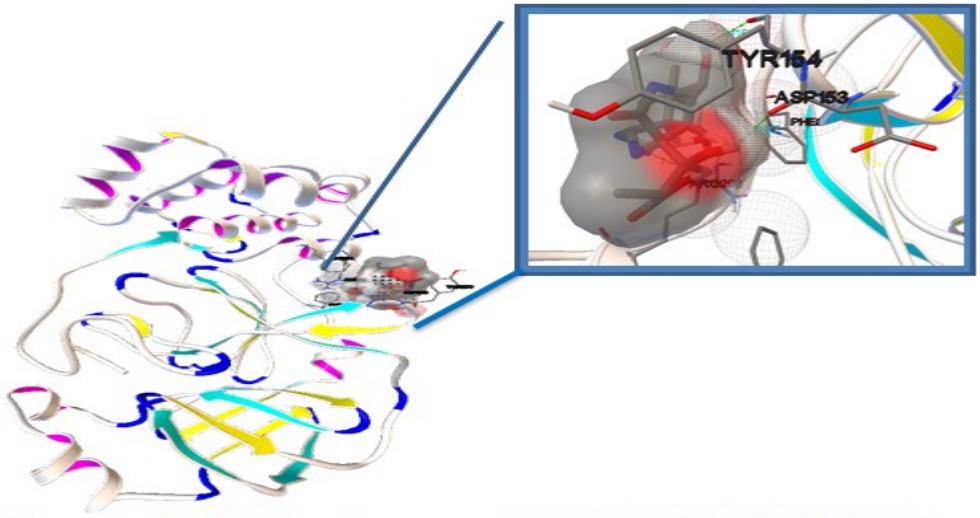
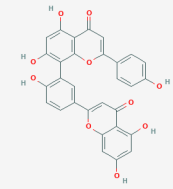
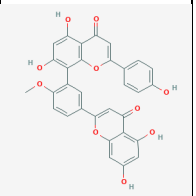
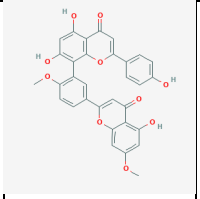
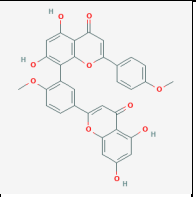
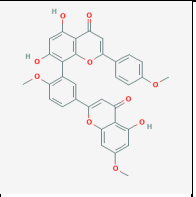
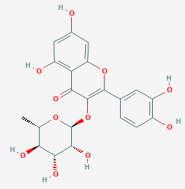
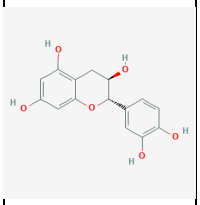
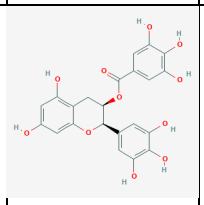
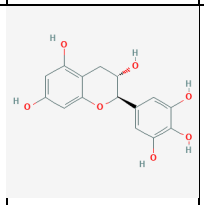
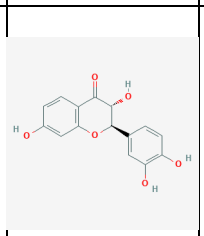
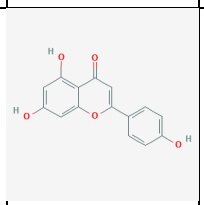
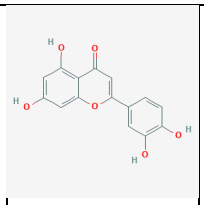
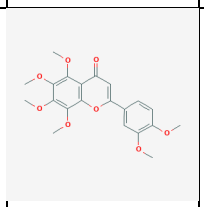
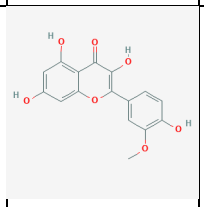
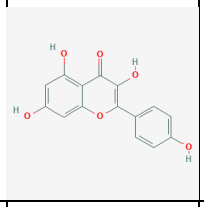
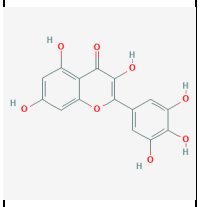
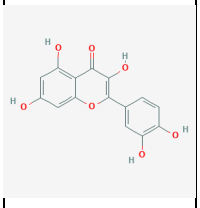


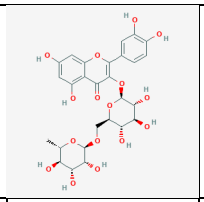
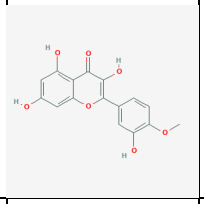
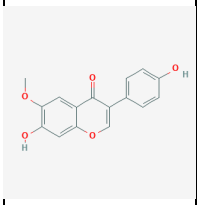
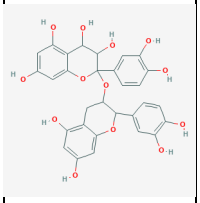
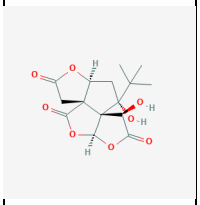
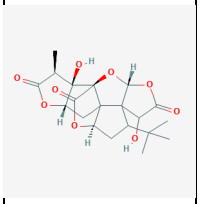
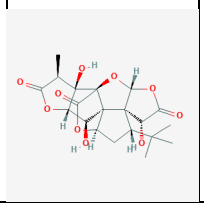
Fig1:- Molecular docking Interaction of COVID-19 main protease protein (PDB ID: 6LU7) with Ginkgolide-J.

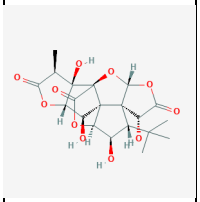
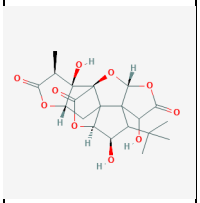
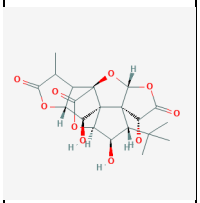
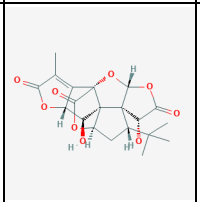
Table 2:- Molecular Docking Analysis Score with crystal structure of COVID-19 main protease Kinase- 6LU7.

No.	Name of Compound	2D structure	Types of Group compound	B.E.	L.E	I.C. (Ki) uM (micromolar)	I.M.E.	vdW + Hbond + desolv Energy	E.E.	F.T.I.E.	T.F.E	U.S.E.	R.M.S.D.	Active residues
1	Amentoflavone		Biflavonoid	-4.9	-0.12	255.36	-7.59	-7.12	-0.47	-3.99	2.68	-3.2	54.42	ARG217, ALA260 ASP263, GLY71, MET17, HIS246
2	Bilobetin		Biflavonoid	-4.43	-0.11	566.49	-7.11	-6.92	-0.2	-2.58	2.68	-2.58	43.57	ARG222, GLN127, ARG298, ARG105, TYR237
3	Ginkgetin		Biflavonoid	-4.47	-0.11	526.49	-7.16	-7.02	-0.13	-4.21	2.68	-4.21	55.43	GLU240, HIS246, PHE181, MET276, THR98, PHE103, SER10, VAL125
4	Isoginkgetin		Biflavonoid	-5.35	-0.13	119.02	-8.04	-8.09	0.06	-3.74	2.68	-3.74	61.27	ALA191, ASN180, PHE181, GLN107, LEU271, ALA285, ARG105
5	Sciadopitysin		Biflavonoid	-4.19	-0.1	851.29	-6.87	-6.77	-0.1	-3.17	2.68	-3.17	79.14	HIS64, ASP153, ARG217, GLY183, PHE103, LYS5, THR196
6	Quercitrin		Bioflavonoid	-2.18	-0.07	25.14	-5.17	-5.06	-0.1	-5.06	2.98	-5.06	59.5	ILE152, TYR237, LEU272, GLY278, LEU75, ASP153,

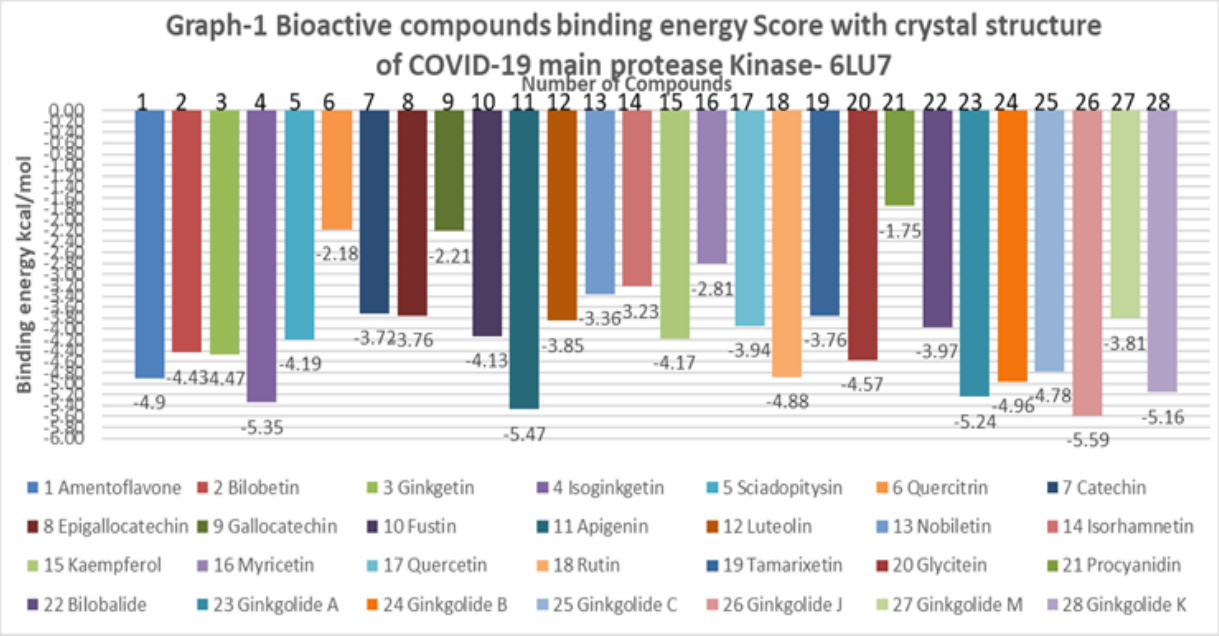
														LYS100
7	Catechin		Flavanol	-3.72	-0.18	1.88	-5.51	-5.26	-0.24	-1.88	1.79	-1.88	63.16	GLY15, LYS97, GLN110, ILE152, GLY71, LEU310, GLN256, GLY23
8	Epigallocatechin gallate		Flavanol	-3.76	-0.17	1.74	-5.85	-5.6	-0.25	-2.13	2.09	-2.13	57.37	TYR239, LEU271, TYR101, ASP155, LEU287, MET235, ASP153, ARG298, LEU282, LA285, GLU178, ASN277, ARG279
9	Gallocatechin		Flavanol	-2.21	-0.07	23.93	-5.79	-5.43	-0.37	-5.63	3.58	-5.63	56.61	ASN63, PHE66, VAL77, TYR154, ASP153, SER301, VAL297, MET235, MET276, ARG298, ASP245, THR199, ASP289
10	Fustin		Flavanonol	-4.13	-0.2	938.81	-5.62	-5.34	-0.28	-1.86	1.49	-1.86	60.54	TYR101, ALA285, LEU287, GLU178, GLU290, GLN127, TYR37, PHE103, LEU271
11	Apigenin		Flavone	-5.47	-0.27	98.63	-5.76	-5.72	-0.04	-0.29	0.3	-0.29	65.57``	ASN119, LEU271, ASN238, LEU287, LYS88, PHE103,

12	Luteolin		Flavone	-3.85	-0.18	1.5	-5.34	-5.2	-0.14	-2	1.49	-2	59.21	ASP33, GLY15, THR93, GLU178, ALA285, LYS97
13	Nobiletin		Flavone	-3.36	-0.12	3.42	-5.45	-5.26	-0.2	-1.53	2.09	-1.53	55.56	MET276, LYS236, ILE152, LEU287, PHE185, ARG40, ARG298, PRO132, THR199
14	Isorhamnetin		Flavonol	-3.23	-0.14	4.26	-5.02	-4.95	-0.08	-2.18	1.79	-2.18	78.22	GLN74, TYR101, LYS5, LEU287, ASP153, TRP218, ARG217, ARG222, TYR239, LEU271, TYR237
15	Kaempferol		Flavonol	-4.17	-0.2	878.5	-5.66	-5.42	-0.24	-1.56	1.49	-1.56	58.86	THR199, LYS5, ASP289, PHE103, PHE185
16	Myricetin		Flavonol	-2.81	-0.12	8.79	-4.89	-4.76	-0.14	-3.13	2.09	-3.13	57.09	MET276, ARG105, GLN110, ASN180, ASP33, PHE103, THR199, GLY278, LEU287
17	Quercetin		Flavonol	-3.94	-0.18	1.29	-5.37	-5.51	-0.22	-2.92	1.79	-2.92	62.28	GLY15, LYS97, GLU178, ASP33, PHE103, ALA285, TYR239, MET17
18	Rutin		Flavonol	-4.88	-0.11	263.4	-7.27	-7.13	-0.14	-5.41	2.39	-5.41	57.48	THR199, LEU271, PRO9, ARG105,

														HIS246
19	Tamarixetin		Flavonol	-3.76	-0.16	1.75	-5.55	-5.45	-0.1	-2.32	1.79	-2.32	58.48	ALA193, ARG188, LYS137, PHE103, ARG279
20	Glycitein		Isoflavone	-4.57	-0.22	445.93	-5.76	-5.53	-0.23	-1.04	1.19	-1.04	58.53	LEU287, GLU166, ALA308, GLU14, ALA7, TYR37, GLY183, ASP33, GLY71
21	Procyanidin		Proanthocyanidin	-1.75	-0.04	51.98	-5.93	-5.81	-0.12	-6.2	4.18	-6.2	79.88	PHE181, ILE249, GLN306, PRO168, GLY138, GLY195, THR196, LYS100, GLY283, ARG4
22	Bilobalide		Terpenic lactone	-3.97	-0.17	1.22	-4.87	-4.85	-0.01	-2.1	0.89	-2.1	67.91	THR26, ASN221, ARG279, LEU287, ASP153
23	Ginkgolide A		Terpenic lactone	-5.24	-0.18	144.46	-6.13	-6.03	-0.11	-1.28	0.89	-1.28	58.13	LEU272, MET276, ILE152, GLU178, LYS88, ARG298
24	Ginkgolide B		Terpenic lacton	-4.96	-0.17	233.01	-6.15	-5.98	-0.17	-1.9	1.19	-1.9	57.18	LEU271, LEU287, THR26, ARG4, LYS5, GLN107, GLY183,

			e											PHE181, MET276
25	Ginkgolide C		Terpenic lactone	-4.78	-0.15	315.48	-5.97	-5.8	-0.17	-2.74	1.19	-2.74	57.43	LEU271, GLY183, PHE181, ASN180, MET276, LEU287, ASP153, ALA285, LYS5
26	Ginkgolide J		Terpenic lactone	-5.59	-0.19	80.06	-6.19	-5.91	-0.27	-0.01	0.6	-0.01	58.61	ARG298, TYR154, ASP153, THR199, SER158, GLU240, HIS246, ASN221, ARG222,
27	Ginkgolide M		Terpenic lactone	-3.81	-0.13	1.63	-5	-4.94	-0.06	9.13	1.19	9.13	58.25	ARG298, LYS269, PHE3, YHR26, LYS5, THR98
28	Ginkgolide K		Terpenic lactone	-5.16	-0.18	165.43	-6.05	-5.94	-0.11	-1.85	0.89	-1.85	57.37	LEU287, ALA7, GLY23, GLN74, GLY71, LYS97, LEU287

[a] B.E ΔG.= Binding Energy in kilocalorie per mole [b]L.E.=Ligand Efficiency [c] I.C.= Inhibition Constant (Ki) in uM (micromolar)
[d] I.E.= Intermolar Energy in kilocalorie per mole [e] vdW + Hbond + desolv Energy= vandervals hydrogen bond dissolve energy in kilocalorie per mole
[f] E. E= Electrostatic Energy [g] F.T.I.E. = Final Total Internal Energy
[h] T.F.E.=Torsional Free Energy
[i] U.S.E.= Unbound System's Energy [j]R.M.S. D= root-mean-square deviation in angstrom (Å)



From this molecular docking analysis study, binding affinity of bioactive compounds with crystal structure of COVID-19 main protease Kinase- 6LU7 follows the given sequence respectively.
GinkgolideJ > Apigenin > Isoginkgetin > GinkgolideA > GinkgolideK > GinkgolideB > Amentoflavone > Rutin > GinkgolideC > Glycitein > Ginkgetin > Bilobetin > Sciadopitysin > Kaempferol > Fustin > Bilobalide > Quercetin > Luteolin > GinkgolideM > Epigallocatechin > Tamarixetin > Catechin > Nobiletin > Isorhamnetin > Myricetin > Gallocatechin > Quercitrin > Procyanidin.

In table 2 and graph 1, docking finding reveal that selected bioactive compounds interact with COVID_19 main protease (PDB ID: 6LU7) very active residues like HIS246, HIS64, GLN127, PHE181, PHE103, ASP153, LYS100, THR26, ARG279, THR93, TYR154, ASN277, GLN306, MET276, SER301, LYS236, and LEU75 through intermolecular hydrogen bond.

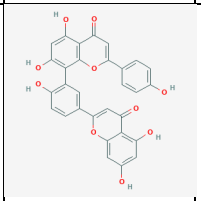
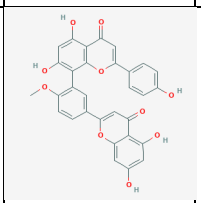
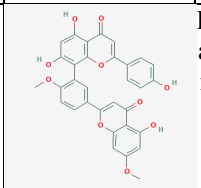
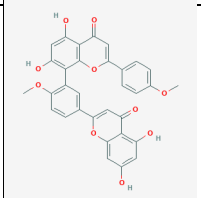
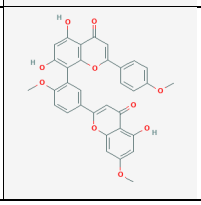
Ginkgolide J have highest binding energy value with main protease and Procyanidin have shown least binding affinity with COVID-19 main protease protein. Its already clearly mentioned in drug likeness rules if compound have more than 3 drug rules violation, it is not considered as drug for treatment. As Procyanidin and Rutin have 5 rules violated in drug likeness rule. Literature review reveals that nelfinavir is the best potential inhibitor against COVID-19 Mpro, based on binding free energy60. Active amino acid sites of protein protease enzyme molecules were identified by Computed Atlas for Surface Topography of Proteins (CASTp) server online61. In this study, it is observed that Hydroxy groups (-OH), ketone groups (=O) and ether groups (-O-) of selected bioactive compounds interact with amino acid -NH, -OH, and -H groups of selected proteins protease enzyme via hydrogen bonding, electrostatic and van der Waals reactions. These compounds can effectively inhibit these amino acids sites during the catalytic process by intermolecular hydrogen bond in key amino acids active site.

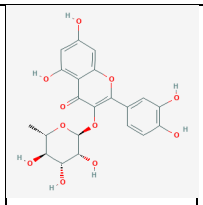
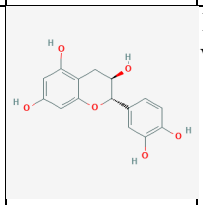
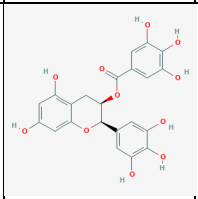
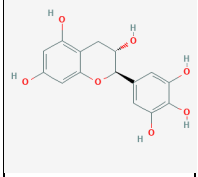
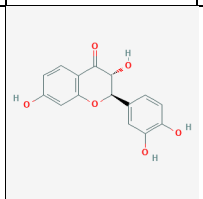
According to drug likeness rule study, ginkgolides series is considered for inhibition treatment studies because they have less drug likeness rule violation. Also, ginkgolide series of compounds may be useful as antiviral drug because it interacts with main protease of COVID-19 virus. All selected compounds are naturally available in plants. These also available in market in form herbal extract medicine supplements. Since the whole world is trying to find out antiviral drug of COVID-19 virus because its fast and uncontrollable tendency of spreading. Under present circumstances inhibition of this virus by these natural bioactive compounds will be a boon to society. Data of research show that this Covid-19 virus is mutating so slowly that its basic strands are fundamentally very similar to each other, eight stains of the coronavirus are identified (may have same active residues) So Ginkgolide series can be helpful. Ginkgolide have same structure but its position and grouping of -OH and -H make different and different names are assigned to them. Till date total 8 types of ginkgolides are identified, similarly 8 main stains of virus are

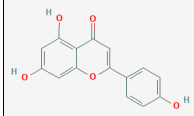
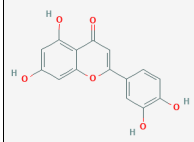
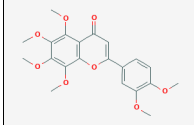
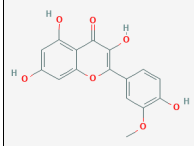
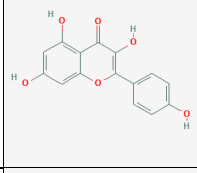
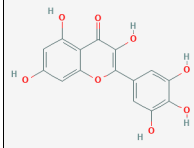
identified. Molecular docking studies reveal that 8 types of ginkgolides can inhibit and interact with this virus. Ginkgolides are low risk herbal drug compounds.

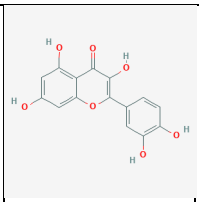
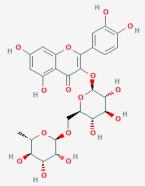
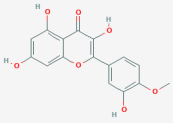
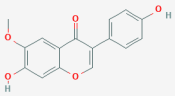
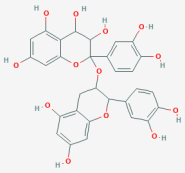
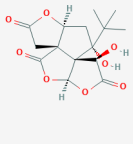
Molecular docking study repeated with crestal structure of COVID chymotrypsin-like protease Kinase- 2GTB results are given in table-3 and in graph-2. COVID crystal structure of SARS chymotrypsin-like protease protein (PDB ID: 2GTB) chain A contain 299 residues c=1474, N=403, O=433, S=22 of molecules with 11349 valance electrons62. Pictorial presentation of Molecular docking Interaction of COVID chymotrypsin-like protease protein (PDB ID: 2GTB) with Ginkgolide-J is given in Figure-2.

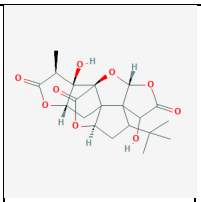
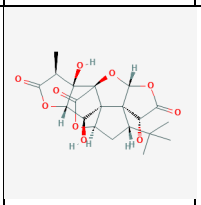
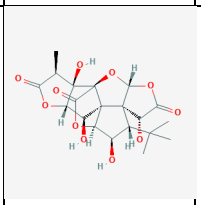
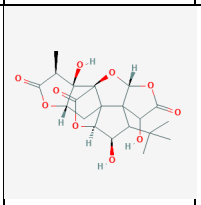
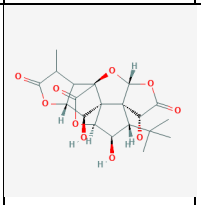
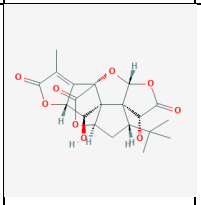
Table 3:- Molecular Docking Analysis Score with crestal structure of COVID-19 chymotrypsin-like protease Kinase- 2GTB.

No.	Name of Compound	2D structure of Compound	Types of Group compound	B.E.	L.E	I.C.(Ki) uM (micromolar)	I.M.E.	vdW+Hbond+desolv Energy	E.E.	F.T.I.E.	T.F.E	U.S.E.	R.M.S.D.	Active residues
1	Amentoflavone		Biflavonoid	-4.6	-0.12	422.81	-7.29	-7.11	-0.18	-3.11	2.68	-3.11	15.55	LEU287, GLY125, TYR154, LYS100, MET276, PRO108, THR292
2	Bilobetin		Biflavonoid	-4.86	-0.12	275.78	-7.54	-7.49	-0.05	-2.71	2.68	-2.71	18.69	THR111, THR292, GLY278, ASP245, ARG105, PRO108, HIS163, MET276
3	Ginkgetin		Biflavonoid	-6.35	-0.15	22.15	-9.03	-8.9	-0.13	-3.1	2.68	-3.1	19.22	THR24, GLN127, ARG298
4	Isoginkgetin		Biflavonoid	-5.05	-0.12	199.13	-7.73	-7.63	-0.11	-3.09	2.68	-3.09	21.31	GLU178, ILE152, MET276, GLY278, GLN110
5	Sciadopitysin		Biflavonoid	-4.87	-0.11	268.39	-7.56	-7.39	-0.17	-4.25	2.68	-4.25	18.32	ASP153, SER139, TYR154, MET276

6	Quercitrin		Bioflavonoid	-3.26	-0.1	4.07	-6.24	-6.18	-0.06	-4.73	2.98	-4.73	23.53	GLN192, GLN273, TYR237, LEU287, MET276, TYR154, ARG298, TYR239, GLY278
7	Catechin		Flavanol	-4.26	-0.2	753.98	-6.05	-5.92	-0.13	-1.91	1.79	-1.91	22	LEU141, SER144, MET276, TYR101, THR111, GLN110, GLN107, LYS102, ASP153, ASP33, PHE103, TYR154
8	Epigallocatechin gallate		Flavanol	-4.28	-0.19	730.26	-6.37	-6.16	-0.21	-2.1	2.09	-2.1	20.89	SER144, ASN277, LEU287, THR199, ASP153, SER144, GLY143, TYR101, PHE294, THR190, GLU166, ILE249
9	Gallocatechin		Flavanol	-3.09	-0.09	5.4	-6.67	-6.56	-0.11	-5.31	3.58	-5.31	22.96	ASN142, SER144, HIS163, MET276, GLN110, ILE152, TRP207, TYR154, ARG298
10	Fustin		Flavanonol	-5.37	-0.26	116.62	-6.86	-6.72	-0.13	-1.93	1.49	-1.93	21.4	LEU141, HIS163, SER144, HIS164, GLU166, MET276, GLY143, THR190

11	Apigenin		Flavone	-6.21	-0.31	28.28	-6.5	-6.5	0	-0.29	0.3	-0.29	20.63	ILE152, THR199, MET276, HIS163, LEU271,
12	Luteolin		Flavone	-5.21	-0.25	152.39	-6.7	-6.51	-0.19	-1.97	1.49	-1.97	21.56	LEU141, SER144, TYR237, LEU271, MET276, PRO108, PHE294, THR199, GLN19, GLU14, GLU240, THR111, LEU272, ILE286
13	Nobiletin		Flavone	-4.91	-0.17	251.4	-7	-7.09	0.09	-1.49	2.09	-1.49	20.81	LEU272, MET276, PHE103, LEU271
14	Isorhamnetin		Flavonol	-3.72	-0.16	1.89	-5.51	-5.28	-0.22	-2.31	1.79	-2.31	20.09	TYR237, PRO9, LYS12
15	Kaempferol		Flavonol	-4.94	-0.24	238.05	-6.43	-6.33	-0.11	-1.48	1.49	-1.48	19.72	GLY143, SER144, HIS163, TRP207, MET276, PHE103,
16	Myricetin		Flavonol	-3.6	-0.16	2.31	-5.68	-5.19	-0.5	-3.32	2.09	-3.32	3.64	LYS5, THR111, ASP153, ASP295, HIS163, GLU55, ASN84, GLU240, ALA7, GLN127, THR285, SER254

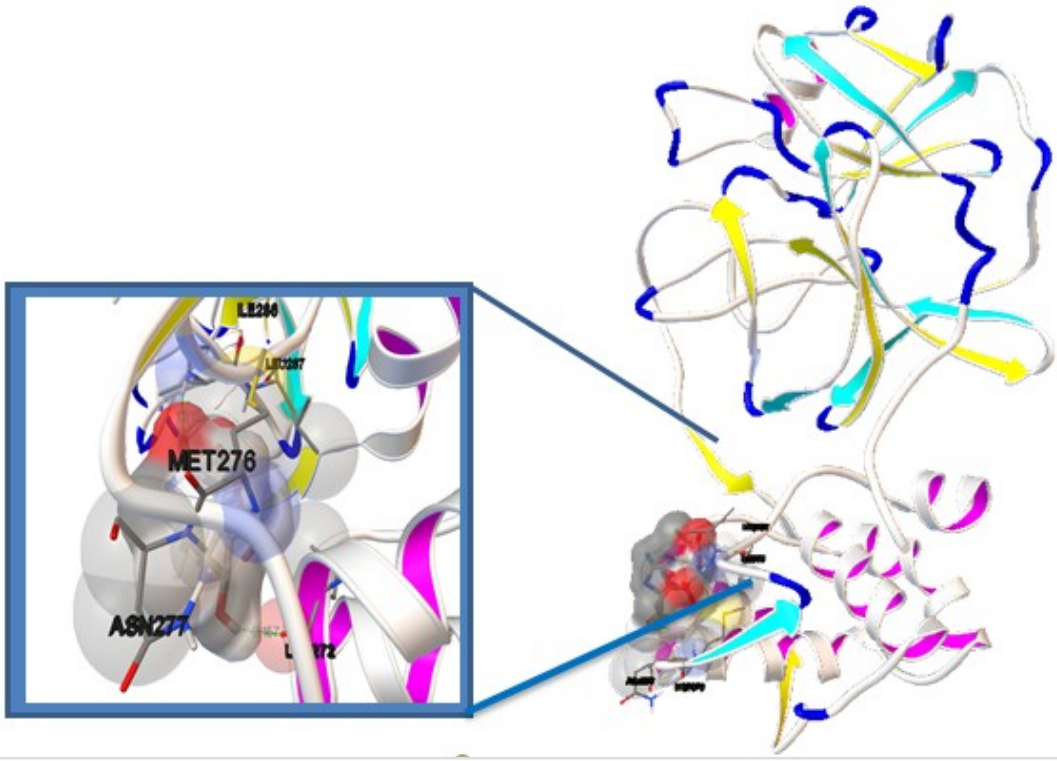
17	Quercetin		Flavonol	-4.44	-0.2	554.94	-6.23	-6.13	-0.1	-2.93	1.79	-2.93	20.3	SER144, HIS163, THR199, MET276, TYR237, THR190, ASP153, ASN203, PHE294
18	Rutin		Flavonol	-2.08	-0.05	29.78	-4.47	-4.46	-0.01	-5.48	2.39	-5.48	23.58	HIS80, ASN274, THR93, ARG217, GLN74
19	Tamarixetin		Flavonol	-4.02	-0.17	1.13	-5.81	-5.71	-0.09	-2.37	1.79	-2.37	21.21	HIS163, THR196, LEU271, ALA7, GLN127, GLY278, THR257, ILE249
20	Glycitein		Isoflavone	-5.23	-0.25	145.8	-6.43	-6.34	-0.09	-0.87	1.19	-0.87	22.19	SER144, GLY11, MET276, PHE294, THR98
21	Procyanidin		Proanthocyanidin	-2.75	-0.06	9.67	-6.92	-6.85	-0.08	-7.16	4.18	-7.16	18.03	MET276, LEU287, LYS5, ASP216, ARG217, GLN127, ARG298, GLU166, ALA7, TYR154, TYR101, ARG105
22	Bilobalide		Terpenic lactone	-4.57	-0.2	448.85	-5.46	-5.32	-0.14	-2.08	0.89	-2.08	18.27	ASN277, GLY278, MET276, SER254, VAL261, LEU287, LYS102, GLN110, ILE249

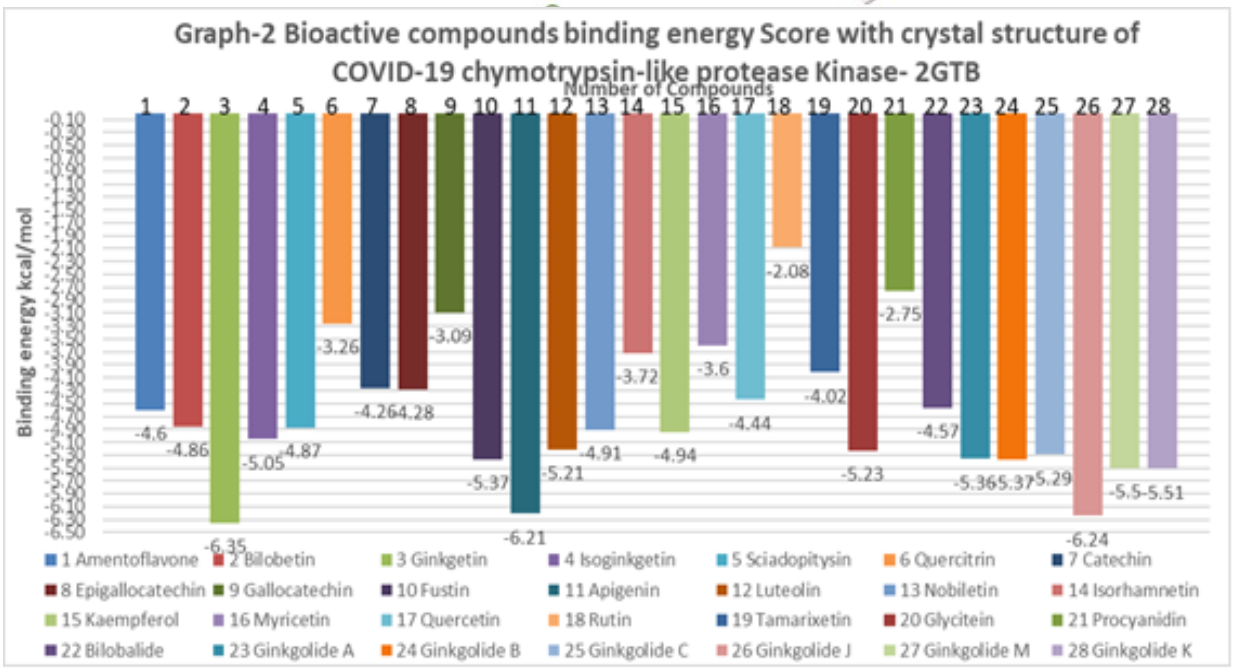
23	Ginkgolide A		Terpenic lactone	-5.36	-0.18	117.91	-6.25	-6.2	-0.05	-1.33	0.89	-1.33	17.73	MET276, ASN228, GLN244,
24	Ginkgolide B		Terpenic lactone	-5.37	-0.18	116.73	-6.56	-6.45	-0.11	-1.91	1.19	-1.91	17.08	ILE286, LEU287, MET276, PHR3, TRP207, GLY278, LEU271
25	Ginkgolide C		Terpenic lactone	-5.29	-0.17	133.23	-6.48	-6.28	-0.2	-2.8	1.19	-2.8	17.29	LEU271, GLY278, ILE286, LEU287, MET276, GLY278,
26	Ginkgolide J		Terpenic lactone	-6.24	-0.21	26.62	-6.84	-6.65	-0.19	-0.21	0.6	-0.21	17.19	LEU272, ILE286, ASP153, GLN110, TYR239 H, MET276, PHE181, GLY2, PHE3, ARG298
27	Ginkgolide M		Terpenic lactone	-5.5	-0.18	92.81	-6.69	-6.59	-0.11	9.26	1.19	9.26	16.92	MET276, TYR154, GLU240, GLN107, LYS97, TYR101
28	Ginkgolide K		Terpenic lactone	-5.51	-0.19	91.64	-6.4	-6.32	-0.09	-1.86	0.89	-1.86	17.3	ILE286, LEU287, MET276, PHE103, GLY278, ASP33, TYR101, ASN277

[a] B.E.= Binding Energy in kilocalorie per mole [b]L.E.=Ligand Efficiency [c] I.C.= Inhibition Constant (Ki) in uM (micromolar)
[d] I.E.= Intermolar Energy in kilocalorie per mole [e] vdW + Hbond + desolv Energy= vandervals hydrogen bond dissolve energy in kilocalorie per mole
[f] E. E= Electrostatic Energy [g] F.T.I.E. = Final Total Internal Energy

	[h] T.F.E.=Torsional Free Energy [i] U.S.E.= Unbound System's Energy [j]R.M.S.D= root-mean-square deviation in angstrom (Å)

Fig 2:- Molecular docking Interaction of COVID SARS chymotrypsin-like protease protein (PDB ID: 2GTB) with Ginkgolide-J.





Docking study show that selected compounds interact with COVID crystal structure of SARS chymotrypsin-like protease protein (PDB ID: 2GTB) via active residues like Active residues are intricate MET276, GLN127, ASP153, TYR154, TYR239, LYS102, ASN277, THR199, LEU271, ILE286, GLN19, ALA7, THR285, THR257, ASP216,

and LYS102. Here, it is interested to find that back bone residues like TYR154, ILE286, ASN277 also interacts with selected bioactive compounds through intermolecular hydrogen bond.

On the basis of binding score, chymotrypsin-like protease- 2GTB protein binding affinity of selected natural bioactive compounds is in following sequence: Ginkgetin > Ginkgolide J >Apigenin > Ginkgolide K > Ginkgolide M > Fustin > GinkgolideB > GinkgolideA > Ginkgolide C > Glycitein > Luteolin > Isoginkgetin > Kaempferol > Nobiletin > Sciadopitysin > Bilobetin > Amentoflavone > Bilobalide > Quercetin > Epigallocatechin > Catechin > Tamarixetin > Isorhamnetin > Myricetin > Quercitrin > Gallocatechin > Procyanidin > Rutin. If we show particular ginkgolide compounds sequences are: Ginkgolide J > Ginkgolide K > Ginkgolide M > GinkgolideB > GinkgolideA > Ginkgolide C > Bilobalide. This sequence is nearly same as observed for COVID-19 main protease Kinase- 6LU7. Because of COVID-19 main protease protein (PDB ID: 6LU7) 96% similarity with the COVID_19 crystal structure of SARS chymotrypsin-like protease protein (PDB ID: 2GTB) and just 4% vary from it63. So, it anticipated that selected bioactive compounds particularly Ginkgolides can inhibit replication of this virus strains.

Conclusions:-

Based on our results, it is concluded that 28 natural bioactive compounds namely Amentoflavone, Apigenin, Bilobalide, Bilobetin, Catechin, Epigallocatechin, Fustin, Gallocatechin, Ginkgetin, Ginkgolide A, Ginkgolide B, Ginkgolide C, Glycitein, Isoginkgetin, Isorhamnetin, Kaempferol, Luteolin, Myricetin, Nobiletin, Procyanidin, Quercetin, Quercitrin, Rutin, Sciadopitysin, Tamarixetin, Ginkgolide J, Ginkgolide M, and Ginkgolide K can effectively interact and bind with two protein strains of Covid-19 virus and can inhibit replication of these strains. As per the drug likeness rule study, Ginkgolides series is considered the best for inhibition treatment because these show least drug likeness rule violation. Ginkgolide series A, B, C, J, K, M, L show high affinity with COVID-19 main protease protein (PDB ID: 6LU7) and COVID-19 chymotrypsin-like protease protein (PDB ID: 2GTB) via hydrogen bonding, electrostatic and vanderwaals interactions These eight Ginkgolides can inhibit replication of 8 stains of Covid-19 virus. Also, Ginkgolides are found to be useful as antiviral drug for treating many symptomatic diseases like lung weakness, asthma, coughing, cancer, ischemic cardiovascular and cerebrovascular diseases and used as protective against the immune system, central nervous system, and ischemic injury. Use of herbal extracts is very common among masses for different reasons of ailments because these have less side effects. Herbal extracts containing Ginkgolides may be useful in controlling COVID-19 virus, detailed in vivo research is required.

Acknowledgements:-

The authors are thankful to Gujarat State Bio-Technology Mission, Gandhinagar for financial support in the form of Major Research Project FAP-1421 to carry out this research work.

1. World Health Organization. Director-General's remarks at the media briefing on 2019-nCoV on 11 February 2020. https://www.who.int/dg/speeches/detail/who-director-general-s-remarks-at-the-media-briefing-on-2019-ncov-on-11-february-2020.
2. Wu Z, McGoogan JM. Characteristics of and Important Lessons from the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases from the Chinese Center for Disease Control and Prevention. JAMA 2020.
3. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 2020; 579:270.
4. Gorbalenya AE, Baker SC, Baric RS, et al. Severe acute respiratory syndrome-related coronavirus: The species and its viruses – a statement of the Coronavirus Study Group. bioRxiv 2020. https://www.biorxiv.org/content/10.1101/2020.02.07.937862v1
5. Tang X, Wu C, Li X, et al. On the origin and continuing evolution of SARS-CoV-2. National Science Review 2020.
6. World Health Organization. Novel coronavirus situation reports -2. January 22, 2020. https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200122-sitrep-2-2019-ncov.pdf
7. Van Doremalen N, Bushmaker T, Morris DH, et al. Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1. N Engl J Med 2020.
8. Li Z, Yi Y, Luo X, et al. Development and Clinical Application of A Rapid IgM-IgG Combined Antibody Test for SARS-CoV-2 Infection Diagnosis. J Med Virol 2020.
9. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA 2020.

10. Carmine Ceraolo and Federico M Giorgi. Genomic Variance of the 2019-nCoV Coronavirus. J Med Virol. Feb 2020
11. Global Initiative on Sharing All Influenza Data (GISAID)
12. Lu et al. Genomic Characterization and Epidemiology of 2019 Novel Coronavirus: Implications for Virus Origins and Receptor Binding. Lancet. Jan 30, 2020
13. Tseng CT, et al. Immunization with SARS Coronavirus Vaccines Leads to Pulmonary Immunopathology on Challenge with the SARS Virus. PLOS ONE 7(8): 10.1371. 2020
14. Gaussian 16, Revision B.01, M. J. Frisch, G. W. Trucks, H. B. Schlegel, et al. Gaussian, Inc., Wallingford CT, 2016.
15. GaussView, Version 6, Roy Dennington, Todd A. Keith, and John M. Millam, Semichem Inc., Shawnee Mission, KS, 2016.
16. Michel F. Sanner. Python: A Programming Language for Software Integration and Development. J. Mol. Graphics Mod., 1999, Vol 17, February. pp57-61
17. Sanner, M.F., Spehner, J.-C., and Olson, A.J. Reduced surface: an efficient way to compute molecular surfaces. Biopolymers, 1996, Vol. 38, (3),305-320.
18. Bajaj, C, Park, S., Thane, A., A Parallel Multi-PC Volume Rendering System, ICES and CS Technical Report, University of Texas, 2002.
19. Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S. and Olson, A. J. Autodock4 and AutoDockTools4: automated docking with selective receptor flexiblity. J. Computational Chemistry 2009, 16: 2785-91
20. Bajaj, C, Pascucci, V., Schikore, D., Fast IsoContouring for Improved Interactivity, Proceedings of ACM Siggraph/IEEE Symposium on Volume Visualization, ACM Press, San Francisco, CA, 1996, 39 - 46,
21. Michel F. Sanner, Daniel Stoffler and Arthur J. Olson. ViPEr a Visual Programming Environment for Python. 10th International Python Conference, February 2002.
22. J. Racine, "The Cygwin tools: a GNU toolkit for Windows," Journal of Applied Econometrics, John Wiley & Sons, Ltd., 2000, vol. 15(3), 331-341.
23. Berman H. M., Westbrook J, Feng Z, et al. The protein data bank. Nucl Acids Res. 2000; 28,235-242.
24. Kim S, Chen J, Cheng T, Gindulyte A, He J, He S, Li Q, Shoemaker BA, Thiessen PA, Yu B, Zaslavsky L, Zhang J, Bolton EE. PubChem 2019 update: improved access to chemical data. Nucleic Acids Res. 2019 Jan 8;47(D1):D1102-D1109.
25. Iman K, Mirza MU, Mazhar N, Vanmeert M, Irshad I, Kamal MA. In silico Structure-based Identification of Novel Acetylcholinesterase Inhibitors Against Alzheimer's Disease. CNS Neurol Disord Drug Targets. 2018 Apr 26;17(1):54-68.
26. Lipinski, C.A., Lombardo, F., Dominy, B.W. and Feeney, P.J, Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv. Drug Delivery Rev., 2001; 46, 3-26.
27. Congreve M, Carr R, Murray C, Jhoti H., A 'rule of three' for fragment-based lead discovery?". Drug Discov. Today. 2003; 8 (19): 876–7.
28. Ricardo Macarrón & Juan I Luengo, Yin and Yang in medicinal chemistry: what does drug-likeness mean?, Future Medicinal Chemistry, 2011;vol. 3, no. 5
29. Morris GM, Goodsell DS, Halliday RS, et al. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. J Comput. Chem., 1998; 19,1639–1662.
30. Pettersen EF, Goddard TD, Huang CC, et al. UCSF Chimera a visualization system for exploratory research and analysis. J Comput Chem, 2004; 25,1605-1612.
31. Liu, X., Zhang, B., Jin, Z., Yang, H., Rao, Z., The crystal structure of COVID-19 main protease in complex with an inhibitor N3,2020
32. National Center for Biotechnology Information. PubChem Database. Amentoflavone, CID=5281600, https://pubchem.ncbi.nlm.nih.gov/compound/Amentoflavone
33. National Center for Biotechnology Information. PubChem Database. Ginkgetin, CID=5271805, https://pubchem.ncbi.nlm.nih.gov/compound/Ginkgetin
34. National Center for Biotechnology Information. PubChem Database. Isoginkgetin, CID=5318569, https://pubchem.ncbi.nlm.nih.gov/compound/Isoginkgetin
35. National Center for Biotechnology Information. PubChem Database. Sciadopitysin, CID=5281696, https://pubchem.ncbi.nlm.nih.gov/compound/Sciadopitysin
36. National Center for Biotechnology Information. PubChem Database. Apigenin, CID=5280443, https://pubchem.ncbi.nlm.nih.gov/compound/Apigenin

37. National Center for Biotechnology Information. PubChem Database. Luteolin, CID=5280445, https://pubchem.ncbi.nlm.nih.gov/compound/Luteolin
38. National Center for Biotechnology Information. PubChem Database. Myricetin, CID=5281672, https://pubchem.ncbi.nlm.nih.gov/compound/Myricetin
39. National Center for Biotechnology Information. PubChem Database. Nobiletin, CID=72344, https://pubchem.ncbi.nlm.nih.gov/compound/Nobiletin
40. National Center for Biotechnology Information. PubChem Database. Quercitrin, CID=5280459, https://pubchem.ncbi.nlm.nih.gov/compound/Quercitrin
41. National Center for Biotechnology Information. PubChem Database. Quercetin, CID=5280343, https://pubchem.ncbi.nlm.nih.gov/compound/Quercetin
42. National Center for Biotechnology Information. PubChem Database. Rutin, CID=5280805, https://pubchem.ncbi.nlm.nih.gov/compound/Rutin
43. National Center for Biotechnology Information. PubChem Database. Tamarixetin, CID=5281699, https://pubchem.ncbi.nlm.nih.gov/compound/Tamarixetin
44. National Center for Biotechnology Information. PubChem Database. Isorhamnetin, CID=5281654, https://pubchem.ncbi.nlm.nih.gov/compound/Isorhamnetin
45. National Center for Biotechnology Information. PubChem Database. Bilobetin, CID=5315459, https://pubchem.ncbi.nlm.nih.gov/compound/Bilobetin
46. National Center for Biotechnology Information. PubChem Database. (-)-Catechin, CID=73160, https://pubchem.ncbi.nlm.nih.gov/compound/Catechin
47. National Center for Biotechnology Information. PubChem Database. (-)-Epigallocatechin gallate, CID=65064, https://pubchem.ncbi.nlm.nih.gov/compound/Epigallocatechin-gallate
48. National Center for Biotechnology Information. PubChem Database. (-)-Gallocatechin, CID=9882981, https://pubchem.ncbi.nlm.nih.gov/compound/(-)-Gallocatechin
49. National Center for Biotechnology Information. PubChem Database. Bilobalide, CID=73581, https://pubchem.ncbi.nlm.nih.gov/compound/Bilobalide
50. National Center for Biotechnology Information. PubChem Database. Ginkgolide A, CID=441293, https://pubchem.ncbi.nlm.nih.gov/compound/Ginkgolide-A
51. National Center for Biotechnology Information. PubChem Database. Ginkgolide-B, CID=6324617, https://pubchem.ncbi.nlm.nih.gov/compound/Ginkgolide-B
52. National Center for Biotechnology Information. PubChem Database. Ginkgolide-C, CID=9867869, https://pubchem.ncbi.nlm.nih.gov/compound/Ginkgolide-C
53. National Center for Biotechnology Information. PubChem Database. Ginkgolide-M, CID=46937025, https://pubchem.ncbi.nlm.nih.gov/compound/Ginkgolide-M
54. National Center for Biotechnology Information. PubChem Database. Ginkgolide J, CID=441296, https://pubchem.ncbi.nlm.nih.gov/compound/Ginkgolide-J
55. National Center for Biotechnology Information. PubChem Database. Ginkgolide K, CID=101553595, https://pubchem.ncbi.nlm.nih.gov/compound/Ginkgolide-K
56. National Center for Biotechnology Information. PubChem Database. Fustin, CID=5317435, https://pubchem.ncbi.nlm.nih.gov/compound/Fustin
57. National Center for Biotechnology Information. PubChem Database. Kaempferol, CID=5280863, https://pubchem.ncbi.nlm.nih.gov/compound/Kaempferol
58. National Center for Biotechnology Information. PubChem Database. Glycitein, CID=5317750, https://pubchem.ncbi.nlm.nih.gov/compound/Glycitein
59. Nakanishi, K.O.J.I. The ginkgolides. Pure Appl. Chem. 1967, 14, 89–114
60. Z. Xu, C. Peng, Y. Shi, Z. Zhu, K. Mu, and X. Wang, "Nelfinavir was predicted to be a potential inhibitor of 2019-nCov main protease by an integrative approach combining homology modelling, molecular docking and binding free energy calculation," vol. 1201, pp. 0–2, 2020
61. G. S. B, L. Xavier, and S. Michael, "Molecular Docking Studies on Antiviral Drugs for SARS," vol. 5, no. 3, pp. 75–79, 2015.
62. Lee, T.-W., Cherney, M.M., Huitema, C., Liu, J., James, K.E., Powers, J.C., Crystal structure of SARS coronavirus main peptidase (with an additional Ala at the N-terminus of each protomer) inhibited by an aza-peptide epoxide in the space group P43212,2006
63. A. Zhavoronkov et al., "Potential 2019-nCoV 3C-like Protease Inhibitors Designed Using Generative Deep Learning Approaches," no. February, 2020, doi: 10.26434/CHEMRXIV. 11829102.V1.