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In-vitro antiviral activity evaluation of Indian medicinal plants against SARS-CoV-2

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Abstract

SARS-CoV-2, the highest mortality rate virus of the 21st century, initially affects the respiratory system, evades immune system fighter cells, and later affects other organs if not eliminated from the body. In absence of any specific pharmacological treatment without adverse side effects, the need to find new therapeutic alternatives is coherent. Ethnomedicine and 'Ayurveda', are holistic approaches that stimulate non-specific immunity by assisting CD4+ and CD8+ T cells to act against the pathogens to ward them off and remove root causes underlying a disease. Various phytochemicals from Indian medicinal herbs are known to act against viral receptors, and their target protein sites and interfere with viral reproduction, and also as potential immune cell enhancers. The current research used LCMS to identify phytochemicals in 4 Indian traditional medicinal herbs and their ayurvedic blend against SARS-CoV-2, through a pre-post treatment strategy in vitro using qRT-PCR. Herein, pre-post treatment of the extracts & their ayurvedic blend inhibited viral load significantly statistically ($p<0.05$), with Piper nigrum, Terminalia arjuna, and the 4 ayurvedic blend exhibiting maximum antiviral activity almost equivalent to standard drug Remdesivir, thereby confirming their anti-SARS-CoV-2 activity which was efficient similar to synthetically derived Remdesivir. Herein, the anti-SARS-CoV-2 activity of 4 herbal extracts and their mixture was evaluated, while their brief role in stimulating the immune system in order to ward off pathogenic organisms is discussed.

Keywords: Immunostimulatory, *Piper nigrum*, *Terminalia arjuna*, qRT-PCR, SARS-CoV-2, Remdesivir, Infectious diseases, Molecular virology.

Introduction

SARS-CoV-2, a large plus sense ssRNA virus and the causative pathogen causing COVID-19, after originating in Wuhan in December 2019 and further spreading to various parts of the globe was the reason for the global pandemic of COVID-19. This virus is known to cause coronavirus-induced pneumonia or acute respiratory distress syndrome (ARDS). This distress is a result of the excessive release of pro-inflammatory cytokines and chemokines by the immune effect or cells in infected humans. Alpha, beta, gamma, and delta are the genera of coronavirus. There is a high resemblance between SARS-CoV and the novel SARS-CoV-2. The S proteins of SARS-CoV-2 are responsible for its rapid transmission in humans[1]. The virulence of SARS-CoV-2 is imparted by the mutations in the receptor-binding domain region of SARS-CoV-2, which can be the reason behind the breach of the observed epidemiological barriers and the rapid spread of the pandemic. Host cell expression of ACE-2 and other proteases also increases susceptibility to contracting SARS-CoV-2 infection. Omicron's spike protein for the human ACE-2 receptor is the main reason behind its virulence[2]. Owing to the mutations, SARS-CoV-2 is more virulent, and even with the lesser availability of the viral entry-receptors, it can efficiently enter the host with greater replication, resulting in high viral loads; and can result in prolonged viral shedding, widespread organ injury, and severe inflammation, thereby leading to increased viral transmissibility and lethality. Thus, considering the mutations which have made the SARS-CoV-2 infection more lethal, it is of utmost importance to build a strong immune system that can be effective enough, when attacked by the virus.

Recent studies on the effect of SARS-CoV-2 on the immune system have reported that the virus affects the immune system when CD4+ and CD8+ cells play a substantial antiviral role in fighting the pathogens[3,4]. CD4+ T cells are known to help in the production of antibodies that are specific to the virus, through T cell–dependent CD8+ T cells are known to be cytotoxic, with an ability to kill virus-infected cells. As per research, in COVID-19 patients, CD8+ T cells are majorly responsible for reducing the viral load and causing inflammatory damage in the pulmonary

interstitium[5]. T helper cells produce pro-inflammatory cytokines via NF-kB signaling[6,7]. In a recent study in 2020, it was seen that in certain cases, an overwhelmed immune response causes apoptosis of the host cell by cytokine release leading to a fatal condition by cytokine release syndrome (CRS)[8]. This phenomenon is more serious among patients with comorbidities like cardiovascular disorders, diabetes mellitus, arterial hypertension, hepatic, etc.[9]. In such cases, it becomes crucial to explore a specific approach where chemically derived drugs do not add to the dangers of the disease that has affected the patient. Thus, *Ayurveda*, being a multi-modal approach to stimulating the immune system cells to eradicate the underlying root cause of the disease, proves beneficial over chemically synthesized drugs with adverse side effects. It can thus be hypothesized that immune-stimulator phytochemicals can play a vital role in the efficient functioning of CD4+ and CD8+ cells.

For the detection of the SARS-CoV-2 virus, qRT-PCR is a widely used diagnostic test. The qRT-PCR protocol for SARS-CoV-2 detection targets the viral sequence with Open reading frame (ORF), RNA-dependent RNA polymerase (RdRp), an envelope protein (E), Spike protein (S) and nucleocapsid (N) genes of SARS-CoV-2[10]. When the nasopharyngeal/ oropharyngeal swabs are collected in VTM tubes from patients suffering from COVID-19 symptoms, the nucleic acid extraction from the patient's samples are extracted and loaded onto a 96 well microtiter plate, consisting of Master mix, wherein the primers, probes, RTase enzyme in the master mix helps in conversion of the viral RNA to cDNA, and thus in further amplification of the target DNA into several copies which can be detected and quantified in real-time using qRT-PCR.

The hypothesis of the current research is to identify phytochemicals in Indian medicinal herbs used traditionally to treat various diseases and test their ability to inhibit SARS-CoV-2 by comparing their efficacy to that of standard drugs used against SARS-CoV-2. Considering several treatments of synthetic drugs available to treat COVID-19 patients and their adverse effects, looking for a holistic and natural approach in the form of ethnomedicine can prove quite beneficial.

Since SARS-CoV-2 was identified at a genetic level, several antiviral drugs were figured out as treatment options for patients suffering from COVID-19. Antivirals such as Remdesivir, Hydroxychloroquine, Chloroquine, Baloxavir, and Favipiravir, were used, of which Remdesivir and Hydroxychloroquine were the most commonly used antivirals in the treatment of COVID-19. These drugs work as nucleotide analog prodrugs, RNA polymerase inhibitors for SARS-CoV-2 as well as Influenza virus[11-17]. As most commonly used drugs mentioned above which were tested on COVID-19 patients have adverse side effects, the risks that come along with drug dosage for treatment of COVID-19 are numerous. Thus, looking for an alternative option to prevent or even treat COVID-19 in a natural and effective way looks promising in terms of avoiding the adverse side effects.

Respiratory disorders primarily affect the respiratory system but are greatly influenced by immunity. And in terms of immunity, the human gut has a vital role in maintaining the immune system. In ancient times, the focus of individuals was on a holistic approach to maintaining immunity, instead of the only treatment of diseases. Since ancient times, '*Ayurveda*' has been looked after, as a holistic approach that instead of the disease, treats the individual as a whole. Ethnomedicine has proved essential in treating several diseases and disorders that are known since ancient times. Herbal supplements, detox treatments, etc. have addressed the root cause of the disease, as well as the individual suffering from it. *Ayurveda* is extremely beneficial in boosting the immune system and treating chronic respiratory diseases simultaneously. To date, respiratory disorders such as asthma, pneumonia, Bronchitis, chronic obstructive pulmonary disease (COPD), respiratory allergies, rhinusitis, hay fever, etc. have been treated using herbal medicines mentioned in *Ayurveda*. Basically, ethnomedicinal drugs and *ayurvedic* herbs help to control infection and/ or disease, by improving the respiratory function and metabolism of an individual. In *Ayurveda*, not only the edible parts of the plants but also the non-edible parts that are usually considered waste, are used as herbal medicines. In recent years, several non-edible parts of the herbal plants have been studied, including the hard shells of *Juglans regia* which were found to be rich in bioactive components found in nature[18]. Herbs with potential phytochemicals that have antiviral effects on virus receptors or their target sites or have the ability to interfere with virus replication in the host can be used as natural antivirals and potent immune enhancers or modulators. Plant-based conventional medicines have been in use for ages. Medicinal plants as a whole are useful for therapeutic and healing purposes, including for cessation of viral proliferation in the body. This is achieved by regulating viral adsorption, binding to host cell receptors, inhibiting viral fusion into the host cell membrane, and intracellular signal modulations[19,20]. Similarly, the binding interaction between a viral protein and a phytochemical ligand derived from herbal extracts may result in the inhibition of the activity of viral proteins[21]. Enough evidence of traditional medicines being used to treat viral infection by enhancing immunity of the body is available in the literature[22-24]. The current research focuses on medicinal herbs such as black pepper, Arjunsal, neem, and giloy. Individual, as well as synergistic antiviral effects of these herbs, have been studied in this study.

Piper nigrum (Black pepper), a commonly found spice in Indian households, is known for its antioxidant, antiviral, anticancer, and antimicrobial activity[25]. It has potential immunomodulatory effects that help in reducing inflammation[26]. Piperine, a bioactive component found in black pepper, is known to possess antiviral activity against Dengue and the Ebola virus. In a study in 2020, piperine was proven to inhibit the Methyltransferase of Dengue and VP35 Interferon Inhibitory Domain (PDB id 3FKE) of the Ebola virus in comparison with the commercial antiviral available for treatment[27]. *Terminalia arjuna* is known for its

antimicrobial as well as antiviral activity. Casuarinin, a tannin present in the *Terminalia arjuna* bark, is capable of antiviral activity against Herpes simplex virus type-2 (HSV 2)[28]. *Azadirachta indica* leaves and bark has been used in several diseases treatment in *Ayurveda*. Research has proven that the biologically active compounds from the neem tree possess antiviral properties. When the neem leaves were investigated for their antiviral activity against SARS-CoV-2, it was known that they can work as RBD-ACE2 inhibitors by blocking viral cell entry. Azadirachtin H, quentin, and margocin were the potential compounds in *Azadirachta indica* that demonstrated viral cell entry inhibition properties[29]. *Tinospora cordifolia* or GiloyGhanvati is a herb that is found as an ingredient in tablets manufactured by Patanjali Ayurved Limited. In a recent study in 2021, zebrafish models were used to determine the effect of GiloyGhanvati in reversing the inflammation caused due to SARS-CoV-2 spike proteins[30]. Apart from the antiviral activity against SARS-CoV-2, GiloyGhanvati has also been found to possess the antiviral effect against HIV, and HTLV by inhibiting the virus to target T helper cells[31]. In another study in 2019, *Tinospora cordifolia* was found to possess phytochemicals with an immunomodulatory activity that improved the phagocyte function in infected humans, without affecting cell-mediated and humoral immunity[32].

As all the herbs mentioned above which are used in this research study have proven to be capable of antiviral, antioxidant, and immunomodulatory effects at an individual level, the individual and synergistic action of these against SARS-CoV-2 can be remarkable. The hypothesis of the current study was to identify phytochemicals in 4 herbal extracts and their ayurvedic blend and determine the antiviral activity of the Indian medicinal herbs compared to Remdesivir as a standard drug using the qRT-PCR method *in vitro*. As secondary metabolites from natural products have a high potential for chemical novelty and biological activity, extensive modern and ancient studies on medicinal plants have resulted in the investigation of new natural compounds with pharmaceutical properties and their application for holistic approach-based treatment. It has been reported that about 50,000 species among the total 500,000 plants in the world are used in conventional medicine. The recent outbreaks of many virus-related new diseases including SARS-CoV-2, H1N1, influenza virus, and MERS-CoV, suggest that bioactive compounds from natural resources against infectious viruses should be constantly studied and developed for the protection of human health. The main objective of using natural plants as a research source is to find bioactive compounds against zoonotic diseases by pathogenic viruses in humans. While screening plants that were used in India traditionally as medicinal herbs to treat respiratory disorders, *Piper nigrum*, *Terminalia arjuna*, *Azadirachta indica*, and *Tinospora cordifolia* were known to be used widely. Considering these ethnomedical resources, identifying the phytoconstituents in these extracts, and screening their antiviral ability, they showed potential viral inhibitory effects against SARS-CoV-2. In this research, we set out to evaluate the antiviral activity against SARS-CoV-2 of 4 extracts produced from medicinal plants frequently used within traditional medicine in India to find possible therapeutic alternatives to face COVID-19 and their brief role in strengthening the immune system in order to ward off the pathogenic organisms is discussed.

Materials and Methods

General experimental procedures: *Piper nigrum* peppercorns, *Terminalia arjuna* stem, *Azadirachta indica* leaves, and *Tinospora cordifolia* stem were procured from D.G. Herbal *Ayurvedic* medicinal shop in Mumbai. The samples were washed using distilled water and oven dried in a hot air oven at 80^{o}C for 48 hrs. Remdesivir (100mg/ml) was used as a standard drug, which was procured from Local hospital pharmaceutical. The medicinal herbs were then ground to a fine powder using a grinder. An ayurvedic extract was made up using a 1:1 proportion of the 5 herbs mentioned above. Lab-grade ethanol was used to prepare a 100% concentration of the herbal ethanolic extracts and their ayurvedic mixture. The ethanolic herb extracts obtained were stored in air-tight glass beakers at room temperature in a shaker with intermittent shaking for 7 days. The ethanolic extracts obtained after 7 days were filtered using a clean muslin cloth and transferred to Eppendorf. 1 mg/ml Eppendorfs were prepared. The extracts were purified by filtering through a syringe filter of pore size 0.2 µM and the solutions were preserved at −20°C until use.

Phytochemical identification: Liquid chromatography - Mass spectrometry (LC-MS) analysis of the ethanolic herbal extracts was carried out using the Thermo/Finnigan Surveyor System consisting of a degasser, autosampler, binary pump, and column heater. The column outlet was coupled to Thermo fleet (LCQ-Fleet) Ion Trap MS equipped with an ESI ion source. Data acquisition and mass spectrometric evaluation were carried out using Data Analysis software (Qual Browser; Thermo Electron, San Jose, CA). For the chromatographic separation, a Phenomenexluna 5-µm C8 column (250×4.6mm) was used. The column was held at 95% of 0.1% acetic acid in water and 5% of 0.1% acetic acid in acetonitrile for 1 min, followed by an 11 mins step gradient from 5% of 0.1% acetic acid in acetonitrile, then 4 mins with 100% of 0.1% acetic acid in acetonitrile. Elution was achieved with a linear gradient from 100% of 0.1% acetic acid in acetonitrile to 5% of 0.1% acetic acid in acetonitrile for 2 mins. The flow rate was 200µl/min and the injection's volume was 5µl. The capillary temperature was 300°C, the nebulizer pressure was 40 psi, and the drying gas flow rate was 15L/min.

***In vitro* qualitative RT-PCR studies:** The antiviral activity of herbal extracts was evaluated by a pre-post-infection treatment strategy, where the positive SARS-CoV-2 nasopharyngeal, oropharyngeal samples were checked in real-time before being treated with the herbal extracts and after being treated with the herbal extracts. Briefly, 2 method parameters were used to study

the antiviral effect of the extracts. In the first method, the herbal extracts were added to the SARS-CoV-2 positive samples before RNA extraction, and in the second way by addition into the SARS-CoV-2 RNA extracted template. 0.2% and 0.4% concentrations were used in the first method where 0.5 ml and 1 ml of the 100% ethanolic herbal extracts respectively, were added into the SARS-CoV-2 positive sample VTMs. For the standard drug Remdesivir, 100mg/ml of the drug was added to the positive sample VTM. The extracts were mixed homogeneously and then the post-herbal extract treatment SARS-CoV-2 samples were added into Q-line RNA extraction 16 deep well plates for further processing in a semi-automated Q-line RNA extraction machine. The obtained RNA template from the RNA extracted deep well plate was then added into the complete applied biosystems CoviPath COVID-19 RT-PCR kit Master mix solution in the 96 well optical bottom plate. For the second method, a concentration of 8% and 20% were used where 2.5µl and 5µl of the 100% ethanolic herbal extracts respectively were directly added into the 96-well optical bottom plate aliquoted with the complete master mix. Positive control (PC) and negative control (NC) were used as per kit instructions. Non-template control (NTC) was set by adding RNase-free distilled water into the complete master mix. One of each PC, NC, and NTC was considered. Biorad CFX96 Real-time PCR machine was set at optimized protocol for processing SARS-CoV-2 samples for the detection, using FAM, VIC, and TEXAS RED channels for SARS-CoV-2 ORF gene, SARS-CoV-2 N gene, and Human origin genes respectively. The experiment was performed in triplicates.

Analyzing data in real-time: The data obtained for pre-treated (untreated) SARS-CoV-2 samples was analyzed by considering the CT (cycle threshold) values for the FAM channel that denotes SARS-CoV-2 ORF gene, VIC channel that denotes SARS-CoV-2 N gene and TEXAS RED channel that denotes the Human origin genes. RFU (Reference fluorescence units) were considered for all the 3 channels. The CT and RFU values were noted for the pre-treated and post-treated SARS-CoV-2 nasopharyngeal, and oropharyngeal samples. The difference in the pre-post treated SARS-CoV-2 samples was used for the determination of the SARS-CoV-2 inhibition activity of the herbal extracts.

Results and discussion

Phytochemical identification and screening from Herbal extracts: On conducting LCMS analysis of the extracts obtained from *Piper nigrum* peppercorns, *Terminalia arjuna* stem, *Azadirachta indica* leaves, *Tinospora cordifolia* stem, and their ayurvedic blends, 12, 7, 13, 16, 15 and 51 phytoconstituents were identified respectively. Phytoconstituents Propanedioic acid, dimethyl ester; Bicyclo [3.1.1] heptane,6,6-dimethyl-2-methylene-,(1S)-; 3-Carene; Cyclohexene; 1,6-Octadien3-ol,3,7- dimethyl-; 2-Methyl-1-ethylpyrrolidin e; 2-Isopropenyl-5-methylhex-4-enal; L-α-Terpineol; (R)-lavandulyl acetate; Pyrrolizin-1,7-dione-6-carboxylic acid, methyl(ester); 7-epi-cis-sesquisabinene hydrate; Stigmasterol; Ursodeoxycholic acid; 2-Cyclohexen - 3-ol-1-one, 2-[1- iminotetradecy l]-; 9-Octadecenami de, n-butyl; Z-5-methyl-6-heneicosen-11-one; 1-Heptatriacotanol; (-)-Spathulenol; Cinnamic acid, 4-hydroxy -3- methoxy-,{5-hydroxy-2-hydroxy methyl; Piperine were identified from ethanolic extracts of *Piper nigrum*. In the ethanolic extracts of *Terminalia arjuna* stem, Gallic Acid; 3,4 Dihydroxy Benzoic Acid; Caffeic Acid; Coumaric Acid; Ellagic Acid; Chlorogenic Acid; Arjunetin were identified. Leaf ethanolic extracts of *Azadirachta indica* showed Betulin; Ginsenoside; Caryophyllene oxide; Soyasaponin I; Ecgonine; Scutellarin; Epicatechin; Icariin; Sesamolin; Lupeol; Andrographolide; 10-Deacetylbaccatin III; 3-Acetyl-11-keto- βboswellic acid; O-chloroacetylcarbamoyl fumagillol; Rutin; Azadirachtin. *Tinospora cordifolia* stem ethanolic extract showed the presence of phytoconstituents Cordiol; Berberine; Syringin; Isocolumbin; Ecdysterone; Cordioside; Cordifolioside A; Tembetarine; Palmitine; Columbin; Tinocordifolin; Octacosanol; Heptacosanol; nonacosan-15-one; makisterone A. Whereas the ayurvedic blend of the 4 extracts mentioned above showed the presence of Gallic Acid; 3,4 Dihydroxy Benzoic Acid; Coumaric Acid; Ellagic Acid; Chlorogenic Acid; Arjunetin; Propanedioic acid, dimethyl ester; 3-Carene; Cyclohexene; 2-Methyl-1- ethylpyrrolidin e; 2-Isopropenyl-5-methylhex-4- enal; L-α-Terpineol; Stigmasterol; Ursodeoxycholic acid; 9-Octadecenami de, n-butyl; 1- Heptatriacotanol; Cinnamic acid, 4-hydroxy -3- methoxy-,{5- hydroxy-2- hydroxy methyl; Piperine; Betulin; Ginsenoside; Caryophyllene oxide; Ecgonine; Scutellarin; Epicatechin; Lupeol; Andrographolide; Soyasaponin I; Rutin; Azadirachtin; Columbin; Octacosanol; Cordiol; Isocolumbin; Ecdysterone; Cordioside; Cordifolioside A; Heptacosanol; nonacosan-15-one; makisterone A.

Evaluating antiviral activity of the herbal extracts *In vitro* using qualitative RT-PCR: The SARS-CoV-2 inhibitory activity of the herbal extracts was evaluated by careful consideration of the difference between the pre and post-treated SARS-CoV-2 samples. As higher amounts of nucleic acids are detected in earlier cycles that are determined with lower CT values and higher RFU values, the focus of this research was on observing the difference between the pre and post-treated SARS-CoV-2 samples. On testing the *Piper nigrum*, *Terminalia arjuna*, *Azadirachta indica*, and *Tinospora cordifolia* medicinal herb extracts individually and all together in an ayurvedic form against SARS-CoV-2 samples, the results obtained showed increased CT values of the SARS-CoV-2 positive samples. Maximum antiviral activity was observed in the 0.4%, and 20% concentrations of *Piper nigrum*, *Terminalia arjuna*, and ayurvedic extracts as mentioned in Table-1, Figure-1, 2, 5. This indicates that the herbal extracts *Piper nigrum, and Terminalia arjuna* at an individual level, and all 4 herbal extracts at a synergistic level, are capable of decreasing the viral load of SARS-CoV-2. As the CT values of all SARS-CoV-2 positive samples fall between 15-23 for both Orf and N genes, the results obtained show that the phytochemicals in the herbal extracts act

on the Orf and N gene in the SARS-CoV-2 viral sequence. RFU values significantly decreased from 6000 to 1500 in SARS-CoV-2 samples treated with herbal extracts. The CT values of samples treated with Remdesivir as a standard drug increased equivalent to those treated with medicinal plants extracts, indicating that these naturally obtained medicinal extracts were as effective antiviral against SARS-CoV-2 as remdesivir; thus the naturally obtained extracts can be used as an alternative to the synthetically derived drugs to treat infections caused by coronaviruses.

Table-1: Pre-post treatment CT and RFU values of SARS-CoV-2 with the herbal extracts, their ayurvedic blend and standard drug remdesivir.

Sample	CT value (Fam)	CT value (Vic)	RFU (Fam)	RFU (Vic)
Positive sample 1	17	15	3800	6000
Positive control	25	23	3000	3400
0.2% *P. nigrum*	23	20	1800	4200
0.4% *P. nigrum*	33	33	1000	2200
8% *P. nigrum*	23	20	2900	5000
20% *P. nigrum*	35	36	900	900
Positive sample 2	19	20	3800	5500
Positive control	25	23	3000	3400
0.2% *T. arjuna*	34	33	1500	3000
0.4% *T. arjuna*	34	39	700	500
8% *T. arjuna*	24	25	1300	1500
20% *T. arjuna*	32	37	700	1400
Positive sample 3	22	21	3200	5200
Positive control	25	23	3000	3400
0.2% *A. indica*	23	22	3900	6000
0.4% *A. indica*	28	27	3000	5100
8% *A. indica*	26	26	2200	4200
20% *A. indica*	27	27	2100	4000
Positive sample 4	22	23	3200	5100
Positive control	25	23	3000	3400
0.2% *T. cordifolia*	25	24	3900	6000
0.4% *T. cordifolia*	26	26	3200	5200
8% *T. cordifolia*	26	26	2000	2600
20% *T. cordifolia*	27	27	2000	4000

Positive sample 5	22	20	3200	5100
Positive control	25	23	3000	3400
0.2% ayurvedic extract	30	29	1300	1500
0.4% ayurvedic extract	34	34	600	1400
8% ayurvedic extract	25	25	2000	4000
20% ayurvedic extract	31	33	1200	1200
Positive sample 6	18	18	3800	5800
Positive control	25	23	3000	3400
100 mg/ml Remdesivir	26	26	2000	2600

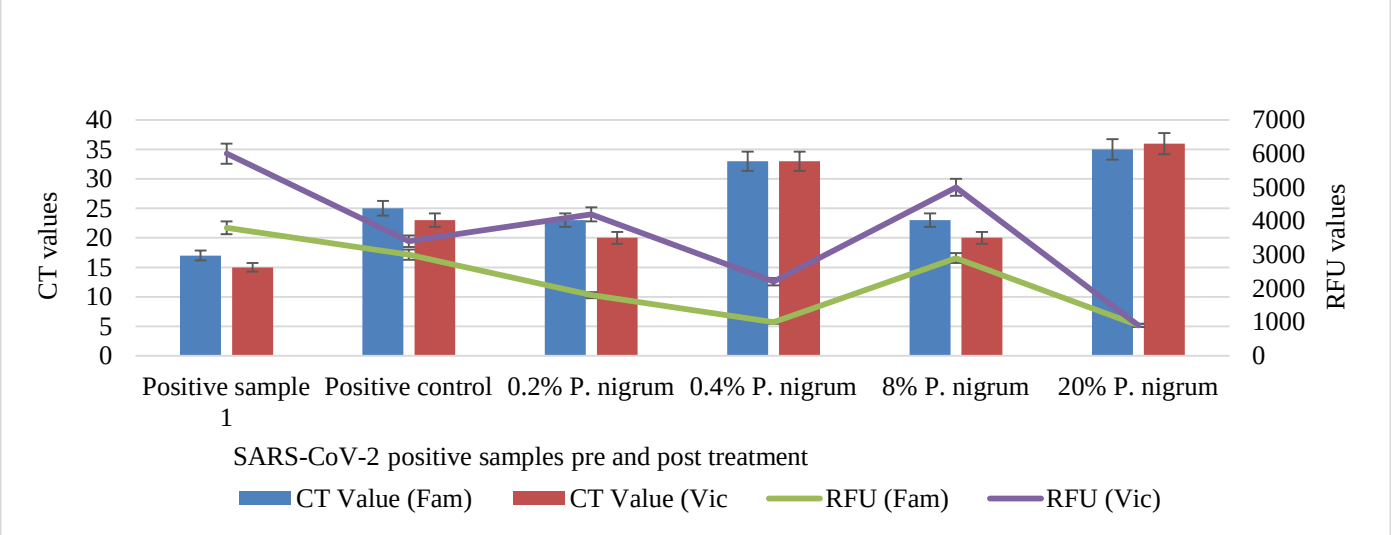

Figure-1: Pre-post treatment CT and RFU values of SARS-CoV-2 with *Piper nigrum.*

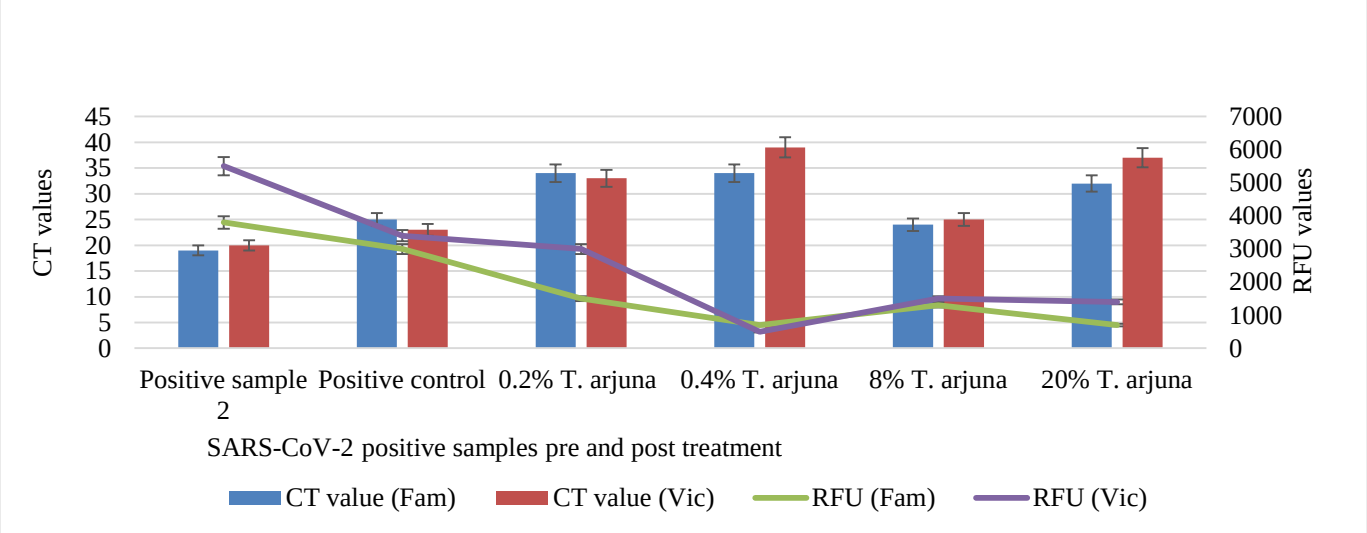

Figure-2: Pre-post treatment CT and RFU values of SARS-CoV-2 with *Terminalia arjuna.*

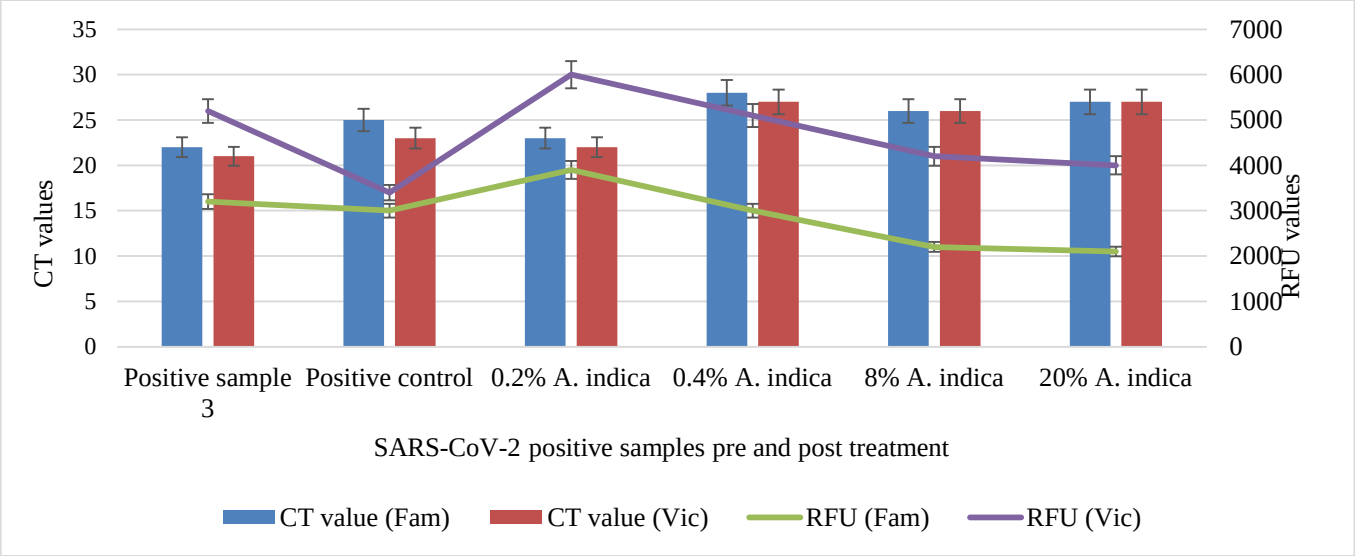

Figure-3: Pre-post treatment CT and RFU values of SARS-CoV-2 with *Azadirachta indica.*

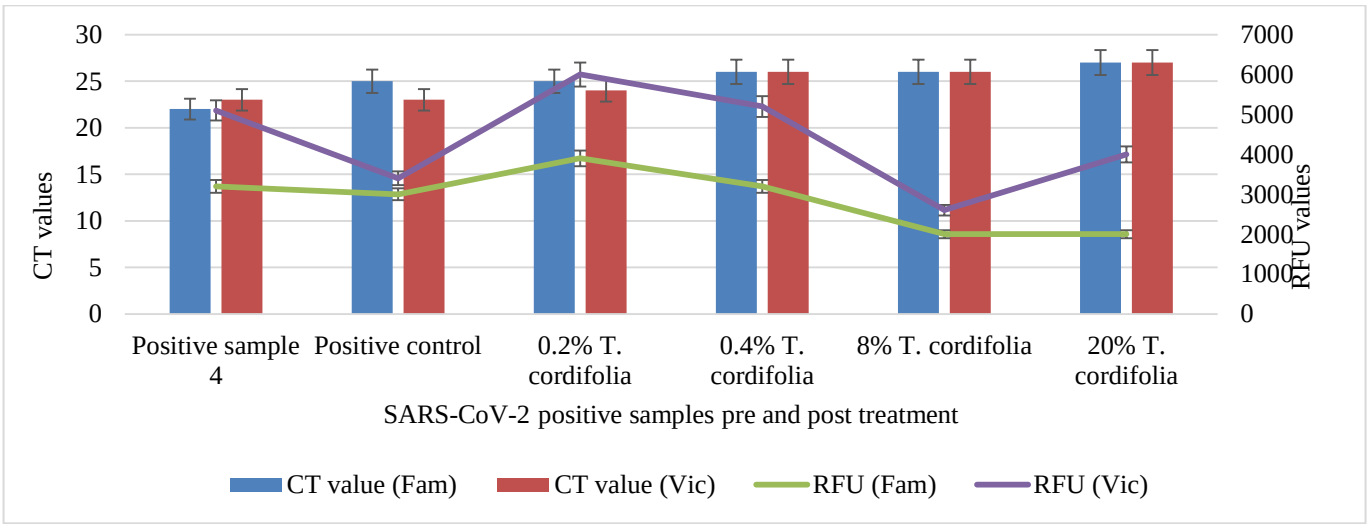

Figure-4: Pre-post treatment CT and RFU values of SARS-CoV-2 with *Tinospora cordifolia.*

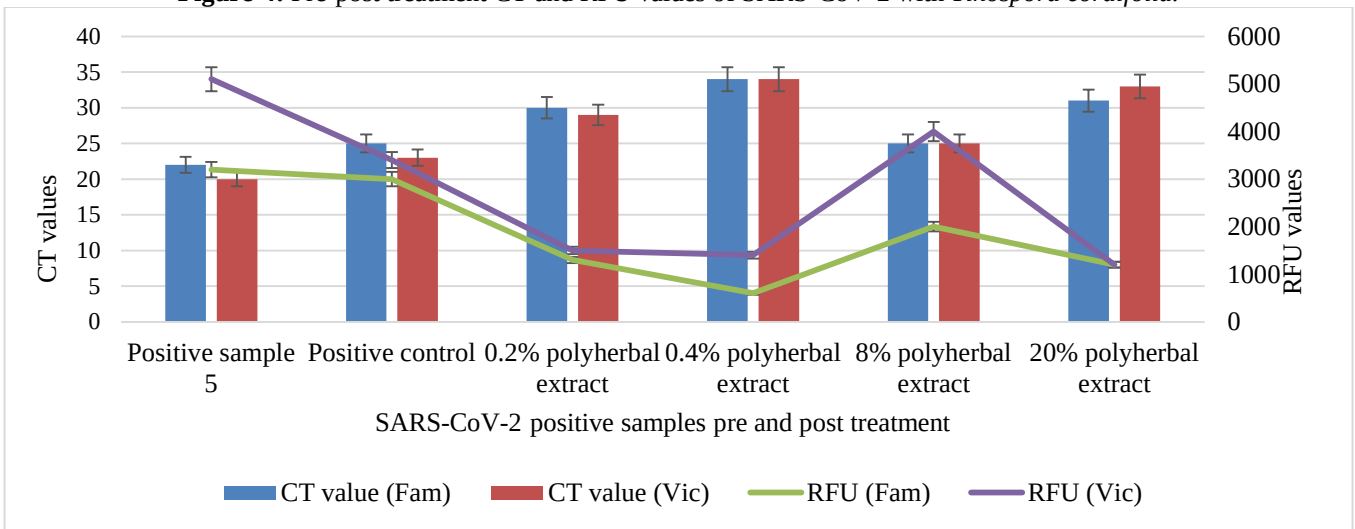

Figure-5: Pre-post treatment CT and RFU values of SARS-CoV-2 with the ayurvedic blend.

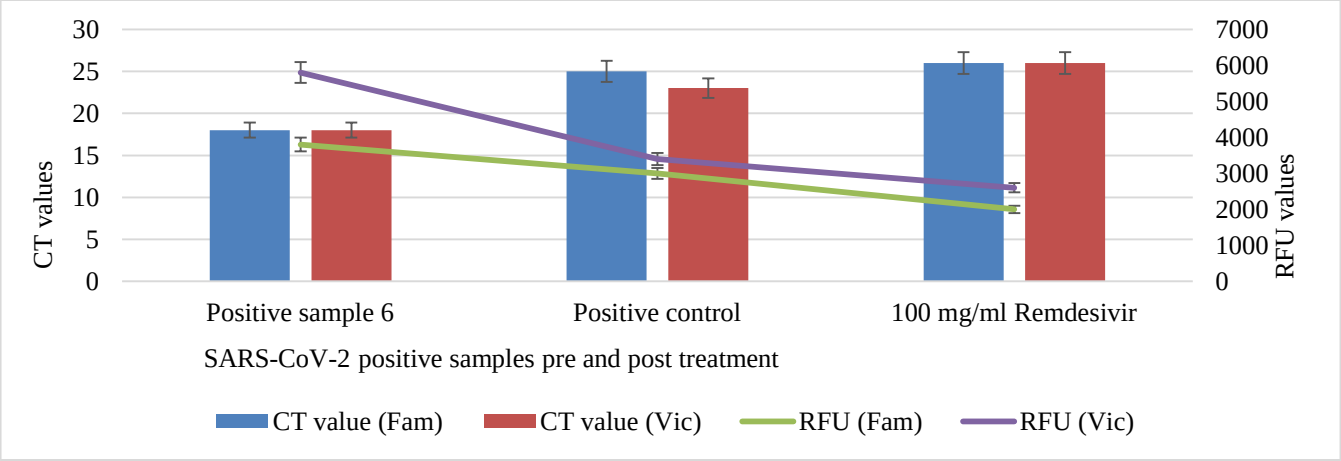

Figure-6: Pre-post treatment CT and RFU values of SARS-CoV-2 with Remdesivir.

Conclusion

This article evaluated the antiviral activity of 4 herbal extracts prepared from 4 medicinal plants and their ayurvedic blend used by indigenous people from India in ancient times to treat respiratory tract infections, which were also found to inhibit the viral load of SARS-CoV-2 *in vitro* by qRT-CR. The current study brings light to the efficacy of these traditional treatments widely used in India. Usually, medicinal herbs cleave the virus and target its viral receptors and achieve antiviral activity against the virus. Our results showed that *Piper nigrum, Terminalia arjuna,* and the ayurvedic blend of 4 herbal extracts have a maximum antiviral effect against SARS-CoV-2 *in vitro,* which is similar to that of Remdesivir. Given the literature review of all the medicinal values of the herbal extracts used in this research and the results obtained in this study, the antiviral and immunomodulatory properties of the extracts pointing towards the CD4+ and CD8+ immune cells stimulating ability in this research were clearly exhibited and confirmed. Thus, this research study investigated the antiviral activity of *Piper nigrum, Terminalia arjuna, Azadirachta indica, Tinospora cordifolia,*and their ayurvedic blend using qRT-PCR studies *in vitro* and illustrated the antiviral and immunomodulatory activity of the medicinal herbs against SARS-CoV-2. A comparison of results obtained of standard drug redelivers and the medicinal plant extracts denoted the antiviral efficacy of the plants being as effective as synthetically derived drugs, without affecting the body along with increased immunity. Our studies thus provided a new class of natural scaffolds that can be developed as potential anti-SARS-CoV-2 agents that inhibit the replication of the virus in the host. These medicinal plants can be used as an alternative to synthetically derived drugs with adverse side effects. Furthermore, human coronaviruses, like MERS, from the same Coronaviridae family share some similar replication mechanisms with SARS-CoV-2, and the development of antivirals against MERS and SARS-CoV-1 shares similar observance as per the literature review[33]. Therefore, the anti-SARS-CoV-2 molecules obtained in this study could also be significantly noteworthy candidates for further investigation against the fatal human coronaviruses, including SARS-CoV-2 and MERS.

Statistical analysis: The results expressed are as the means ± SD of three independent experiments. Two-tailed unpaired student's t-test was used for the analysis of the statistical differences between sample groups in all experiments, assuming equal variances. Statistical significance was accepted at $p < 0.05$.

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