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Formulation and Development of Polyherbal Tea Formulation Intended for Immunity Boosting for The Treatment of Covid-19

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Mirza Prince[1*], Rajendirn K[2]

[1]Bachelor of pharmacy, T.john college of pharmacy,Bangalore-560083 India.

[2]Deparment of pharmcognoy, T.john college of pharmacy,Bangalore-560083 India.

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ABSTRACT

The evaluation of immunity-boosting activity was carried out on the powdered ashwagandha (*Withania somnifera*), cinnamon, ginger. This study aimed to evaluate the immunity-boosting activity of ashwagandha, cinnamon, ginger against covid-19, also to evaluate the immunity-boosting activity of prepared Herbal Tea formulation against hydroxychloroquine as a standard. The immunity-boosting activity of powdered ashwagandha, cinnamon, ginger was determined by docking study, by inhibition of SARS CoV-2 main protease. The powdered ashwagandha, cinnamon, ginger showed significant inhibition of SARS CoV-2 main protease. This result provides valuable information that ashwagandha, cinnamon, ginger hold great promise as highly effective as an immunity-boosting agents.

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I. INTRODUCTION

Immunity boosters are products that claim to be able to support the immune system. A healthy immune system protects us by first creating a barrier that stops those invaders, or antigens, from entering the body. And if one slips by the barrier, the immune system produces white blood cells and other chemicals and proteins that attack and destroy these foreign substances.

During the current pandemic scenario, the use of medicinal plants is the major need for optimizing the quality of life through better immunity to get a healthy life. As mentioned in ancient science, the person can strengthen his or her immunity by consuming medicinal herbal plants or herbal products. Indians are practice using many herbal plants as a part of their daily life. Mainly our society considered medicinal herbs to be natural, safe, cheap, easily accessible without any harmful toxic and side effects, and also they have high therapeutic effects and pharmacological importance.

Herbs have been used alone or in amalgamation with other herbal drugs as a polyherbal formulation. US FDA has approved many Phyto-compounds, extracts, essential oils; decoctions, powders, tinctures, poultices as well as raw herbs form important parts of drugs. Phyto-compounds like terpenoids, flavonoids, polyphenols, quinones, tannins, coumarins, terpenes, lectins, polypeptides, and saponins are the herbal constituents that provide more than 50% of modern therapeutic and pharmacological effects. These components play a major and important role in signal transduction, mitoses (cell division), and apoptosis (cell death) too. Medicinal plants–based Phytomedicine leads, guide, and contribute to the concoction of newer drugs.

Drugs affecting the immune system are termed immunomodulatory or adaptogenic. Some repress the system and are of value in, for example, preventing rejection of transplanted organs, and others are stimulatory and can be used to help combat viral infections such as AIDS or assist in the treatment of cancer. Until relatively recently such herbal drugs were largely ignored by Western orthodox medicine, although they have always featured in traditional Chinese and Indian medicine in seeking to achieve homeostasis concerning bodily functions. Now, however, ginseng leads the market in herbal sales in Europe and the US, and Echinacea spp., used by native N. American 15 Indians, ranks around fifth in the US herb

market sales and is widely used in Europe, with 800 preparations being quoted as available in Germany.

Ashwagandha is used similarly to ginseng is used in Asia. It is an excellent adaptogenic herb and increases the body's overall ability to adapt to and resist stress. Ashwagandha is one of the deep immune strengthening plants. A powerful building and strengthening plant, ashwagandha has been highly esteemed and revered for centuries in its native land of India, and is rapidly gaining recognition even in the west as its remarkable qualities become known. The herb Ashwagandha can be an effective therapeutic and preventive drug in the prevention of COVID -19 Infection; the natural compounds from Ashwagandha have the potential to be an immune-modulatory booster. The phytoconstituents may target the main SARS-CoV-2's enzyme for splitting proteins; known as the main protease (Mpro) that plays a key role in mediating viral replication. This is an attractive drug target for this virus, and as humans don't naturally have this enzyme, compounds that target Mpro are likely to have low toxicity.

Ashwagandha can be an alternative to Hydroxychloroquine (HCQ), an anti-malarial drug, as a potential COVID-19 preventive.

Docking is most commonly used in the field of drug design. It is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets to in turn predict the affinity and activity of the small molecules. A binding interaction between a small molecule (Ligand) and an enzyme protein may result in activation or inhibition of the enzyme. If the protein is a receptor, then ligand binding may result in agonism or antagonism. Most drugs are small organic molecules, and docking may be applied to (i) Hit Identification: Docking combined with scoring function can be used to quickly screen a large database of potential drugs in silico to identify molecules that are likely to bind protein target interest. (ii) Lead Optimization: Docking can be used to predict where and in which relative orientation a ligand binds to proteins (also referred to as the binding mode or pose). This information may in turn be used to design more potent and selective analogs. (iii) Bioremediation: Protein-ligand docking can be used to predict pollutants that can be degraded by enzymes. To perform a docking screen, the first requirement is a structure of the protein of interest. Usually, the structure has been determined using a 16 biophysical technique such as X-ray crystallography, or less often, NMR spectroscopy. This proteins structure and a database of the potential ligands serve as inputs to a docking program.

I.I Aim: To formulate and develop a polyherbal formulation for immunity boosting the potential for the treatment of covid 19.

I.II Objective:

- To formulate and develop the polyherbal formulation.
- To perform docking studies of phytoconstituents against SARS CoV-2 main protease.
- To perform In-vitro immune assay and anti-viral studies.

II. MATERIALS AND METHODS

II.I Ashwagandha: Drug consists of dried roots of *Withania somnifera* (Linn.) Dunal (Syn. Physalis Somnifera Linn., P. Flexuosa Linn., P. Arborescence DC.); Fam. Solanaceae. The Plant is widely distributed in North-Western India, Bombay, Gujrat, Rajasthan, Madhya Pradesh, Uttar Pradesh, Punjab plains and extends to the mountain regions of Himachal Pradesh and Jammu.

- **Chemical constituents:**

The majority of the constituents are withanolides (steroidal lactones with ergostane Skeleton) and alkaloids. These include Withanone, Withaferin Withanolides I, II, III, A, D, E, F, G, H, I, J, K, L, M, WS-I, P and S, withasomidienone, withanolide C", and alkaloids" viz., cuscohygrine, anhydride, tropine, nseudotropine, anaferine, 1sopellatierine, 3-tropyltigloate (Kalra, et al., 2017). Total alkaloids about 0.2%.

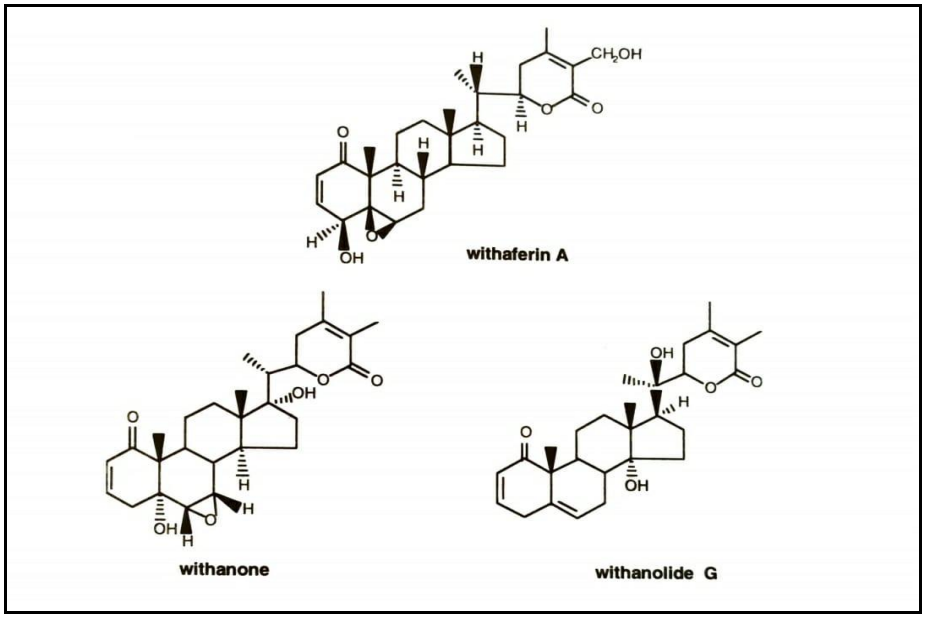

Figure No. 1: chemical constituents of ashwagandha

- **Uses:**

Roots of the plant show antitumor and radiosensitizing effects in animal models Total alkaloidal fraction of the root extract exhibits hypotensive, bradycardic, and Respiratory stimulant activities in dogs. It shows relaxant and antispasmodic effects against several plasmalogens on intestinal, uterine, bronchial, tracheal, and Blood vascular muscles. Withanolides possess remarkable antibacterial, Antitumour, 28 antiarthritic, and immunosuppressive properties and protective effects against carbon tetrachloride-induced toxicity.

Figure No.2: Ashwagandha plant

Figure No. 3: Ashwagandha root

II.II Ginger: The drug consists of the dried rhizomes (scraped or unscraped) of Zingiber Qficinale (Willd.) Rosc. (Syn. Z. MissionisWall.. Z. BlancoiMassk. Z. Majus Rumph., Amomum zingiber Rosc., Curcuma longifolia Wall. Cat): Fam. Zingiberaceae. The plant is universally known and widely cultivated all over the warm parts of India.

➢ **Chemical Constituents**

Major:

• Oleoresin (-5.3 -8.6%) comprising of nonvolatile pungent principles (gingerolsmainly [6}-gingerol), non-pungent substances (fats and waxes), and volatile oil

• Volatile oil (-1.5 – 2.2%) containing sesquiterpene hydrocarbons viz..azingiberene, B-sesquiphellandrene and ar-curcumin as major constituents (The composition of volatile oil varies according to origin" 10- and changes upon storage").

• Lipids (-6-8%)

• Proteins (-10%)

• Starch (~ 40-60%)

Minor:

Numerous monoterpene and sesquiterpene hydrocarbons and Their oxygenated derivatives in volatile other pungent principles viz., shogaols (anhydro-gingerols, generally absent in fresh ginger").Paradols, gingerdiols, gingerdiacetates. Gingerdiones" 6-gingersulfonic Acid. Gingerenones and a number of diarylhepatanoids": Diterpenes": gingerglycolipids A. B, & C".

Figure No.4: Dried Ginger

➢ **Pharmacology**: Ginger and its constituents act as digestive aids: possess antiulcer" Cholagogic and antiemetic properties and increase gastro-intestinal Motility which may be due to the antiserotoninergic activity 38.3 of the Drug. Inhibition of prostaglandin synthesis 40-42 by the constituents of Ginger is thought to play a role in the anti-inflammatory activity 43.44 Exhibited by the drug. Ginger is also known to possess hypolipidaemic/ antiatherosclerotic, antidiabetic" and cardiotonic properties. In Clinical trials, ginger seems to be of use in treating motion sickness and rheumatic disorders.

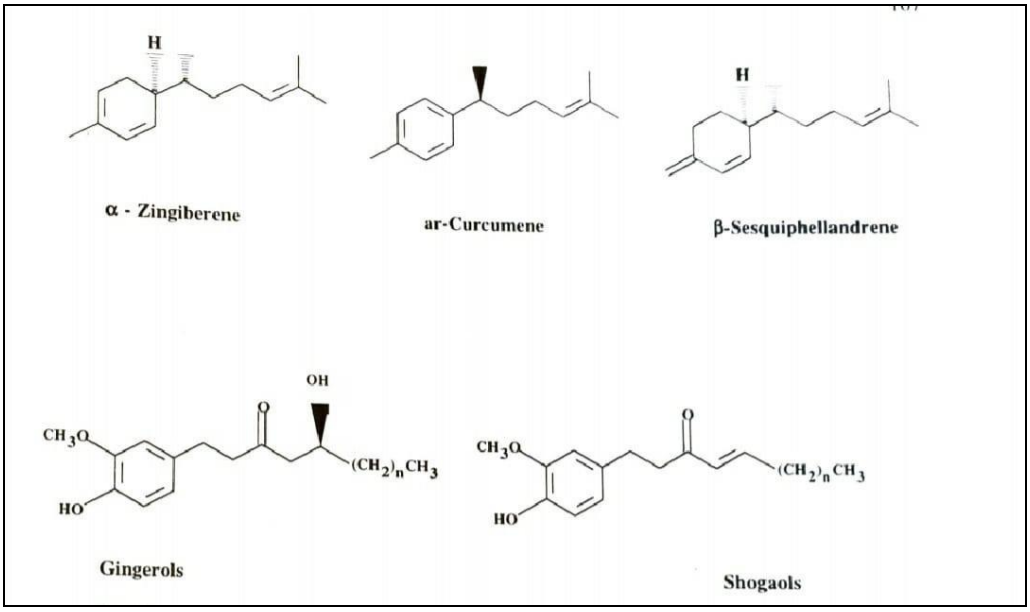

Figure No. 5: chemical constituents of ginger

II.III Cinnamon bark, Kalmi- Dalchini, Ceylon Cinnamon Cinnamon consists of the dried inner bark of the shoots of coppiced trees of Cinnamomum Zeylanicum Nees belonging to the family Lauraceae. It should not contain less than 1.0% of volatile oil.

- **Chemical constituents:**

Cinnamon bark contains about 0.5 to 1% of volatile oil,1.2% of tannins, mucilage, calcium oxalate, starch, and a sweet substance known as mannitol. The volatile oil is the active constituent of the drugs. It is light yellow in color and changes to red on storage. Bark yields 14 to 16 % of 90% alcohol-soluble extractive.

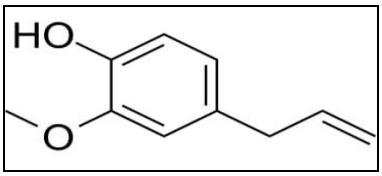

Figure No. 6: Eugenol

Cinnamon oil contains 60 to 70% of cinnamaldehyde, 5 to 10% eugenol, benzaldehyde, cumin aldehyde, and other terpenes like phellandrene, Pinene, cymene, caryophyllene, etc. Cinnamon oil is yellow to red.

➢ **Uses:**

The bark is used as a carminative, stomachic, and mildly astringent. It is also used as a flavoring agent, stimulant, and aromatic and antiseptic. Commercially, it is used as a spice and 29 condiments, and also in the preparation of candy, denitrifies, and perfume.

Figure No. 7: Cinnamon bark

II.IV Tea

➢ **Synonyms**: Camellia Thea, BIOLOGICAL SOURCES: It contains the prepared leaves and leaf buds of Thea Sinensis belonging to the family Theaceae.

➢ **Chemical constituents**: Tea leaves are considered a rich source of caffeine. It is extracted from tea dust and tea leaves waste or sweepings. It also contains theobromine and theophylline in minor quantities. The color of tea leaves is due to gallotannic acid. the agreeable odor is due to the presence of a yellow volatile oil. Tea leaves also contain an enzymatic mixture called these.

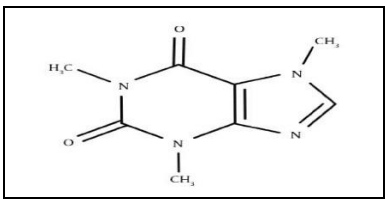

Figure No.8: Caffeine

➢ **Uses**: CNS Stimulant in the form of beverage and also as a diuretic. Numerous studies have shown that a variety of teas may boost your immune system, fight off inflammation, and even ward off cancer and heart disease. While some brews provide more health advantages

than others, there's plenty of evidence that regularly drinking tea can have a lasting impact on your wellness.

Figure No. 9: Dried tea leaves

II.V METHODS

II.V.I Formula of herbal tea powder

All the plant materials were dried and finely powder. The finely powdered raw material was passed through sieve no 40 and then 16.7 gm of each of the four ingredients were mixed. a powder mixture of 2 gm was packed in tea bags.

Table No.1: Formula of tea powder

Sr. No.	Ingredients	Quantity taken	Catagory
1	Ashwagandha	16.7gm	Anti-Viral
2	Cinnamon	16.7gm	Anti-Viral
3	Ginger	16.7gm	Anti-Viral Anti-fungal
4	Tea	16.7gm	Anti-oxidants

II.V.II Evaluation Test:

A: Bulk Density: Bulk Density of the powder was measured by measuring the ratio of the mass of the powder and its bulk volume.

Bulk density =mass of powder/bulk volume

B: Tapped Density: The tapped density was attained after mechanically tapping a container containing the powder sample.

Tapped density = weight of sample/tapped volume

C: Angle of repose: Under the static balance, the Angle between the slope of a powder pile and the horizontal plane was measured.

Angle of repose(Ɵ)= tan^-1(h/r)

h= height of pile in cm, r= average radius of circle in cm

D: Loss on drying: weighed of empty Petri dish and took 2gm of powder sample into it. placed this Petri dish in a hot air oven for 1 hour and calculated the weight of Petri dish frequently. Repeated the same procedure until the weight of the Petri dish became equal and noted down the constant reading of loss on drying of herbal formulations.

%LOD= (Weight of water in sample/ weight of dry sample)×100%

E: Total ash value: Weighed accurately about 2gm of a powered drug in a tarred silica crucible. Incinerated at a temperature not exceeding 450°C for 4 hours, until free from carbon, cooled and weighed.

% Total ash value= (weight of total ash/ weight of crude drug taken)×100

F: Water-soluble extractive value: Took 5 gm of the powder sample of the herbal drug in a conical flask and 90ml of water and added 10ml of chloroform and kept it for magnetic stirring for 6 hours then placed it for 18 hours. Filtered it and took 25 ml of filtrate from that evaporated it.

G: Alcohol soluble extractive value: Took 5gm of the powder sample of herbal drug mixture in a conical flask added 100ml of alcohol into it kept it for magnetic stirring for 6 hours then placed it for 18 hours filtered and took 25 ml of filtrate from that evaporated it.

II.V.III In vitro cytotoxicity assay: Determination of mitochondrial synthesis by MTT assay Principle: The ability of the cells to survive a toxic insult has been the basis of most cytotoxicity assays. This assay is based on the assumption that dead cells or their products do not reduce tetrazolium. The assay depends both on the number of cells present and on the mitochondrial activity per cell. The cleavage of MTT to a blue formazan derivative by living cells is a very effective principle on which the essay is based. 35 The principle involved is the cleavage of tetrazolium salt MTT (3-(4,5 dimethyl thiazole-2 yl)- 2,5-diphenyl tetrazolium bromide) into a blue-colored product (formazan) by mitochondrial enzyme succinate dehydrogenase. The numbers of cells were found to be proportional to the extent of formazan production by the cells used.

- **Procedure**: i. The cell culture was centrifuged and the cell count was adjusted to 1.0x105 cells/ml using DMEM medium containing 10% FBS. ii. To each well of a 96 well flat bottom microtitre plate, 100µl of the diluted cell suspension (approximately 10,000 cells/well) was added. iii. After 24 hours, when the cell population was found adequate, the cells were centrifuged and the pellets were suspended with 100 microliters of different test sample concentrations prepared in maintenance media. The plates were then incubated at 37oC for 48 hrs in a 5% CO2 atmosphere, and microscopic examination was carried out and observations recorded every 24 hours. After 48 hours, 20 microlitres of MTT (2mg/ml) in MEM-PR (MEM without phenol red) was added. iv. The plates were gently shaken and incubated for 2 hours at 37oC in a 5% CO2 atmosphere. v. The 100 microlitre of DMSO was added and the plates were gently shaken to solubilize the formed formazan. vi. The absorbance was measured using a microplate reader at a wavelength of 540nm. The percentage cell viability was calculated using the following formula and the concentration of drug or test samples needed to inhibit cell growth by 50% values were generated from the dose-response curves.

% Cell Viability = (Mean OD of individual Sample / Mean OD of control)×100

II.V.IV Docking study: The three-dimensional crystal structure of COVID – 19 protein called SARS – CoV – 2 main protease receptor cocrystallized with 6-(ethylamino) pyridine-3-carbonitriles (PDB ID: 5R82, Resolution: 1.31 Å) was retrieved from the protein data bank. The protein was prepared using the protein preparation wizard of the epic module of Schrödinger suite 2019-4. The initial protein structure is a monomer, having similar binding sites that were removed with deleting waters, refining bond orders, and addition of hydrogens. Missing chain atoms are added by using the Prime module of Schrödinger suite 2019-4. Protein minimization was performed using Optimized Potentials for Liquid Simulations 3 molecular force field with root mean square difference (RMSD) of crystallographic heavy atoms kept at 0.30 Å. A grid box was generated to define the centroid of the active site. All the compounds were docked into the catalytic pocket of COVID-19 by using the Glide module of Schrödinger suite 2019-4 in extra precision (XP) mode. The binding modes with the best glide G score were selected.

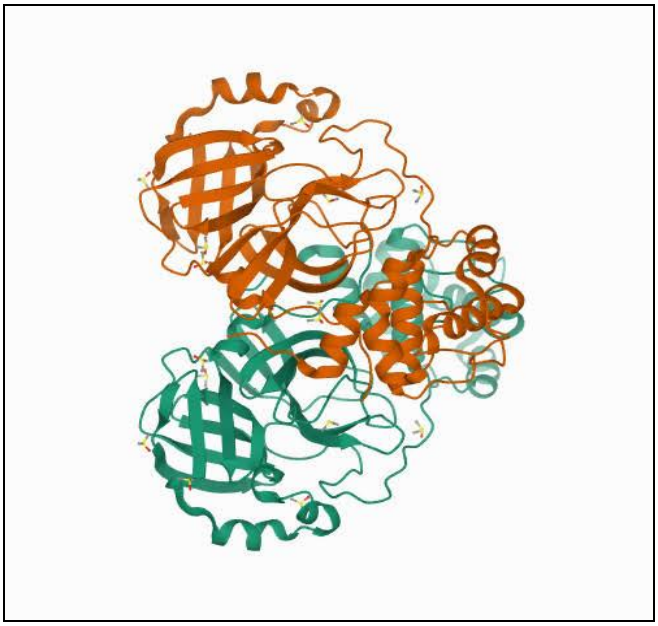

Figure No. 10: SARS CoV-2 main protease

III. RESULTS AND DISCUSSION

Table No. 2: Results of evaluation test

Sr. No.	Evaluation test	Values
1	Bulk density	0.32 gm/ml
2	Tapped density	0.38 gm/ml
3	Angle of repose	17.13
4	Loss on drying	48.75%
5	Total ash value	1.91
6	Water soluble extractive value	4.8%
7	Alcohol soluble extractive value	5.2%

Table No. 3: In-Vitro cytotoxicity studies

Sr. No.	Sample description	Vero IC50 µg/m
1	A	86.68145
2	B	78.88046
3	C	119.5279

III.I Docking study results:

It is demonstrated that some of the chemical constituents from ginger-like 8-Gingerol (−5.88), 10-Gingerol(−5.82), are significantly active against COVID19 with a significant Glide score more when compared to the currently used drug hydroxychloroquine (−5.47). The above compounds have a good affinity to the receptor due to their more lipophilic character and also due to hydrogen bonding.

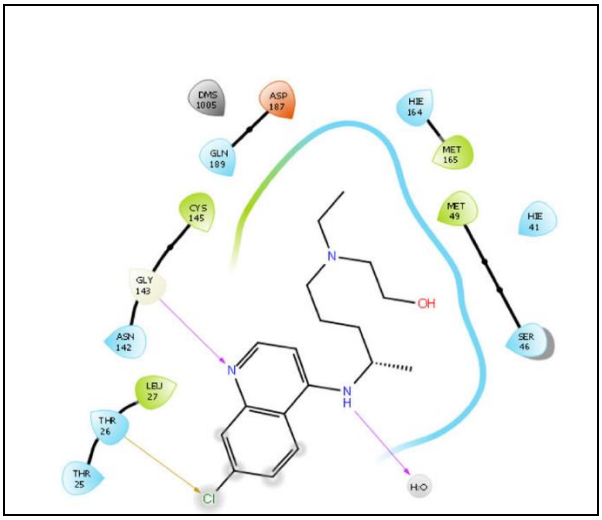

Figure No.11: Ligand interaction of Hydroxychloroquine with SARS CoV – 2 main protease

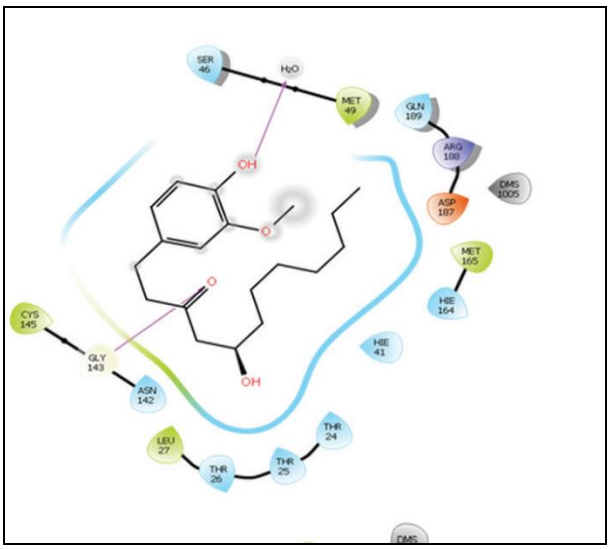

Figure No. 12: Ligand interaction of Gingerol with SARS CoV – 2 main protease

The phytoconstituents may target the main SARS – CoV-2's enzyme for splitting proteins, known as the main protease (Mpro), that plays a key role in mediating viral replication. This is an attractive drug target for this virus, and as humans don't naturally have this enzyme, compounds that target Mpro are likely to have low toxicity. Ashwagandha can be an alternative to the anti-malarial drug Hydroxychloroquine (HCQ) as a potential COVID-19 preventive.

DISCUSSION:

In the present experiment, an herbal formulation was made to prepare an effective immunity booster against COVID-19 by using various ingredients shown in table 1. Many of these ingredients are also having antiviral activity such as ashwagandha, cinnamon, and ginger. Tea is also rich in natural antioxidants, and ginger has anti-inflammatory and antimicrobial properties. Common possess antifungal properties along with flavour. The prepared formulation was evaluated for various phytochemical properties and satisfactory results were obtained. The formulation proved to be beneficial and had excellent activity against CORONA viruses.

Conflict of Interest	None
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Authors' Contribution	All authors conceived and designed the study. Mirza Prince conducted the experiments, analyzed the data and wrote the paper. All authors contributed to manuscript revisions. All authors approved the final version of the manuscript and agreed to be held accountable for the content therein.
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	Author Name – Mirza Prince Bachelor of pharmacy T.John college of pharmacy, Bangalore-560083
Image **Author -2**	Dr. Rajendrian K M.Pharm, Ph.D Associate Professor, Department of Pharmacognosy T.John college of pharmacy, Bangalore-560083