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A Druggability study to Probe immunomodulatory Unani plants as active Inhibitors of Pseudotyped Particle Cell Entry

Research Article

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Abstract

Medicinal herbs, plants and their products show revolutionized effect in modern therapeutic system. Industries used their activity as antioxidant, antitoxin, antimicrobial, immune booster and many others and use them accordingly as the prime agent to produce cosmetics, food, drug synthesis etc. Unani is one of the traditional therapeutic systems which use medicinal herbs mostly for making their herbal medicines. There are several immunomodulatory herbs along with their phytochemicals are present to serve their best. Bioassay is the method of analysis to find out the potency or concentration of any substance when effect on living things. Sars Cov-2 virus and its fatal outbreak is a real threat to modern society. To perform in vitro procedure to screened out small molecule inhibitors of this malicious pseudo typed particle cell entry, Dr. Whittaker's group use this bioassay system like PubChem AID: 1479144, PubChem AID: 1479149, PubChem AID: 1479150 and PubChem AID: 1494158. This in silico study is to show the potency of the phytochemicals of traditional herbs of Unani, as if inhibitors by means of machine learning with the help of WEKA. The ADMET study and molecular docking with Mpro (6lu7 chain A) is also done to test the druggability and binding affinity of the qualified phytochemicals.

Keywords: Druggability, Bioassay, Immunomodulatory, Molecular docking, PubChem AID, Unani, ADMET, WEKA.

Introduction

In the present day scenario, the viruses are taking responsibility for a huge number of contagious and hard-to-cure diseases in the human body. Current and emerging forms of all these horrible human pathogenesis can cause a severe threat to modern healthcare, particularly when unavailable of preventive antivirals therapies or vaccines (1).

Covid-19 is a world pandemic, it affects more than 24 million world population. Almost 83 lakh people heard the troll and 16 million recovered. It sparked in Wuhan city in China (2) and engulfed all the countries around the world very quickly. Many countries suffered huge fatalities, according to a survey held in the last week of March 2020 in the USA, 72% of Americans felt that they were impacted by this highly infectious virus (3). In India, the socio-economic stratum (4) faced great challenges. Many vaccines and medicines are trying to stop its empowerment with their best but no one can succeed to stop its lash of virulent effects yet (5).

Covid-19 which is referred to as SARS–Cov2 (Severe Acute Respiratory System Corona Virus) belongs from the order Nidovirales, Coronaviridae family, and under betacoronavirus genera (6). These Coronaviruses exist for 10,000 years in birds and bats etc. as their natural hosts (7). Out of its many species, seven coronaviruses are there which infects people referred to as HCov among them 229E, NL63, OC43, HKU1 (8) are common coronaviruses, and SARS-Cov, MERS-Cov, and SARS-Cov-2 are caused fatal situations. SARS-Cov spread pandemic in around 2003 and MERS-Cov in around 2012 (9) before SARS-Cov-2. To fight against this virus pandemic the body's immune system provides a good shield. To build this shelter immunomodulatory holistic and less side effect treatment is required in the present day.

Alternate medicine of the Indian system of therapy has a significant signature from ancient times. It is traditional, widely studied by experience and practice. In India Ministry of AYUSH (Ayurveda, Yoga and Naturopathy, Unani, Siddha, and Homeopathy) has many wings of drugs that are developed on the base of herbs and trees. Many of these plants have a good immunomodulatory effect which has traditional use. This therapeutic system is found by traditional experience and practice. The herbs used in this system of therapy are available in India and the sub-continent. Many of us consume these herbs as spices and dietary elements.

It makes astonished when great affluent countries with their much modern healthcare enrichments have been much affected by COVID 19 and have seen much

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mortality rates, in comparison to the less affluent Southeast Asian nations and India (10).

Unani is a genre of AYUSH, it was introduced in India during the Mughal Empire. Nurtured in a combination of Greek-Arabic methods derived from the teachings of Hippocrates, this therapeutic method contains antiviral, immunomodulatory, and herbal remedies for various respiratory and infectious diseases. Phytochemicals are the biological compound of a plant that helps in flowering, budding, medicinal, toxicity, excretion. Secondary metabolic phytochemicals (11) are here for their pharmacological abilities.

This work is to find out best phytochemicals from the secondary metabolites of immunomodulatory medicinal plants which are recommended by AYUSH's Unani wing, with the help of computational data mining.

Materials and Methods

Selection of Unani Immunomodulatory Plants and Phytochemicals: (12)

Through a careful study of the literature, 25 medicinal plants acting as immunomodulators following the Unani therapeutic method were selected and enlisted in table 1 with its Name, Unani name, scientific name, family, parts, Therapeutic potentials, and used in Unani medicines. The secondary metabolites were searched out with the help of Dr. Duke database, from these herbs and enlisted in supplementary table 1 along with its structure, PubChem CID, and Canonical Smiles.

Figure 1: Study trail details

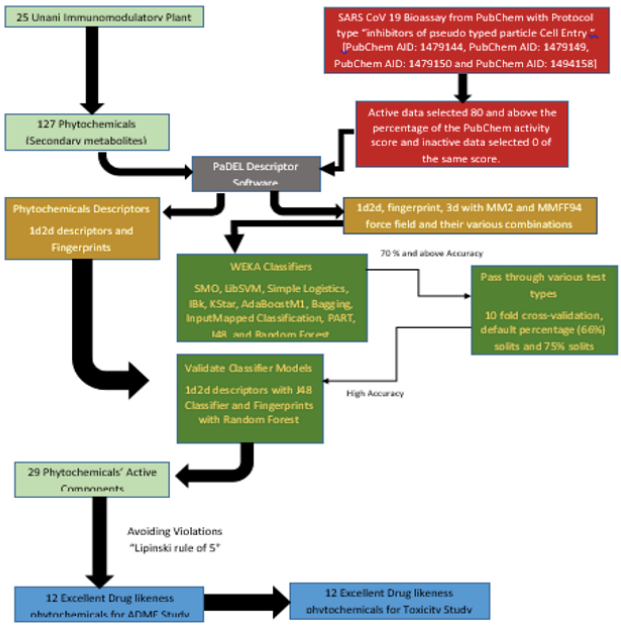


Table 1: List of Unani immunomodulatory Plants with their Unani name, Scientific name, Family, Parts used, Therapeutic potential and use in Unani Medicines

Sl. No	Name	Unani Name	Scientific Name	Family	Part Uses	Therapeutic Potencial	Unani Medicine use
1	Ashwagandha	Asgand	Withania somnifera (L.) Dunal	Solanaceae	Root	adaptogenic, antibiotic, aboritifacient, aphrodisiac, astringent, anti-inflammatory, diuretic and sedative properties, antioxidant protection and stimulates the activation of immune system	For strength, vigor and rejuvenation. adaptogenic, antiarthritic, antispasmodic, antiinflammatory, nervine tonic, nerve soothing, antioxidant,immunomodulator, free radical scavenger, anti-stress and anticancer agent
2	Neem	Nim	Azadirachta indicia .Juss.	Meliaceae	Neem oil , Flowers, leaves, seeds	antiplasmodial,antitrypanosomal, antioxidant, anticancer, antibacterial, antifungal, antiviral, nematicidal, antiulcer, spermicidal, anthelminthic, antidiabetic, anti-implantation, as well as immunomodulating, contraceptive, molluscidal, insecticidal, antifeedant, insect repellant	Effect on male fertility, female fertility, Antioxidant, hepatoprotective and cancer Chemopreventive, Insecticidal, Antidiabetic .
3	Grit Kumari	Elwa	Aloe vera (L.) Burm.f.	Asphodelaceae	Leaves	Asthma, Cardiac Activity, Haemodynamic Activity, Laxative Effect, Immunomodulatory Effect, Antifungal Activity, Antioxidant Activity, Anti-inflammatory Activity, Antidiabetic Effect	Qabz (constipation), Zofe Ishteha (Loss of appetite), Qarhae Medi (peptic ulcers), irritable bowel syndrome, Qaulanj (Colitis) as well as, Zeequn Nafas (asthma), Ziabetus Shakri (diabetes), Sartan (Cancer) and Taqwiyate Manat (enhancement of the immune system), and externally for eczema, dermatitis, sunburn
4	India Madder	Fowah	Rubia cordifolia Hochst. exA.Rich.	Rubiaceae	Leaves	heating, alexiteric, antidysenteric, antipyretic, analgesic, anthelmintic	Cough, freckles of skin, hepatic obstruction, indigestion, inflamed parts, ulcers and fractures, mental agony, obstructions in the urinary passage and paralytic affections

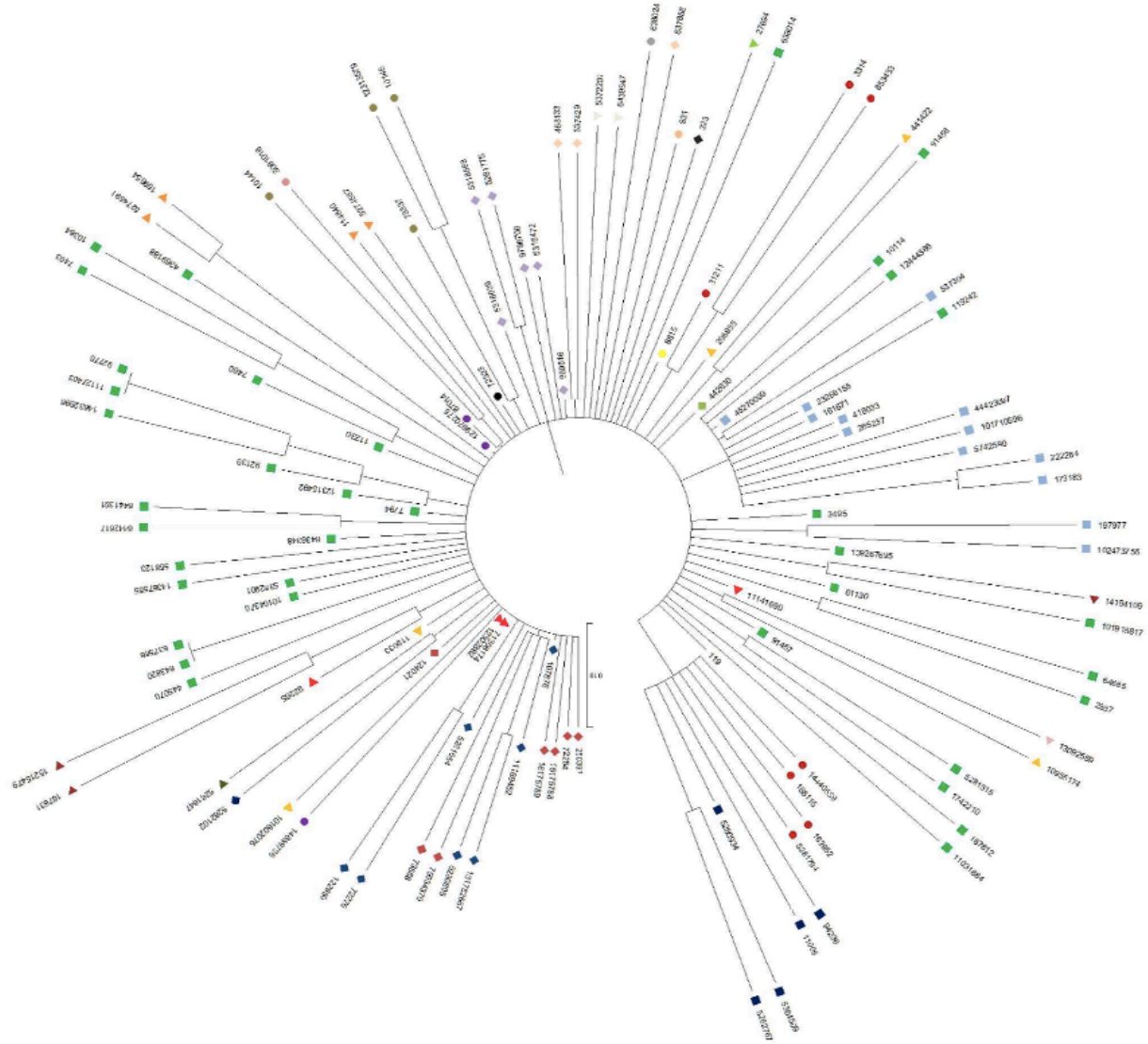
5	Black Cumin	Kalonji	Nigella sativa L.	Ranunculaceae	Seeds	antibacterial, antifungal, anti-inflammatory, antitumor, antitussive, galactogogue and immunomodulatory	Retention of Urine, Amenorrhoea, Agalactorrhoea, Flatulence, Weakness of Stomach, Cough, Asthma and in Cold & Coryza as medicated steam inhalation
6	China Rose	Gudhal	Hibiscus rosa-sinensis L.	Malvaceae	Flower	Antioxidant activity, antibacterial activity, antidiabetic effect , Hepatoprotective effect , Ameliorative effect Cardioprotective effects, Renal function, Antifertility effect	Epilepsy, leprosy, bronchial catarrh and diabetes aphrodisiac, menorrhagic, oral contraceptive, laxative
7	Turmeric	Haldi	Curcuma longa L	Zingiberaceae	Rhizome	Anti-inflammatory activity, skin diseases, Central nervous system diseases, eye diseases	Inflammation, skin, eye, central nervous system, respiratory, cardiovascular, gastrointestinal, urogenital and metabolic disorders
8	Indian Banyan	Bargad	Ficus benghalensis L.	Moraceae	Whole plant	Anti-inflammatory Activity, Anti-stress and Anti-allergic, Anti-microbial Activity,Antifungal, Immunomodulatory Activity,	Anti-tumour, Insulin raising effect, asthma, chloroform extract, Wound Healing,
9	Safed Musli	Musli safed	Chlorophytum borivilianum Santa pau&R.R.Fern.	Asparagaceae	Root	Sexual stimulant activity, Aphrodisiac activity,Spermatogenic activity, Anti hypertensive activity	It is used in sterility as well as in oligospermia to increase sperm count. It is also used in premature ejaculation. It is beneficial in paralysis, tetanus, bell's palsy and stomatitis, besides it is helpful in increasing body weight
10	Shatavari	Satavar	Asparagus racemosus Willd.	Asparagaceae	Root	Antioxidant activity, Anti-diarrhoeal effects, Antilithiatic effect, Anticancer, Antioxitocin, Antiinflammatory activity, Antidiabetic, Insulin secretory activity, Antiulcer, Antimicrobial activity, Immunomodulators	Respiratory system, neurodegenerative disorders, CNS ACTIVITY cancer, ulcer, diabetes, heart diseases, male and female infertility and postmenopausal syndrome
11	White Water Lily	Neelofer	Nelumbo nucifera Gaertn	Nelumbonaceae	Rhizome, seeds	Anti-ischaemic activity, antioxidant activity, hepatoprotective activity, anti-inflammatory activity, anti-fertility activity, anti-arrhythmic activity, anti-fibrosis activity, antiviral activity, antiproliferative activity, antidiarrhoeal activity, psychopharmacological activity, diuretic activity, antioxidant activity, antipyretic activity, immunomodulatory activity, hypoglycaemic activity, aldose reductase inhibitory activity, antibacterial, aphrodisiac activity, antiplatellet activity, cardiovascular activity, anti-obesity activity, lipolytic activity, hypocholesterolaemic activity, hepatoprotective activity, anticancer activity	Strangury, vomiting, leprosy, skin disease and nervous exhaustion demulcents for haemorrhoids and are beneficial in dysentery, chronic dyspepsia, and have nutritive, diuretic and cholagogue activities
12	Mulethi	Asluss us	Glycyrrhiza glabra Torr.	Fabaceae	Bark and root	Anti-tussive & expectorant activity, Antioxidant activity, Skin lightening and skin tightening activity, Anti-inflammatory activity, Anti-viral effects, Anti-bacterial Activity, Anti-malarial activity, Anti-viral effects, Immunostimulatory effects, Memory enhancing activity,	Sorethroat, cough and bronchial catarrh, antiseptic

13	Indian long pepper (Pipli)	Filfil daraz	Piper longum Blume	Piperaceae	Fruit and leaves	Immunomodulatory, Antiasthmatic, Anti-inflammatory, Antiamoebic, Antibacterial	Laxative, antidiarrhoeic, antidysentric, asthma,bronchitis,abdominal complaints,fever,leprosy,insomnia, jaundice, hiccoughs, tubercular glands,
14	Haritaki	Halela	Terminalia chebula Willd.	Combretaceae	Fruits	Antibacterial activity, Antifungal activity, Antiamoebic and immunomodulatory activities, Antimutagenic and anticarcinogenic activities, Antioxidant activity, Antiviral activity, Antidiabetic and retinoprotective activities, Antianaphylactic and adaptogenic activities, Antiulcerogenic activity, Radioprotective activity, Cardioprotective activity, Hepatoprotective activity, Chemomopreventive activity, Hypolipidemic and hypocholesterolemic activities, Antispermatogenic activity	Antimicrobial, antioxidant, antihyperglycemic, anticancer and protective effects on various vital organs such as nerves, heart, kidney and liver
15	Papaya	Papita	Carica papaya L.	Caricaceae	Leaves, seeds	Antimicrobial activity, Anthelmintic activity, Antimalaria, Antifungal activity, Antri-amoebic, Antifertility activity, Immunomodulatory activity,	Carminative, emmenagogue, vermifuge, abortifacient, treatment of ringworm and psoriasis, anti-fertility agent in males,bleeding piles and enlarged liver and spleen Jaundice,urinary complaints and gonorrhoes(infusion), dressing wounds, anti-bacterial, vermifuge, in colic, fever, beriberi, abortion(infusion), asthma(smoke)
16	Amla	Amla	Emblica officinalis Gaertn.	Phyllanthaceae	Fruit	Antioxidant, Hepatoprotective, Hypolipidemic, Nephroprotective, Metabolic Syndrome, Cardioprotective, Immunostimulant, Immunostimulant, Antimicrobial	Longevity, improve digestion, constipation, fever, cleanses the blood, decreases cough, eases asthma, strengthens the heart, benefits the eyes, encourages hair growth, invigorates the body, and augments the intellect, dyspepsia, gastritis, hyperacidity, constipation, colitis, hemorrhoids, hematuria, menorrhagia, anemia, diabetes, cough, asthma, osteoporosis, premature graying of hair, weakness and fatigue
17	Giloy	Gilo	Tinospora cordifolia (Willd.) Miers ex Hook.f. & Thomson	Menispermaceae	Stem & root	Anti-diabetic activity, anti-periodic activity, anti-spasmodic activity, anti-inflammatory activity, anti-arthritic activity, anti-oxidant activity, anti-allergic activity, anti-stress activity, anti-leprotic activity, anti-malarial activity, hepatoprotective activity, immunomodulatory activity and anti-neoplastic activity.	Induced anemia, Boosting of phagocytic, ability of macrophages influence the cytokine production, mitogenicity, stimulation and activation of I5.mmune effecter cells antibacterial activity against urinary pathogens was found to increase in foot pad thickness Significant increase in the WBC counts and bone marrow cells reveals stimulatory effect on haeomopoetic system eosinophil count and increase hemoglobin percentage in HIV/AIDS patients, anti hiv and anti cancer
18	Broad Leaf Plantain	Bartang	Plantago major Bert.	Plantaginaceae	Seeds		

19	Chamomile	Babboona	Matricaria chamomilla Blanco	Asteraaceae	Flowers	Cardiovascular effects, Anti spasmodic, Anti ulcer, Choleretic, Anxiolytic, Sedative, Uterine Tonic, Anti-inflammatory, Anti allergic, Anti bacterial,Anti fungal, Anti viral, Anti neoplastic, Anti oxidant	Arthritis, Ear ache, Episodic fevers, Indigestion, Ammenorrhoea, Inflammation, Neurological disorders, Stomachic, Jaundice
20	Aam	Aam	Mangifera indica Thwaites, Enum.	Anacardiaceae	Fruits	Antioxidant, Antibacterial, Hepatoprotective, anticancer, Immunomodulator, Anti asthmatic, Anti-inflammatory, Antihyperglycemic, Analgesic, Anthelminthic	Help to prevent colon cancer, Calming inflammation, Fruit Juice is act as a restorative tonic used in heat strok
21	Tulsi	Rehan	Ocimum sanctum L.	Lamiaceae	Leaf	Analgesic Activity, Antiasthmatic Activity, Antibacterial Activity, Anticancer Activity, Anticataleptic Activity, Antiemetic Activity, Antifertility activity, Antifungal Activity, Anti-helminthic Activity, Antihyperlipidemic Activity, Antiinflammatory Activity, Antioxidant Activity, Antiplasmodial Activity, Anti-spasmodic Activity, Antistress Activity, Antithyroidic Activity, Antianxiety Activity, Antidepressant Activity, Demulcent/Stimulant/ expectorant., Eye Disease, Hepatoprotective Activity, Immunomodulatory Activity, Memory Enhancer Activity, Neuroprotective Activity, Radio-protective Activity	Antidiabetic, antioxidant, antistress, antihyperlipidemic and antibacterial, neuropsychological disorders, Eye Disease, Neuro, Memory Enhancer
22	Ginger	Zanjabeel	Zingiber officinale Roscoe	Zingiberaceae	Rhizome	Antioxidant activity, Anticancer, antimutagenic, Antimicrobial activity	Flatulence, nausea, indigestion, intestinal infections and certain types of food poisoning, high blood pressure, inhibits platelet aggregation, motion and morning sickness, against vomiting associated with pregnancy, arthritis
23	Khayer	Kath	Acacia catechu (L.f.) Willd.	Fabaceae	Bark	Anti-bacterial activity, Anti mycotic activity, Anti-oxidant activity, Anti-diarrhoeal activity, Immuno modulatory activity, Antisecretory and Antiulcer activity, Anti-pyretic activity Anti-diarrhoeal activity	Spongy Gums, Bleeding Gums, Stomatitis, Leucoderma, Urinary Disorder, Diabetes, Leprosy, Psoriasis, Syphilis
24	Darchini	Darchini	Cinnamomum zeylanicum Blume	Lauraceae	Bark	Anti diabetic, Anti oxidant, anti cancerous activity, Anti parasitic activity, Anti microbial activity	It is used in stomach and cardiac disorder like palpitation, hiccups, indigestion and flatulence. It is also used as tonic for stomach and liver. Further it finds its use as an antidote as well as sexual tonic.
25	Bahera	Balela	Terminalia bellirica (Gaertn.) Roxb.	Combretaceae	Fruits	Analgesic activity, Anti diarrhoeal activity, Antihypertensive Effect, Anti salmonella activity, Anti-Spasmodic and Bronchodilatory Properties, Anti-microbial activity, Antimicrobial and Toxicity Studies, Antioxidant activity, Wound healing activity, Acute and Sub-acute Toxicities, Hepatoprotective activity, Immune, Antimutagenic Activity,	Hepatitis, bronchitis, asthma, dyspepsia, piles, diarrhoea, coughs, hoarseness of voice, eye diseases and scorpion-sting; used as a hair tonic

ClassyFire (online software) was used here for chemical nature of those secondary metabolites and a dendrogram was designed by Mega X for their representation. (Figure 2).

Figure 2: Circular Dendrogram of Chemical Classification of the listed Phytochemicals



Benzenoids

- Phenols
- Anthracenes
- Naphthalenes
- Phenol ethers

Lipids and lipid-like molecules

- Prenol lipids
- Steroids and steroid derivatives
- Fatty Acyls
- Lactones
- Benzodioxoles
- Naphthopyrans
- Tetrahydroisoquinolines

Phenylpropanoids and polyketides

- Cinnamic acids and derivatives
- Coumarins and derivatives

Organic oxygen compounds

- Organooxygen compounds

Organic acids and derivatives

- Carboxylic acids and derivatives

Organoheterocyclic compounds

- Isoquinolines and derivatives
- Benzopyrans
- Oxanes

Alkaloids and derivatives

- Aporphines
- Aristolactams
- Protoberberine alkaloids and derivatives
- Blanks

Phenylpropanoids and polyketides

- Tannins
- Diarylheptanoids
- Flavonoids
- Macrolides and analogues

Exploration of Descriptors from SARS-CoV 19 bioassay

4 datasets [PubChem AID: 1479144, PubChem AID: 1479149, PubChem AID: 1479150 and PubChem AID: 1494158] were selected from PubChem Bioassay (13) and a bioassay table (Supplementary table 3) was created. The unspecified and inconclusive compounds were discarded from this table of active-inactive bioassay because they show uncertainty in their biological activities. All the active tabulated data, were based on 80 and above the percentage of the PubChem activity score and inactive tabulated data were selected based on 0 of the PubChem activity score. As a whole total of 110 tabulated records were tabulated as Active and Inactive elements. PaDEL- Descriptor software was used with this table, to generate descriptors with these active and inactive elements.

Using PaDEL – Descriptor software various descriptors and their combinations were generated (1d2d, fingerprint, 3d with MM2 and MMFF94 force field and their various combinations) to clarify the suitable training data set using Weka software.

Machine Learning Model designed by WEKA

Suitable Model selection

In this stage, various combinations of the PaDEL Descriptors were processed and compared by all well-known Classifiers of WEKA (SMO, LibSVM, Simple Logistics, IBk, KStar, AdaBoostM1, Bagging, InputMapped Classification, PART, J48, and Random Forest). Based on their accuracy percentage (70 percent and higher) the suitable (Figure 3 and Table 2) descriptors along with their classifier methods were selected.

Validation of suitable model

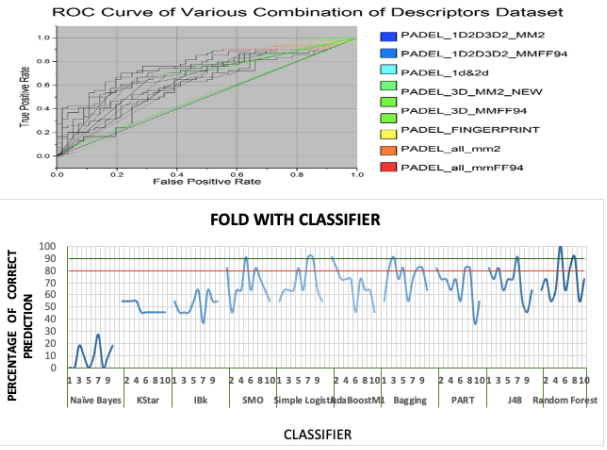
Multiple suitable classifiers along with their descriptors were selected and were performed multiple test options like 10 fold cross-validation, default percentage (66%) splits and 75% splits to validate the trusted model. After a vivid observation of the accuracy percentage of datasets, the best results were selected to validate the trusted model (Random Forest and J48, two methods of the decision tree, were considered as the deserving classifier here.

Exploration of Active Phytochemicals Components

The phytochemicals descriptors were generated by the PaDEL- Descriptor software, according to the descriptor used in the trusted model in Weka. Trusted models were applied to phytochemical descriptors to select the active phytochemical components.

Different descriptor combinations were created from table 2 active-inactive bioassay of SARS Cov 19. (Such as 1d2d, fingerprint, 3d with MM2 and MMFF94 force field, 1d2d+fingerprint, 1d2d+ fingerprint+ 3d(mm2 force field), 1d2d+fingerprint+3d(mmff94 force field),all_mm2 (1d2d+3d with mm2 force field), all _mmff94 (1d2d+3d with mmff94 force field), fingerprint+3d(mm2 force field), fingerprint+3d(mmff94 force field)). Well-known classifier were selected from Weka software and applied on all descriptors for a cumulative study. 3d with MM2 and MMFF94 force field were not in the table 4 because of their inconclusive prediction. Weka Test type options was 10fold cross validation.

Figure 3: ROC Curve of Various Combinations of Descriptors and Accuracy of different Classifications (Percentage of Correct prediction of 10 Fold Cross Validation)



From this upper stated study four descriptors were selected for the Test and the type of test were 10 fold cross-validation, default percentage (66%) splits and 75% splits to validate the trusted model on the basis of accuracy percentage. According to upper stated study 1d2d, Fingerprint,All_MM2 (1d2d+3d with mm2 force field) and all _mmff94 (1d2d+3d with mmff94 force field) were selected as most suitable descriptors depending on the accuracy rate and J48, Random Forest, SMO and Simple Logistic were selected as suitable WEKA classifier. For choosing trusted model prediction test all suitable classifier applied on all suitable descriptors with test type 10 fold cross-validation, default percentage (66%) splits and 75% splits. According to Heat map of Figure 4 (a), the trusted model is selected

Fingerprint descriptor with J48 classifier, 1d2d descriptor with Random Forest and all_mm2 descriptor in SMO classifier environment were choose as trusted model. Though all_mm2 descriptor showed better performance according to the Figure 4(b) but its active instance were very lower then 1d2d and Fingerprint descriptor models. Therefore fingerprint with J48 classifier and 1d2d with Random Forest Classifier model were selected for active phytochemicals prediction.

Smiles of 127 phytochemicals (secondary metabolites) were applied in PaDEL Descriptor software for generated fingerprint and 1d2d descriptors of the phytochemicals. Those stated descriptors were applied with the trusted models for prediction of active phytochemicals. After a cumulative study 29 active phytochemicals were found as active and applied for druggability study (14,15,16).

10 fold Cross Validation

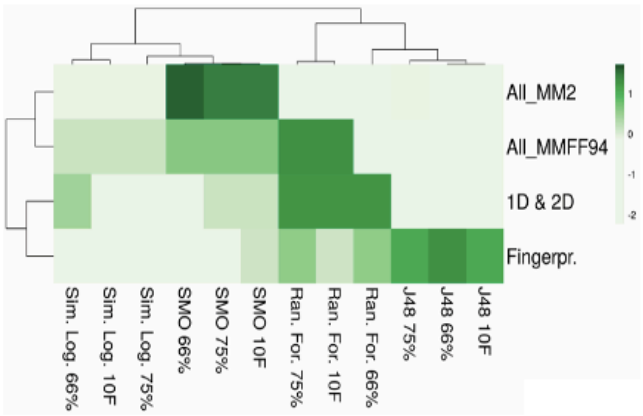
Table 2: Cumulative Study of SARS-Cov 19 descriptors with well-known Classifier of Weka software

Dataset	SMO	LibSVM	Simple Logistic	IBk	KStar	AdaBoostM1	Bagging	Input Mapped	PART	J48	Random Forest
PADEL_1D2D3D2_MM2	66.55	50.91	69	50.82	49.09	61.82	63.82	50.91	68.27	69.55	68.55
PADEL_1D2D3D2_MMFF94	67.27	50.91	69	52.18	49.09	60.18	63.82	50.91	68.45	70.36	68.09
PADEL_1d&2d	67.18	50.91	67.09	68.36	49.09	63.73	62.36	50.91	69.18	70.64	69
PADEL_3D_MM2_NEW	50.91	50.73	45.73	50.91	50.91	50.91	49.36	50.91	50.91	50.91	50
PADEL_3D_MMFF94	49.55	54.18	54.55	49.18	45.45	48.18	51.45	50.91	50.91	50.91	50.64
PADEL_FINGERPRINT	68.27	56.09	62.82	64.73	66.27	59.27	65.64	50.91	64.91	64.64	70.18
PADEL_all_mm2	70.91	50.91	71.73	57	49.09	61.27	62.73	50.91	63.82	65.82	69.73
PADEL_all_mmFF94	70.27	50.91	69.55	55.18	49.09	61.91	61.82	50.91	65.45	65.18	69.64

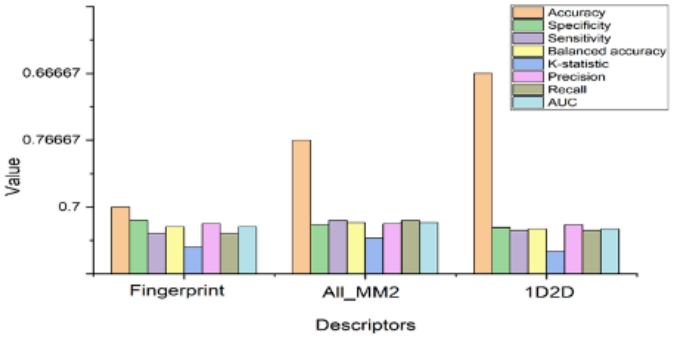
Tester: weka.experiment.PairedCorrectedTTester -G 3,4,5 -D 1 -R 2 -S 0.05 -result-matrix "weka.experiment.ResultMatrixPlainText -mean-prec 2 -stddev-prec 2 -col-name-width 0 -row-name-width 25 -mean-width 2 -stddev-width 2 -sig-width 1 -count-width 5 -print-col-names -print-row-names -enum-col-names"
Analysing: Percent correct
Datasets: 8
Result sets: 11
Confidence: 0.05 (two tailed)
Sorted by: -
Date: 8/29/21, 12:53 AM

Database	Contain (Force Field)
PADEL_1D2D3D2_MM2	1D and 2D and 3D with MM2 force field
PADEL_1D2D3D2_MMFF94	1D and 2D and 3D with MMFF94 force field
PADEL_1d&2d	1D and 2D
PADEL_3D_MM2_NEW	3D with MM2 force field
PADEL_3D_MMFF94	3D with MMFF94 force field
PADEL_FINGERPRINT	Fingerprint
PADEL_all_mm2	1D and 2D and 3D with MM2 force field an
PADEL_all_mmFF94	1D and 2D and 3D with MMFF94 force field

Figure 4: (a)Heat Map basis on accuracy percentage (Test type 10 FOLD Cross Validation AND 75% splits and 66% (default) splits and (b)Comparison between 10 Fold Cross validations of selected suitable active Descriptors



(a)



(b)

Drug-Likeness Assessment and ADME prediction Avoiding Violations

After machine learning, 29 phytochemicals were selected as Active Phytochemicals components according to SARS- CoV 2 bioassay and the basis of molecular weight (MW), lipophilicity (log P), hydrogen bond recipients (HBA), and phytochemical hydrogen bond donors (HBD) was determined and brought under the SWISS ADMET for filtration of the rules of "Lipinski rule of 5.

In this study, Lipinski's 5 rules were applied only for filtration purposes. Other transgressions from Table such as Ghose, Weber, Egan and Mugge were not applied here, thus yielding 12 top phytochemicals.

ADME Prediction

ADME is an essential phase of drug discovery as it helps to find out the effective and eventually safe to discover optimal necessary balance of properties for discovery.

Table 3: Filtration Table of Drug likeness of 29 active phytochemical

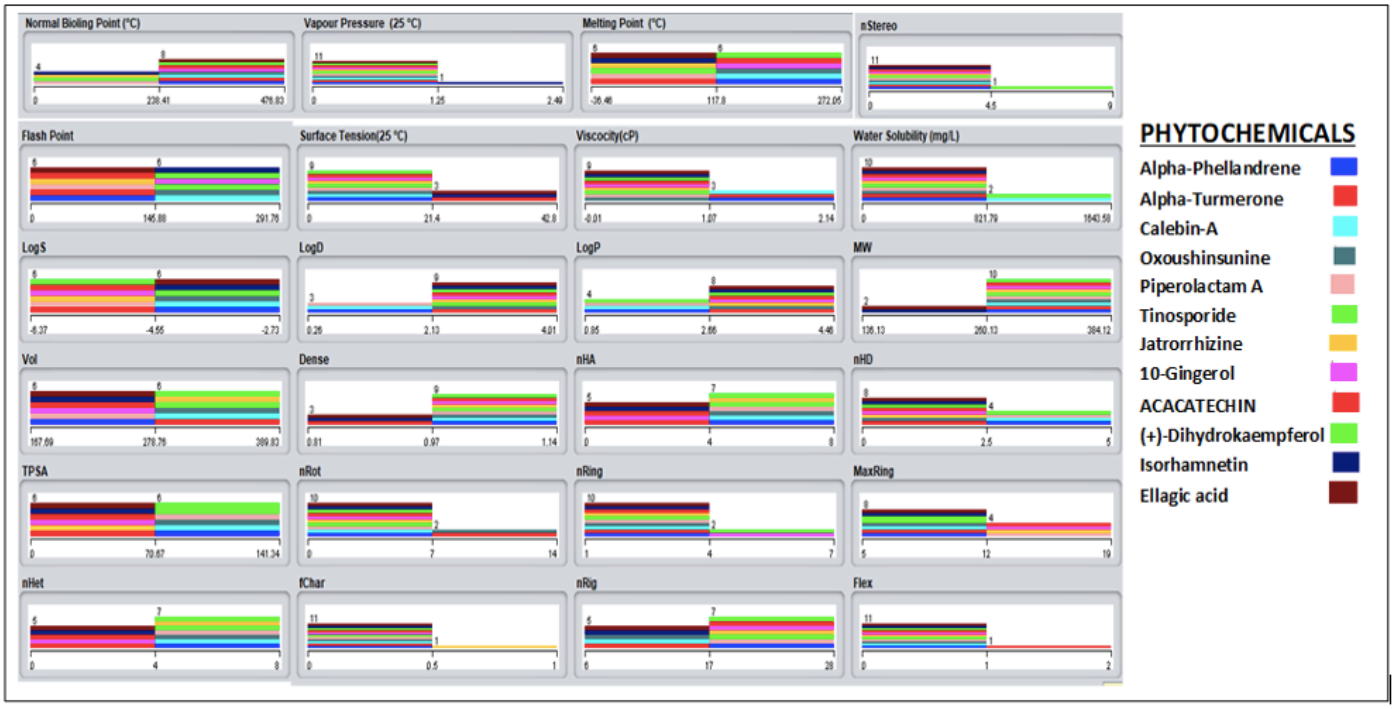
Sl.No	Phytochemicals	Drug likeness				
		Lipinski violations	Ghose violations	Veber violations	Egan violations	Muegge violations
1	alpha-Phellandrene	0	1	0	0	2
2	alpha-Turmerone	0	0	0	0	1
3	Calebin-A	0	0	0	0	0
4	Oxoushinsunine	0	0	0	0	0
5	Glycyrrhizinate	3	3	1	1	4
6	Piperolactam A	0	0	0	0	0
7	Chebulagic acid	3	3	1	1	5
8	Terminalic acid	3	3	1	1	4
9	β-Gentiobiose	2	1	1	1	4
10	Terflavin A	3	3	1	1	5
11	Terchebulin	3	3	1	1	5
12	Chebulagic Acid	3	3	1	1	5
13	Chebulic acid	2	0	1	1	3
14	Tinosporide	0	0	0	0	0
15	Jatrorrhizine	0	0	0	0	0
16	Aucubin	1	1	1	1	2
17	Mangiferin	2	1	1	1	3
18	10-Gingerol	0	0	1	0	1
19	ACACATECHIN	0	0	0	0	0
20	(+)-Dihydrokaempferol	0	0	0	0	0
21	Isorhamnetin	0	0	0	0	0
22	Rutin	3	4	1	1	4
23	Kanamycin-6"-uronic acid	3	3	1	1	5
24	Procyanidin	3	2	1	1	3
25	β-Glucogallin	1	1	1	1	2
26	(+)-Chebulic acid	2	0	1	1	3
27	Chebulinic acid	3	3	2	1	4
28	Corilagin	3	2	1	1	4
29	Ellagic acid	0	0	1	1	0

In this study, the molecular features of 12 drug able phytochemicals were described ADME (Absorption, Distribution, metabolism, Distribution, and Execution) with the help of online soft wares like SWISS ADMET. All the Physicochemical properties shown in Figure 5 by graph and few main properties are in table 4. Table 4 also shows lipophilicity and water solubility as well as pharmacokinetics.

Table 4: ADME details about 12 best Phytochemicals (Physiochemical Properties, Lipophilicity, Water Solubility and Pharmacokinetics)

Sl.No	Phytochemicals	SWISS ADME								
		Physiochemical Properties						Lipophilicity	Water Solubility	Pharmacokinetics
		MW	Rotatable bonds	H-bond acceptors	H-bond donors	MR	TPSA	Consensus Log P	Class	absorption GI
1	Alpha-Phellandrene	136.23	1	0	0	47.12	0	2.97	Soluble	Low
2	Alpha-Turmerone	218.33	4	1	0	70.88	17.07	3.59	Soluble	High
3	Calebin-A	384.38	9	7	2	103.89	102.29	2.88	Moderately soluble	High
4	Oxoushinsunine	275.26	0	4	0	76.67	48.42	2.88	Moderately soluble	High
5	Piperolactam A	265.26	1	3	2	79.28	62.32	2.85	Soluble	High
6	Tinosporide	374.38	1	7	1	89.54	98.5	1.56	Soluble	High
7	Jatrorrhizine	338.38	3	4	1	97.33	51.8	2.31	Moderately soluble	High
8	10-Gingerol	350.49	14	4	2	103.78	66.76	4.62	Moderately soluble	High
9	ACACATECHIN	290.27	1	6	5	74.33	110.38	0.85	Soluble	High
10	(+)-Dihydrokaempferol	288.25	1	6	4	72.73	107.22	0.99	Soluble	High
11	Isorhamnetin	316.26	2	7	4	82.5	120.36	1.65	Soluble	High
12	Ellagic acid	302.19	0	8	4	75.31	141.34	1	Soluble	High

Figure 5: Physiochemical Properties of 12 selected drauggable Phytochemicals



Toxicity estimates and docking

Toxicity analysis (17,18) is performed using the ProtoxII web tool, based on molecular conformation based on pharmacophore, fragment propensity and machine learning models. Organ toxicity including hepatotoxicity and 4 different toxicological endpoints were observed (eg mutagenicity, cytotoxicity, immunotoxicity and carcinogenicity). After analysis of toxicity molecular docking was performed.

Table 5: Predicted toxicity by Protox II such as Toxicity Class (oral toxicity), Hepatotoxicity (Organ toxicity) with toxicity endpoints (Carcinogenicity, Immunotoxicity, Mutagenicity and Cytotoxicity), of the 12 druggable phytochemicals

Sl.No	Phytochemicals	Toxicity Class	Hepatotoxicity	Carcinogenicity	Immuno toxicity	Mutagenicity	Cytotoxicity
1	Alpha-Phellandrene	6	Inactive	Inactive	Inactive	Inactive	Inactive
2	Alpha-Turmerone	6	Inactive	Inactive	Inactive	Inactive	Inactive
3	Calebin-A	4	Inactive	Inactive	Active	Inactive	Inactive
4	Oxoushinsunine	3	Inactive	Active	Active	Active	Inactive
6	Piperolactam A	4	Active	Active	Active	Active	Inactive
14	Tinosporide	3	Inactive	Inactive	Active	Inactive	Active
15	Jatrorrhizine	3	Inactive	Inactive	Active	Active	Inactive
18	10-Gingerol	3	Inactive	Inactive	Active	Inactive	Inactive
19	ACACATECHIN	6	Inactive	Inactive	Inactive	Inactive	Inactive
20	(+)-Dihydrokaempferol	4	Inactive	Inactive	Inactive	Inactive	Inactive
21	Isorhamnetin	5	Inactive	Inactive	Active	Inactive	Inactive
29	Ellagic acid	4	Inactive	Active	Inactive	Inactive	Inactive

Toxicity Class 1: fatal, if LD50 > 4 swallowed
Toxicity Class 2: fatal, if 50>= LD50 > 4 swallowed
Toxicity Class 3: fatal, if 300>= LD50 > 50 swallowed
Toxicity Class 4: fatal, if 2000>= LD50 > 300 swallowed
Toxicity Class 5: fatal, if 2000>= LD50 > 5000 swallowed
Toxicity Class 6: fatal, if 5000>= LD50 swallowed (nontoxic)

LD50 it is lethal for 50% animals in a dose group while the dose is applied as test substance

According to table 5, four phytochemicals who belong to class 5 and 6 and inactive in all toxicity group, were virtually screened by autodock vina using Pyrx software where 6lu7 chain a (Covid 19) was used as macro molecule. According to the supplementary table 4, phytochemicals who possess lowest binding affinity between -6 to -7 was selected for molecular docking. Acacatechin and Isorhamnetin were two phytochemicals, selected for molecular docking (19) by auto dock software (20) as they possess desire binding affinity, against Macromolecule 6lu7 chain a.

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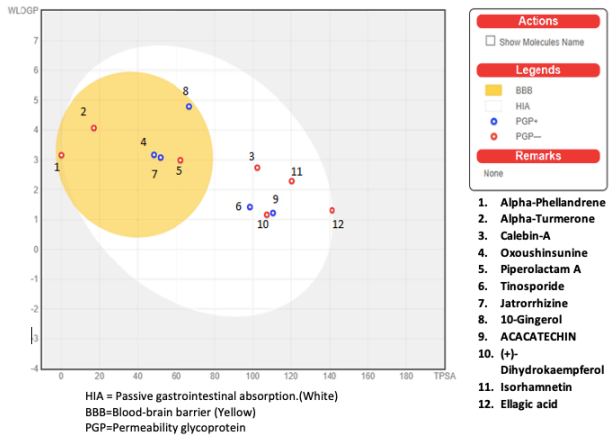
Table 6: Metabolic and Elimination of top druggable Phytochemicals (21)

Phytochemicals	Inhibitors					Elimination (ml/mi /kg)	
	CYP1A2 inhibitior	CYP2C19 inhibitior	CYP2C9 inhibitior	CYP2D6 inhibitior	CYP3A4 inhibitior	CL (Clearance)	T12 (Half-Life
Alpha-Phellandrene	No	No	No	No	No	12.66	0.617
Alpha-Turmerone	No	No	No	No	No	14.305	0.804
Calebin-A	No	Yes	No	No	Yes	12.621	0.957
Oxoushinsunine	Yes	Yes	Yes	No	No	11.173	0.121
Piperolactam A	Yes	Yes	Yes	No	No	2.096	0.48
Tinosporide	No	No	No	No	No	14.938	0.044
Jatrorrhizine	Yes	No	No	Yes	No	10.517	0.497
10-Gingerol	No	Yes	Yes	No	No	12.323	0.823
ACACATECHIN	No	No	No	No	No	17.911	0.853
(+)-Dihydrokaempferol	No	No	No	No	No	6.523	0.767
Isorhamnetin	Yes	No	No	No	No	6.991	0.922
Ellagic acid	Yes	No	No	No	No	2.346	0.863

Result

Table 6 contained the Metabolic and Elimination section. Metabolic section referred the inhibitors and elimination section referred Clearance and half-life (T1/2) (22). Clearance signify the ability of body to clear drugs from plasma (21). Half-life of elimination is connected with drug doses. Table 6 also showed interaction between phytochemicals the inhibitors. ACACATECHIN, Alpha-Phellandrene, Alpha-Turmerone, (+)-Dihydrokaempferol and Tinosporide were not into interaction with any of the CYP inhibitors. Isorhamnetin and Ellagic acid were not into interaction with any of the CYP inhibitors (23) accept CYP1A2 others were interacted with two or more inhibitors.

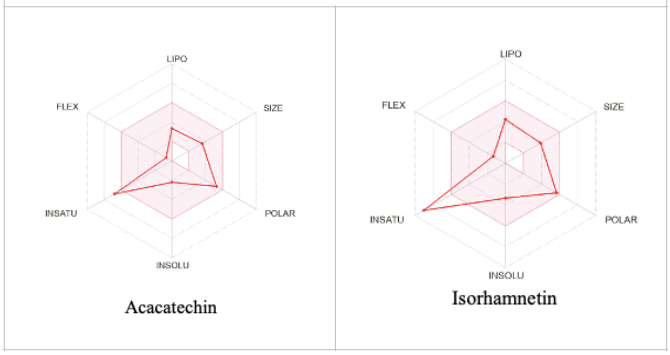
Figure 6: Boiled Egg (WLOGP and TPSA (topological polar surface area graph) representation of 12 selected Phytochemicals along with the Acacatechin and Isorhamnetin.



INSOLU = insolubility in water by log S scale
INSATU = Insaturation as per fraction of carbons in the sp3 hybridization
FLEX = flexibility as per rotatable bonds
LIPO = Lipophilicity as XLOGP3
SIZE = size as molecular weight
POLAR = polarity as TPSA (topological polar surface area)

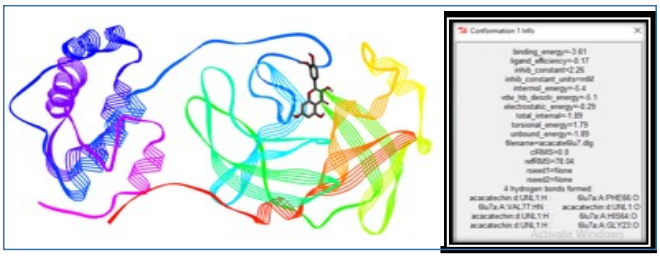
Figure 6 contained the Boiled-Egg plot of TPSA and WLOGP. The plot showed that the absorption in Brain and Gastrointestinal of 12 top selected phytochemicals. According to this plot Acacatechin and Isorhamnetin both phytochemicals which were selected for docking they belongs to HIA (Passive gastrointestinal absorption) category.

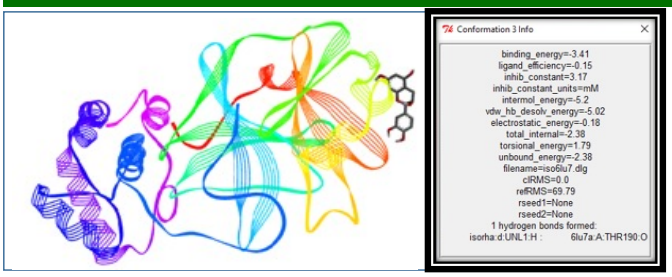
Figure 7: Bioavailability Radar of Acacatechin and Isorhamnetin (24,25)



Bioavailability Radar of Acacatechin and Isorhamnetin was showed in figure 7, The pinkish zone of the radar contained the optimal properties range prime physiochemical features those were lipophilicity (XLOGP3): range −0.7 to +5.0, size (MW): range150 to 500 g/mol, polarity (TPSA): range 20 to 130 Å^2, solubility (log *S)* lower than 6, Instauration as per fraction of carbons in the sp3 hybridization was 0.25 or high, and flexibility: 9 or less than 9 rotatable bonds. According to Figure 7 (Bioavailability Radar of Acacatechin and Isorhamnetin) indicators of Insaturation as per fraction of carbons in the sp3 hybridization were out of optimal range.

Figure 8 a) complex structure of Acacatechin and 6tlu7 and b) complex structure of Isorhamnetin and 6lu7

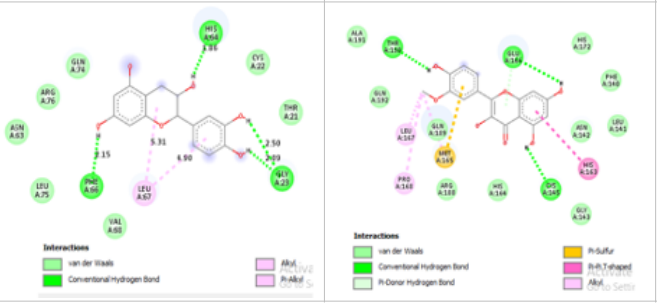




The techniques of molecular docking always target the best matches between a small molecule or ligands and macro molecule or the receptors. Here 6lu7 which is a main protease of Covid 19 virus, which has chain A that is 3C-like proteinase and chain B(N-[(5-METHYLISOXAZOL-3-YL)CARBONYL]ALANYL-L-VALYL-N~1~-((1R,2Z)-4-(BENZYLOXY)-4-OXO-1-{[(3R)-2-OXOPYRROLIDIN-3-YL]METHYL}BUT-2-ENYL)-L-LEUCINAMIDE). For this study chain of 6lu7 was selected.

Figure 9: Protein and ligand interaction

a) Acacatechin and 6lu7 chain a	b) Isorhamnetin and 6lu7 chain a



Molecular docking was performed separately for two separate small molecule acacatechin and isorhamnetin.

In the case of small molecule Acacatechin, ligand bind with the Macro molecule 6lu7 chain A with binding affinity -3.61 Kcal/Mol with Inhibition constant of 2.26 uM and the RMSD value of 78.04.

Table 7: Ligands with binding affinity, Inhibition Constant, RMSD and involve amino acids in the interaction

Ligand	RMSD	Binding affinity as per AutoDock result (Kcal/Mol)	Inhibition Constant (K_i)	Amino acid involves in this interaction
Acacatechin	78.04	-3.61	2.26 uM (micromolar) [Temperature = 298.15 K]	Conventional Hydrogen bond
				66A PHE, 64A HIS, 23A GLY, 23A GLY
				Alkyl bond
				67A LEU
Isorhamnetin	69.79	-3.41	3.17 uM (micromolar) [Temperature = 298.15 K]	Conventional Hydrogen bond
				190A THR, 166A GLU, 145A CYS
				Alkyl bond
				167A LEU, 168A PRO, 189A GLN

RMSD = Root Mean Square Deviation; GLN= Glutamine. GLU= Glutamic acid, PHE = Phenylalanine, LYS= Lysine, CYS= Cysteine, LEU= Leucine, THR= Threonine, HIS= Histidine,GLY= Glycine;

In second case in terms of isorhamnetin, it binds with -3.41 Kcal/Mol with 6lu7 chain A with the RMSD value of 69.79 and 3.17uM inhibition constant.

Acacatechin bind with 6lu7 chain A with conventional hydrogen bond at Atom number 66 with Phenylalanine, atom number 64 with Histidine and bind twice with atom number 23 with Glycine. It also bind with alkyl bond at atom number 67 with Leucine..

Isorhamnetin bind with 6lu7 chain A with conventional hydrogen bond at atom number 190,166,145 with Threonine, Glutamic Acid and Cysteine respectively. It also has alkyl bond at atom number 167,168,189 with Leucine, Proline and Glutamine respectively. With Methionine atom number 165 a pi sulfur interaction is there and pi –pi T shaped interaction with Histidine at atom number 163 is also there.

According to the supplementary table number 2, Acacatechin and Isorhamnetin both phytochemicals are coming from *Acacia catechu.*

Conclusion

This is an *in silico* study where most desired phytochemicals of Unani therapeutic potential were find out, which has medicinal and immunomodulatory properties and may play an efficient part as an inhibitor against 6LU7 chain A. Elimination of all the active toxicity are (by Protox-II software) done by this search and opted Acacatechin and Isorhamnetin phytochemicals as a ligand for the autodock purpose with 6LU7 chain A. The observation of autodock, the moderate quality of binding affinity. Work of dynamic simulation and wet laboratory needed for the completeness of this study.

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