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Antiviral efficacy of *Andrographis paniculata* and andrographolides: A narrative reviewKumarappan Chidambaram[✉]

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ABSTRACT

The rise of emerging infectious diseases has become notably prominent due to ecological changes and mutations in pathogens. The respiratory illness outbreak caused by the COVID-19 pandemic has spread globally. Natural products contain numerous structures and biological activities, offering ample options for discovering new antiviral drugs with unique targets and mechanisms. *Andrographis paniculata* has been utilized in Indian Ayurvedic, Swedish, Traditional Thai, and Chinese medicine to alleviate coughs, colds, and influenza symptoms. Early-stage laboratory studies indicate that this herbal extract may reduce inflammation and fever, and boost the body's natural defenses against viruses, potentially leading to symptom relief. This review aims to systematically present clinical trial data about antiviral herbal formulations derived from *Andrographis paniculata*, delineating the antiviral effects of both natural and synthetic derivatives, along with *in silico* analyses.

KEYWORDS: Ayurveda; *Andrographis paniculata*; Antiviral formulations; Andrographolides; COVID-19

1. Introduction

Viral infections are a major cause of human diseases, requiring global prevention. Despite advancements in immunization and pharmaceutical research, the lack of preventive vaccines and effective treatments remains a challenge, with natural products as a promising source for drug discovery. The augmentation of viral resistance to current antiviral drugs and therapeutic approaches represents a looming challenge that the global population is anticipated to confront in the foreseeable future. Plants are a major source of new chemical entities to develop novel therapeutic agents[1]. Pure compounds like andrographolide, curcumin, glycyrrhizinic

acid, and several plant extracts of *Azadirachta indica* have shown promising antiviral activities against several viruses. They should be investigated further for their therapeutic potential. Medicinal plants and their naturally active phytochemicals are important and powerful resources with diverse antiviral properties. Plants of Indian origin have provided several novel leads and are likely to yield newer chemical entities. Medicinal botanicals represent a promising avenue for the exploration of therapeutic candidates targeting coronavirus disease 2019 (COVID-19). Natural compounds derived from plants, having undergone rigorous evaluations for safety and efficacy, and being globally accessible, particularly in the context of the prevalent burden of respiratory infections, can play a pivotal role in addressing the ongoing pandemic caused by COVID-19[2].

Andrographis paniculata (*A. paniculata*) Nees (Acanthaceae), a widely cultivated medicinal plant in China, is found throughout Southeast Asia and India. In India and China, *A. paniculata* is often used to treat respiratory diseases, including upper respiratory tract infections (URTIs), inflammation, and recurrent viral infections[3,4]. In China, andrographolide and dehydroandrographolide are called “natural antibiotics”[5]. Both compounds are the main active components in *A. paniculata*, which contribute to the anti-infectious properties of the plant. Dehydroandrographolide has been found to enhance innate immunity of the intestinal tract by upregulating the expression of human β -defensin-2 by modulating the p38

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MAPK signaling pathways[6]. Antiviral Chinese and Indian herbal medicines have advantages in preventing and treating influenza virus infection[7,8].

Therefore, natural products with or without chemical modification might offer great potential for developing new lead compounds as antiviral agents. The highly pathogenic avian influenza SARS-CoV2 and H5N1 viruses have recently become a worldwide public health threat, and current antiviral therapies have limited activity against emerging, resistant influenza viruses. Therefore, effective drugs with novel targets against SARS-CoV-2, influenza A, Ebola, and H5N1 strains, should be developed. SARS-CoV-2 is a new viral disease, so there is an urgent need to develop antiviral agents for this new coronavirus disease. This review summarizes earlier studies on the efficacy of *Andrographis* extract on the common cold and its traditional use for COVID-19-associated symptoms and infections.

Most studies corroborate the hypothesis that supplementation with *A. paniculata* stimulates and regulates the immune system. The *A. paniculata* extract and its andrographolide derivatives have been discovered as natural lead chemicals. These compounds have recently been specifically chosen and assessed using *in silico* and *in vitro* models to evaluate their potential in the management of COVID-19. This narrative review integrates clinical, *in silico*, and *in vitro* data to establish a scientific basis for the antiviral potential of andrographolides extracted from *A. paniculata* and related synthetic analogues. The review aims to elucidate the antiviral properties of *A. paniculata*, andrographolides, and their synthetic counterparts, focusing primarily on clinical and preclinical (*in vitro* and *in silico*) investigations, to stimulate further scientific inquiry and expedite the advancement of novel antiviral medications derived from *A. paniculata* and andrographolides.

2. Antiviral phytoconstituents from *A. paniculata*

A. paniculata is one of the most known medicinal plants used in Ayurveda to treat an array of diseases[9]. Several diterpenoid lactones and flavonoids were isolated from *A. paniculata*[10]. The considerable interest in employing *A. paniculata* for symptomatic relief in acute respiratory tract infections is evidenced by a substantial volume of randomized clinical trials conducted and documented, as indicated in a systematic review and meta-analysis[11]. Andrographolide is a main phytoactive compound of *A. paniculata* that has been revealed to possess antiviral activity against numerous viruses[12–14]. Andrographolides and its derivatives have anti-infection, anti-tumour, and immunoregulatory effects. Numerous analogues of andrographolide exhibiting immunostimulatory properties have been identified. Andrographolide has been documented to possess immunoregulatory activities, manifesting varied effects in distinct

immune disease models. Its role as a modulator of altered immune responses is evident, showcasing its potential to influence immune-related processes[15]. In recent years, pharmaceutical chemists have synthesized large numbers of andrographolide derivatives, which bear significant antiviral effects[16,17]. A wide range of antiviral studies have been conducted on crude extract and its active constituents by researchers, especially in Asia, following reports about the immunomodulating properties possessed by this plant, mostly according to traditional medical practitioners in Ayurveda.

Similarly, Complementary and Alternative Medicine systems such as Traditional Chinese Medicine and various preparations of *A. paniculata* were reported to be effective for the common cold in adults[18]. Thus, andrographolide may have wide-ranging cellular effects, with several pathways affected, resulting in various antiviral effects. A recent molecular docking study has shown that andrographolide derivatives led to the strongest binding to the hemagglutinin active site and inhibited neuraminidase H1N1[19]. Andropanolide B has been shown to have weak *in-vitro* antiviral activity against the respiratory syncytial virus[20]. 14-Dehydroandrographolide succinic acid monoester demonstrated the protease inhibitory activity possibly by suppressing the proteolytic cleavage of envelope glycoprotein gp160 of HIV[21]. Another study shows that 14-deoxy-11,12-dehydroandrographolide impairs H5N1 influenza replication at least in part by restraining the nuclear export of viral ribonucleoprotein complexes, and the inhibition of viral replication leads to a subsequent decrease of the intense proinflammatory cytokine/chemokine expression[22]. Hence, andrographolide and its derivatives are effective against various respiratory viral infections, and they could be used alone or in combination with other medications to treat SARS-CoV-2 infection.

3. Antiviral and immunoregulatory activity of *A. paniculata*

For decades, andrographolide and its derivatives have been routinely utilized in China and India to treat respiratory infections. *A. paniculata* ethanol extract inhibited the simian retrovirus titer at 1 µg/mL like the positive control Lamivudine[23]. *A. paniculata* significantly reduced inflammation and oxidative stress in both *in vivo* and *in vitro* models of acute lung injury. This effect appears to be mediated by inhibiting NLRP3-mediated pyroptosis through the suppression of ROS production *via* the Akt/Nrf2 pathway[24]. A very recent virtual screening study suggested that andrographolide, neoandrographolide, and 14-deoxy-11,12-didehydroandrographolide from *A. paniculata* could effectively interact with important targets of SARS-CoV-2, such as 3-chymotrypsin-like protease (3CL^{pro}), Spike, and papain-like protease (PL^{pro})[25]. *A. paniculata* potentially

inhibited H5N1 virus replication by upregulation of *IFN- β* and *TNF- α* mRNA expressions in MDCK cells[26]. Panossian *et al.* concluded that *Andrographis* and Kan Jang influence the activation and proliferation of immunocompetent cells *in vitro*. They stimulate the production of key cytokines and immune activation markers[27]. A novel benzyl amino derivative of andrographolide exhibited the highest *in vitro* antiviral potency against H3N2 compared to all reported compounds. This novel compound was approximately 1.5 times more potent than Lianbizhi and other andrographolide analogues currently used clinically in China. Significantly, the adamantyl derivative exhibited the lowest toxicity, demonstrated by higher TC_{50} and TI values compared to Lianbizhi against the H3N2 virus[28].

4. Antiviral formulations from andrographolide

Restomune, a formulation made from the whole plant *A. paniculata*, demonstrated immunostimulant properties and had a highly promising HIV inhibitory effect, which provides solid ground for conducting further clinical studies. It is believed that Restomune acts against the host cellular proteases that mature HIV viral glycoprotein and help viral fusion of host cells. Restomune administration causes viral replication inhibition, acts against one or multiple host cellular proteases, and has anti-inflammatory properties[29]. Similarly, Xiyanping injection is produced by the sulfonation process of andrographolide from *A. paniculata*. A recent retrospective cohort method-based clinical study in 2019 on Xiyanping injection has proven its clinical outcomes on community-acquired pneumonia, whose results showed the Xiyanping injection had better effects and was more economical than conventional treatments[30,31]. Another Traditional Chinese Medicine, Xingnaojing, positively affected patients with respiratory infections and pneumonia, which can prevent inflammatory mediators and improve pulmonary function[32–34]. The China Food and Drug Administration has approved Xiyanping injections, which contain andrographolide sulfonate, for the treatment of respiratory infections. Additionally, combining these injections with piperacillin and sulbactam significantly improved the clinical efficacy of pulmonary infection treatment in elderly patients[35]. Table 1 presents a compilation of herbal remedies and prescriptions commonly used for respiratory infections, including SARS-CoV-2[36–53]. Recent clinical studies have explored the potential of *A. paniculata* extract and its formulations for treating early COVID-19 in patients without high-risk comorbidities, capitalizing on its ability to inhibit SARS-CoV-2 replication.

5. Clinical trials of *Andrographis* formulations on respiratory infections and COVID-19

Several randomized controlled trials (RCTs) examining the effects of *A. paniculata* formulations on URTIs were searched through August 2021 in the Cochrane Library, Scopus, MEDLINE/PubMed, and Embase databases. Based on the literature search, this review found 13 clinical trials (including randomized trials) of *Andrographis* formulations for URTIs (Table 2). These studies revealed that *A. paniculata*-based formulations were considerably better than placebo at reducing the frequency and severity of respiratory symptoms in patients. *Andrographis* extracts have been shown to alleviate the symptoms of colds and respiratory symptoms in a few well-designed studies. It may also have the added benefit of aiding in preventing respiratory infections. Only a few minor side effects were reported, suggesting that these *Andrographis*-based formulations could be used with conventional therapy to treat respiratory illnesses. More study is needed to compare this herbal treatment to conventional medications for respiratory infections to assess the potential of *Andrographis* formulations as a supplement or an alternative to standard antiviral treatment.

Based on the results of preclinical evidence and multiple clinical trials, *Andrographis* formulations are well tolerated and may be useful in alleviating symptoms of URTIs; nevertheless, the evidence allows for a potential application in the management of COVID-19 early symptoms. With no serious adverse events observed, the evidence and data from clinical trials suggest that this herbal therapy may have immunomodulatory and antiviral effects against COVID-19-related illnesses and symptoms. These unique properties of andrographolide and *A. paniculata* extract can be tested as an anti-coronaviral drug. The various targets of SARS-CoV-2 for andrographolide and its derivatives are depicted in Figures 1 and 2. However, future *in vivo* research may be warranted to verify these andrographolide analogs as better SARS-CoV-2 antivirals.

The information regarding recent clinical trials of *A. paniculata* extract and its formulation in patients with COVID-19 is presented in Table 3. A randomized, controlled pilot study assessed the use of CIM-MEG19 (a standardized *A. paniculata* formulation) as an add-on treatment for mild to moderate COVID-19 patients. The study evaluated the efficacy and safety of CIM-MEG19 in the management of COVID-19, clinical recovery, and outcomes related to hospitalization duration. Additionally, the investigation sought to identify any signs of increased severity due to potential drug-drug interactions between CIM-MEG19 and the standard of care. The study revealed an excellent safety profile for CIM-MEG19, with a notable reduction in the severity of the infection and a halt in the progression of the disease. These findings suggest that CIM-MEG19 holds promise as a potential natural drug for the treatment

Table 1. Efficacy and indications of *Andrographis paniculata* (*A. paniculata*) and its bioactive compounds-based formulations commonly used in practice as immunomodulatory or antiviral agents against URTIs and COVID-19.

Type of traditional medicine	Formulations	Ingredients	Efficacy	Indications	Reference
TCM	DAS injection (potassium or a combination of potassium and sodium salt)	Dehydroandrographolide succinate	Immunostimulatory, anti-infective and anti-inflammatory effect	Viral pneumonia and URTIs	[5]
TCM	Xiyanping injection	Andrographolide sulfonate	Antiviral effect and improve immunity	Bronchitis, viral infection, and SARS-CoV-2	[30,31,36]
TCM	Chuanxinlian Tablet	Andrographolide	Antiviral	Acute respiratory tract infections	[37]
TCM	Andrographolide total ester sulfonate injection	Andrographolide sulfonate	Anti-antitussive, expectorant, and anti-inflammatory activity, as well as bronchodilatation effect	Acute respiratory tract infections and influenza	[38]
Ayurveda	Extract of <i>A. paniculata</i> (KalmCold)	Andrographolide	Antiviral	URTIs	[39]
Swedish herbal medicine	SHA-10 extract (Kan Jang)	Standardized <i>A. paniculata</i>	Anti-cold	Uncomplicated common colds	[40–43]
TCM	Andrographolide (Lian-Ju-Gan-Mao capsules)	Chuanxinlian (herba andrographis)	Anti-cold	Common cold with wind-heat syndrome	[44]
Canadian herbal medicine	Restomune	The whole plant of <i>A. paniculata</i> extract	Antiviral and immunomodulatory activity	SARS-CoV-2	[45,46]
TCM	Lianbizi injection, Yanhuning injection, and Chuanhuning injection	14-dihydroxyandrographolides 14-dehydroxy-11, 12-didehydroandrographolides	Immunomodulatory	Antiviral and anti-SARS-CoV-2	[47,48]
Swedish herbal medicine	Kan Jang	A fixed combination of <i>A. paniculata</i> and <i>Eleutherococcus senticosus</i> (<i>E. senticosus</i>)	Antiviral	Reduces symptoms of URTIs	[49]
Ayurveda	AP-Bio or KalmCold capsules (SAPE extract)	14-deoxy-11,12-didehydroandrographolide (< 15% by HPLC, USP and BP)	Immunomodulatory	Modulates the immune functions	[50]
Swedish herbal medicine	Kan Jang	A fixed combination of <i>A. paniculata</i> and <i>E. senticosus</i> (14-deoxy-11,12-didehydroandrographolide)	Antiviral	Reduces the severity of inflammatory symptoms	[51]
Swedish herbal medicine	Kan Jang	A fixed combination of <i>A. paniculata</i> (SH10) and <i>E. senticosus</i> (SHE3) (14-deoxy-11,12-didehydroandrographolide)	Antiviral (Influenza)	Rapid recovery and reduces the risk of post-influenza complications	[52]
Swedish herbal medicine	Kan Jang	A fixed combination of <i>A. paniculata</i> (SH10) and <i>Acanthopanax senticosus</i> (andrographolide)	Safety study	Safe during viral treatment	[53]
Ayurvedic medicine	CIM-MEG19	Standardized <i>A. paniculata</i> formulation [andrographolide (1), neo-andrographolide (2), 14-deoxy-11,12-didehydroandrographolide (3), and andrograpanin (4)]	Anti-COVID	Excellent safety profile, and halts the disease advancement/ progression	[54]
Ayurvedic medicine	ImmuActive	Curcumin3 Reduct [®] ODN 100 mg; <i>A. paniculata</i> extract >90% 50 mg; Resvenox [®] (resveratrol >95%) 50 mg; zinc monomethionine (eq. elemental zinc) 10 mg; 5 <i>L</i> -selenomethionine (eq. elemental selenium) 40 mcg; 6 BioPerine [®] 3 mg	Anti-COVID	Safe adjunct treatment for effectively managing COVID-19	[55]
Swedish herbal medicine	Kan Jang	A fixed combination of <i>A. paniculata</i> and <i>E. senticosus</i> (14-deoxy-11,12-didehydroandrographolide)	Anti-COVID	Virus clearance, decreases pro-inflammatory symptoms and cytokine	[56]

TCM: Traditional Chinese Medicine; URTIs: upper respiratory tract infections; HPLC: high-performance liquid chromatography; BP: The British Pharmacopoeia; USP: The United States Pharmacopoeia; SAPE: standardized *A. paniculata* extract.

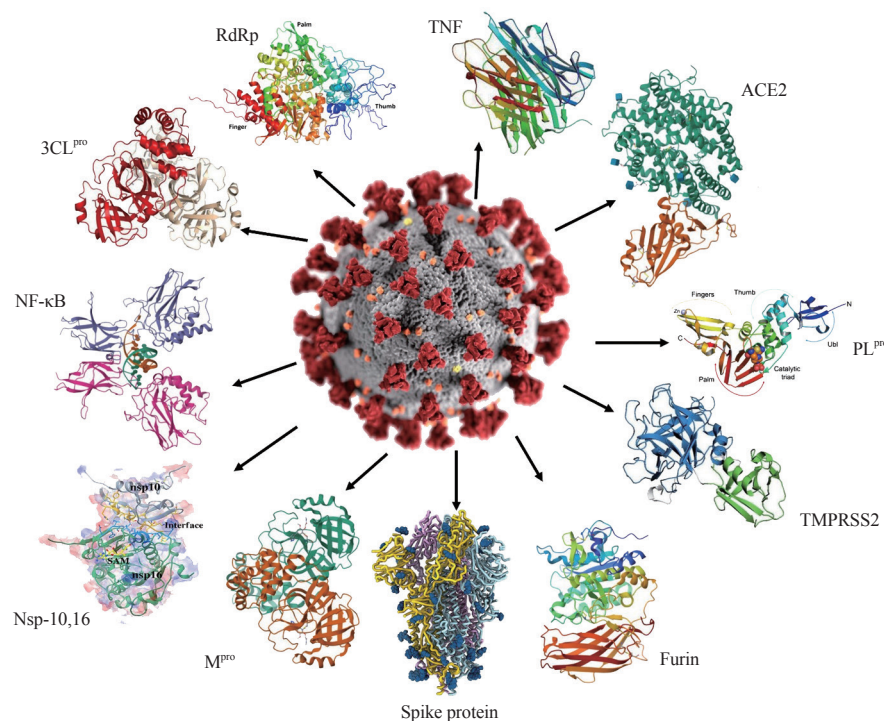


Figure 1. The structure of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the possible molecular targets. Protein structures are obtained from the NCBI Structure Database (<https://www.ncbi.nlm.nih.gov/structure>). TNF: tumor necrosis factor; ACE2: angiotensin-converting enzyme 2; PL^{pro}: papain-like protease; TMPRSS2: transmembrane protease, serine 2; M^{pro}: main protease; Nsp-10, 16: nonstructural protein 10, 16; NF-κB: nuclear factor kappa B; 3CL^{pro}: 3-chymotrypsin-like protease; RdRp: RNA-dependent RNA polymerase.

of COVID-19[54]. The efficacy and safety of *A. paniculata* extract in patients with mild COVID-19 were subject to further evaluation in another RCT. This double-blind RCT, conducted in adults with mild COVID-19 and without risk factors for severe disease, indicated promising effects of the oral treatment regimen of *A. paniculata* extract. These effects included the prevention of pneumonia, a reduction in the duration of viral shedding, and suppression of inflammation. Adverse drug reactions were reported to be mild, self-limited, and infrequent. However, the study was constrained by a small sample size, and it recommended that further studies with adequate power be undertaken to ensure conclusive results[55].

A nutritional supplement based on *A. paniculata* (ImmuActive) underwent an evaluation for efficacy and safety through a randomized, double-blind, placebo-controlled study in COVID-19 patients. The study results suggest that ImmuActive may serve as a beneficial and safe adjunct treatment, effectively mitigating symptoms associated with COVID-19 infection. Furthermore, supplementation with ImmuActive was found to significantly reduce the duration of hospitalization and the time required for viral clearance as determined by RT-PCR testing. Nevertheless, conducting studies in larger populations and among patients with varying degrees of severity may be instrumental in establishing the broader benefits of ImmuActive as an adjunct therapy for COVID-19 infection[56]. The APFaVi trial, a double-blind,

randomized, placebo-controlled study, was conducted to assess the effectiveness of *A. paniculata* extract treatment in individuals with mild to moderate COVID-19 who were concurrently receiving favipiravir therapy. The findings of the study indicated that *A. paniculata* extract did not exhibit additional clinical and virological benefits in patients with mild to moderate COVID-19 who were undergoing favipiravir treatment. Nevertheless, the early reduction of interleukin-1 β (IL-1 β) was noted after treatment with *A. paniculata* extract, suggesting a potential advantage in preventing cytokine storms in severe cases of COVID-19. Further investigation is warranted to explore this aspect[57]. Kan Jang, which comprises a fixed combination of *A. paniculata* and *Eleutherococcus senticosus*, is recognized for its antiviral, immunomodulatory, and anti-inflammatory properties. Recent clinical research on Kan Jang has demonstrated its effectiveness in the acute phase of mild COVID-19, significantly alleviating the severity of inflammatory responses and proinflammatory cytokine levels when compared to a placebo[58].

A randomized controlled trial in Thailand evaluated the powdered form of *A. paniculata* (ages 18-60) compared with favipiravir for treating mild COVID-19 patients over 10 days. The study found *A. paniculata* to be as effective as favipiravir in terms of clinical severity, X-ray findings, and viral load reduction. Additionally, *A. paniculata* offers potential advantages in affordability and accessibility, supporting its use as a first-line treatment for mild

Table 2. Summary of clinical trials on *Andrographis* and its formulations against URTIs.

Type of study and design	Experimental intervention (type, dose, and duration)	Control intervention (type, dose, and duration)	Adverse effect	Observations	Reference
A single-center, randomized, three-way crossover design study	80, 160, or 320 mg of DAS infusion	No control or placebo used in this study	No serious adverse events except stomachache	Has nonlinear pharmacokinetic characteristics	[5]
Randomized controlled trial	10 mg/kg XYP injection for 28 days	Standard symptomatic treatments (oxygen therapy, antiviral, antibiotics and immunomodulators)	No severe adverse effects	Significantly reduces the time to cough relief; fever resolution and virus clearance	[36]
Randomized double-blind placebo controlled clinical trial	KalmCold one capsule containing 100 mg of actives, after breakfast and dinner, twice a day for 5 days	Placebo capsules after breakfast and dinner for 5 days	Vomiting, epistaxis, urticaria and three with diarrhoea, nausea or lethargy	<i>A. paniculata</i> extract was found to be 2.1 times or 52.7% more effective than the placebo	[39]
Randomized double-blind study	<i>A. paniculata</i> dried extract (1200 mg/day) for 5 days	Placebo for 5 days	No adverse effects	Effective in reducing the prevalence and intensity of the symptoms in uncomplicated common cold	[40]
Randomized double-blind placebo controlled clinical trial	Standardized <i>Andrographis</i> extract daily for three months	2 tablets of a placebo per day for three months	No significant adverse effects	Relative risk of URTIs 2.1 times lower (95% CI: 1.32–3.33) in the active group when compared with placebo	[41]
A double blind, placebo-controlled, non randomized trial	Kan Jang three times daily for 5 days	Placebo three times daily for 5 days	No significant adverse effects	Relieves the inflammatory symptoms of sinusitis and URTIs	[42]
A placebo controlled, double-blind study	4 Kanjang tablets thrice daily for 5 days	4 placebo tablets thrice daily for 5 days	Two cases of urticaria	Kanjang tablets are very well suited as self-treatment in uncomplicated common cold	[43]
Randomized, double-blind, placebo-controlled phase II clinical trial	Lian-Ju-Gan-Mao capsules (0.28 g, 0.56 g and 1.12 g) 3 times per day for 3 consecutive days	Placebo (1.12 g) 3 times per day for 3 consecutive days	Not available	Provides high-quality evidence on the efficacy and safety of Lian-Ju-Gan-Mao capsules in treating CCWHS	[44]
Randomized controlled study	53 patients in Group A: 2 Kan Jang tablets 3 times a day for 10 days; 41 patients in Group B: Immunal 10 drops 3 times a day for 10 days	39 patients in Group C (control group): Lavish warm drinks, throat gargles, antiseptic nose drops, and paracetamol at a dose of 500 mg 3 times a day in cases of fever and severe headache	No side effects or adverse reactions	Less severe symptoms (nasal secretion and nasal congestion) of the disease and fast recovery time	[49]
Open-label study	200 mg of standardized <i>A. paniculata</i> extract daily for 30 days	One capsule in the morning and one in the evening every day for 30 days (Standardized <i>A. paniculata</i> extract + HPMC)	No treatment related adverse effects	A significant increase in the T cell IFN- γ and IL-4, and a decrease in IL-2	[50]
Randomized quadruple-blinded, placebo-controlled, two-parallel-group phase II study	6 doses of Andrographolides capsules (60 mg) daily for 5 consecutive days	Placebo capsules for 5 consecutive days	No adverse events	Reduces the recovery duration and URTIs symptoms	[51]
Two randomized, controlled study	2 tablets 3 times a day for 3–5 days	Amantadine 0.05 g: thrice on the first day; twice on the second and third day; and once at the fourth day	No observed adverse effects	Quick recovery, and reduces the risk of post-influenza complications	[52]
Open randomized, phase I clinical study	Kan Jang tablet 60 mg, 3 times per day for 10 days	Ginseng mixture in a dose of 20 mL 2 times a day (85.7 mg of ginsenoside, equivalent to 3 g of the dried plant per day)	No reproductive toxicity	The daily dose employed is safe for the treatment of the common cold	[53]

DAS: dehydroandrographolide succinate; XYP: Xiyanping; LJGMC: Lian-Ju-Gan-Mao capsules; CI: confidence interval; CCWHS: common cold with wind-heat syndrome; HPMC: hydroxypropyl methylcellulose.

COVID-19[59]. A prospective, double-blind RCT investigated the efficacy and safety of *A. paniculata* ethanolic extract (APE) compared to placebo in asymptomatic or mildly symptomatic COVID-19 patients over 5 days. APE treatment significantly improved recovery rates (80.7%) compared to placebo. While APE alleviated headache and olfactory loss symptoms faster than placebo, it did not reduce overall COVID-19 progression. Mild diarrhoea was the most common side effect associated with APE and resolved within a few days. No hepatic or renal toxicity was observed[60]. A retrospective study assessed the effectiveness of two herbal medicines containing *A. paniculata* (alone or combined with *Boesenbergia rotunda*) for clearing SARS-CoV-2 in patients with mild COVID-19. These herbal capsules significantly reduced the median time to clear the virus (9 days) compared to other treatments (11-13 days). While no pneumonia was observed, one participant in the *A. paniculata* group developed hyperkalemia. The study suggests *A. paniculata* with or without *Boesenbergia rotunda* might be a potential alternative for mild COVID-19, especially when access to favipiravir is limited. However, large-scale clinical trials are

necessary to confirm their efficacy and safety for a broader patient population[61].

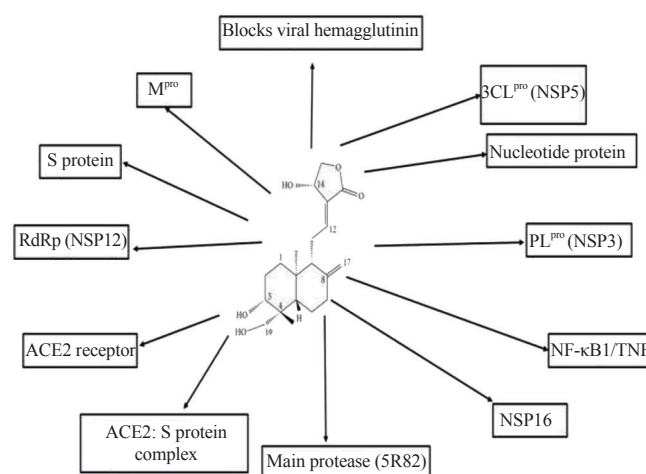


Figure 2. Various anti-SARS-CoV-2 targets (enzymes, receptors, structural and non-structural proteins) of andrographolides.

Table 3. Summary of clinical trials on *Andrographis* and its formulations against COVID-19.

Type of study and design	Experimental intervention (type, dose, and duration)	Control intervention (type, dose, and duration)	Adverse effect	Observations	Reference
Randomized controlled pilot study	Standardized <i>A. paniculata</i> extract formulation (0.2 g twice a day) add on therapy	Standardized <i>A. paniculata</i> extract formulation, standard of care + without antiviral	No adverse effect	CIM-MEG19 therapy is safe and effective in mild-to-moderate COVID-19 patients as an add-on regimen	[54]
Randomized controlled trial	<i>A. paniculata</i> extract (60 mg andrographolide, thrice a day for 5 days)	Standard supportive care (oxygen therapy, antivirals and antibiotics)	None had evidence of hematologic, hepatic or renal impairment	Prevention of pneumonia, shortening of viral shedding period, and inflammatory suppression	[55]
A randomized, double-blind, placebo-controlled study	ImmuActive 500 mg capsule containing <i>A. paniculata</i> extract >90% 50 mg	Active (ImmuActive) for 28 days	No adverse events	Reduces the COVID-19 infection symptoms	[56]
Phase 2/3 randomized, double-blind, and placebo-controlled trial	<i>A. paniculata</i> extract capsule containing 20.53 mg of andrographolide, 3 capsules, 3 times daily for 5 days	200 mg of Favipiravir for 1-4 days	No serious adverse events; mild hepatitis was recovered on day 28	Immunomodulatory effects in severe COVID-19; prevents cytokine storm (interleukin-1 β)	[57]
Randomized, quadruple-blind, placebo-controlled trial	Kan Jang capsules (1 560 mg) 2 capsules 3 times per day for 2 consecutive weeks with supportive treatment of Paracetamol	Placebo capsules per day for 2 consecutive weeks with supportive treatment of Paracetamol	No adverse events reported	Reduces the risk of progression, duration, virus clearance, and days of hospitalization, relieves inflammatory symptoms, and decreases pro-inflammatory cytokine IL-6 level	[58]
Randomized controlled trial	<i>A. paniculata</i> (400 mg per capsule containing 11.35 and 24 mg of andrographolide) 180 mg/day for 5 days	Favipiravir at a dose of 3 600 mg on day 1 and 1 600 mg/day on days 2 to 5	No adverse events reported	The study supports the decision to use <i>A. paniculata</i> powder as the first treatment for mild COVID-19	[59]
A double-blinded randomized control trial	<i>A. paniculata</i> ethanolic extract at 180 mg/day for 5 days (containing 60 mg of andrographolide)	No positive control group	Mild diarrhea, no hepatic or renal toxicity observed	Reduces the symptoms of olfactory loss	[60]
A retrospective study	Four <i>A. paniculata</i> capsules thrice daily; one capsule of <i>Boesenbergia rotunda</i> extract capsule once daily, a combination of both capsules	Favipiravir tablets (200 mg) for 5 days	Hyperkalemia	Quicker viral clearance compared to favipiravir	[61]

6. Antiviral activity of semi-synthetic andrographolide analogues

Many derivatives of andrographolide are effective in patients with URTIs. A novel derivative, 14- α -lipoyl andrographolide (AL-1) has been highly active against influenza A viruses, including the H5N1 strain. AL-1 could thus be used in combination with other antiviral medicines for the treatment of respiratory tract infections. Furthermore, AL-1 may not easily induce drug resistance due to its various modes of action. In addition, the study found that AL-1 is more effective against the human influenza virus than andrographolide and dehydroandrographolide succinate, which have been used in clinical trials for decades. Andrographolide sulfonate is a highly water-soluble sulfonation derivative of andrographolide that has been widely utilized in treating bronchitis and tonsillitis. It possibly suppressed the severity of lipopolysaccharide-induced acute lung injury by attenuating the expression of neutrophil-derived proteases[62]. Therefore, synthetic and semi-synthetic derivatives of andrographolides might be meaningful for treating SARS-CoV-2-related respiratory tract infections (Supplementary Figure 1).

7. COVID-19, *A. paniculata* and andrographolides

COVID-19 is a kind of coronavirus pneumonia that produces severe acute respiratory illness caused by the coronavirus 2[63]. Effective antivirals are still in high demand for treating COVID-19 and other virus-mediated respiratory illnesses. One of the key causes for poor clinical outcomes in severe COVID-19 patients is the lack of effective antivirals against SARS-CoV-2. Identifying novel treatments with all clinical trials and determining side effects and other factors in this emergency scenario is tough. Drug discovery and repurposing methodologies are being pursued to find possible therapeutic agents.

Nevertheless, following well-conducted clinical trials, remdesivir had marginal clinical efficacy, and cost-effectiveness and accessibility remain major concerns[64]. As a result, a novel approach should be used to develop a new therapeutic drug with immunomodulatory and antiviral properties against COVID-19. During the 2003 SARS outbreak, the effectiveness of treatment based on traditional medicinal plants was reported[65,66]. Hence, the scientific community has already begun investigating medicinal herbs as potential therapy options for COVID-19 based on their history and traditional applications[67]. The occurrence of highly pathogenic avian influenza viruses capable of infecting and killing humans highlights the urgency for a new and efficient strategy for the treatment of diseases caused by the virus. Plants have long been utilized in folk medicine as a good source of antiviral drugs to treat viral infections[68]. *A. paniculata* is a widely used medicinal herb in Asia, particularly in China, India, Malaysia, and Thailand. Traditional medicine currently employs *A. paniculata* to treat the

common cold, diarrhoea, and fever caused by various infectious causes and a health tonic[69]. Andrographolide, a diterpene lactone of the isoprenoid family with broad-spectrum antiviral activities, is a key bioactive component of *A. paniculata*. Recent research demonstrates that andrographolide effectively protects chicken macrophages from the harmful effects of infectious bronchitis virus. It also prevents cell death and inflammation, both in cell culture and in chicken embryos. These findings suggest that andrographolide could be a promising treatment for infectious bronchitis virus[70].

7.1. Anti-COVID effects of *Andrographis* and andrographolides *in vitro*

Andrographolides from *A. paniculata* have demonstrated promising *in vitro* antiviral activity against SARS-CoV-2 in various cell models. Post-infection treatment of *A. paniculata* and andrographolide in SARS-CoV-2-infected Calu-3 cells significantly inhibited the production of infectious virions, as determined by the plaque assay. In conclusion, this study provided experimental evidence favouring *A. paniculata* and andrographolide for further development as a monotherapy or in combination with other effective drugs against SARS-CoV-2 infection[71]. In a study by Shi *et al.*, the antiviral effect of andrographolide and its fluorescent derivative, the nitrobenzoxadiazole-conjugated andrographolide (andro-NBD), was assessed through a protease activity assay. Both compounds exhibited the ability to suppress the activities of the main protease (M^{pro}) in both 2019-nCoV M^{pro} and SARS-CoV M^{pro} in a manner dependent on Michael addition. Given that andrographolide is already used in clinical practice with an acceptable safety profile and possesses diverse pharmacological activities, the study suggests the need for extensive investigation into the potential of andrographolide in suppressing 2019-nCoV and its application in COVID-19 therapy. The investigation further showed that andrographolide as well as its derivative andro-NBD inhibit the activity of major protease (M^{pro}) and hence likely suppress the replication of 2019-nCoV and SARS-CoV. A further study revealed that andrographolide and andro-NBD derivative inhibit the activity of M^{pro} and are thus likely to impair the replication of SARS-CoV and 2019-nCoV. Further, they also found that andro-NBD strongly inhibited the activity of 2019-nCoV[72].

The inhibitory activity of C-12 dithiocarbamate andrographolide analogues against SARS-CoV-2 M^{pro} was initially assessed through an *in vitro* enzyme-based assay. The obtained results were then compared to those of rutin, a known SARS-CoV-2 M^{pro} inhibitor. Out of the twenty-one andrographolide analogues tested (3a-u), including their parent compound (1) and precursor (2), compounds 3k, 3l, 3m, and 3t demonstrated notable inhibitory activity against SARS-CoV-2 M^{pro} , surpassing the effectiveness of the known inhibitor rutin. These findings highlight the potential of these andrographolide analogues as candidates for further optimization to enhance their anti-SARS-CoV-2 M^{pro} potency[73]. Recently, extracts from *A. paniculata* were found to exhibit anti-HCoV-OC43 activity, with the antiviral efficacy showing a moderately strong positive linear relationship

($r = 0.7938$) with the andrographolide content in deep eutectic solvent extracts when compared to methanol extracts. The study demonstrated that the acidic deep eutectic solvents extracts of *A. paniculata*, along with andrographolide, contributed to anti-HCoV-OC43 replication activities, demonstrating the collaborative effect of these phytocompounds[74].

Andrographolide and its semi-synthetic derivatives were assessed for their anti-SARS-CoV-2 activities using SARS-CoV-2 (B.3 strain) inoculated Vero E6 cells in a plaque reduction assay. Andrographolide (1), 14 β -andrographolide (9), and 16a demonstrated a dose-dependent inhibition of SARS-CoV-2 replication, with NT₅₀ values in the low micromolar range, which is consistent with previous findings. Importantly, these derivatives exhibited minimal cytotoxicity to AREC32 and Vero E6 cells. This work establishes a promising strategy for combating SARS-CoV-2 infections with novel COVID-19 therapies[75]. Moreover, andrographolide inhibited virus-induced cytopathic effects, like remdesivir. This inhibition extended to the entry of SARS-CoV-2 into Vero E6 cells, exhibiting a high selective index. This emphasizes the importance of confirming the anti-SARS-CoV-2 activity of potential antiviral compounds. The selection of Vero E6 cells for primary screening was practical due to their observable and measurable cytopathic effects[76].

A polyherbal formulation named NOQ19, containing andrographolide, exhibited remarkable antiviral efficacy against SARS-CoV-2-infected Vero E6 cells, resulting in a reduction in viral load. Various concentrations of NOQ19 were employed to inhibit the formation of plaques. Despite these promising findings, it is imperative to conduct further clinical trials to assess the safety and efficacy of NOQ19 in human subjects. Additionally, preclinical studies are needed to ascertain a potentially beneficial therapeutic approach against COVID-19[77]. Further study investigated the safety and anti-SARS-CoV-2 activity of *A. paniculata* in Vero E6 cells. The study revealed that, with *in vitro* anti-SARS-CoV-2 activity, *A. paniculata* showed no cytotoxicity in Vero E6 cells and exhibited a favorable IC₅₀. However, the researchers suggested the necessity for further investigations to demonstrate the *in vivo* anti-SARS-CoV-2 activity and emphasized the importance of exploring the potential of this plant in clinical trials[78].

A recently published review article delves into the comprehensive scientific data on *A. paniculata* and its related compounds, focusing on their *in silico* and *in vitro* antiviral properties within the context of COVID-19. The review highlights the effectiveness of various extracts of *A. paniculata* and andrographolide in inhibiting the production of infectious virions, suggesting a potential synergy with modern drugs. The authors propose that this herb could serve as a promising source of lead compounds for drug discovery in the post-infectious treatment of COVID-19, emphasizing its potential as a therapeutic option rather than a prophylactic measure[79]. The study observed an absolute inhibition of infectious virion production in the post-treatment approach with *A. paniculata* extract (100 μ g/mL) and andrographolide (12.5 μ M). These findings indicate the potential of these compounds as promising anti-SARS-CoV-2

agents. Notably, andrographolide demonstrated potent antiviral efficacy in Vero E6 cells during the post-infection phase, exhibiting a favourable cytotoxicity profile. The information gleaned from this study suggests andrographolide as a candidate for combination therapy with other Food and Drug Administration-approved agents, presenting an effective treatment strategy for COVID-19. The feasibility of this rationale should undergo further evaluation. In conclusion, the authors propose andrographolide as a novel natural candidate for combating SARS-CoV-2 infections[80,81]. A systematic review and meta-analysis identified that among the compounds investigated for their effects on the SARS-CoV-2 strain, andrographolide exhibited the highest selectivity index and antiviral effect. Andrographolide, known for its broad-spectrum antiviral activity, stood out in terms of its potential efficacy and safety profile[82].

7.2. Anti-COVID effects of *Andrographis* and andrographolides in *in silico* studies

To search for potential coronavirus therapeutic drugs as soon as possible, several natural compounds have been subjected to target-based [3CL^{pro}, RNA-dependent RNA polymerase (RdRp), PL^{pro}, NSP, M^{pro}, and ACE2] high throughput virtual ligand and toxicity screening using various bioinformatics tools. A virtual ligand screening of a series of natural compounds (such as andrographolides, flavonoids, and phenolic acids) with antiviral, immunomodulatory, and anti-inflammatory effects revealed that they had a high binding affinity to 21 different target proteins, indicating that they could be useful in treating SARS-CoV-2 infection[83]. Supplementary Table 1 presents the detailed results from the *in silico* prediction of andrographolide and its numerous (natural and synthetic) derivatives. Andrographolide was further tested against two key enzymes of SARS-CoV-2, namely 3C main proteinase and RdRp. The result indicated that andrographolide could only inhibit the SARS-CoV-2 3C main proteinase as strongly as lopinavir (the standard medicine), which has been recommended as the drug of choice to treat SARS-CoV-2 patients[84]. Several andrographolide compounds were also predicted against structural and non-structural proteins of SARS-CoV-2. The binding free energy data from their study suggest that neoandrographolide could be used as a cost-effective drug analogue for treating COVID-19 infection in developing countries[85].

A computational analysis including molecular interaction, pharmacokinetic, and toxicity profiles was performed on 16 semisynthetic andrographolide derivatives against 5 key SARS-CoV-2 enzymes, including the M^{pro}. Andrographolide derivatives 14 and 15 emerged as the most promising candidates for further development as a treatment for SARS-CoV-2 infection. However, the study concluded that further molecular dynamic studies and pharmacological screenings are essential before these derivatives can be considered a viable drug for COVID-19[86]. Another *in silico* paper shows that andrographolide has good solubility,

pharmacodynamic properties, and target accuracy and is a potent inhibitor of SARS-CoV-2 M^{pro}[87].

Interestingly, another combination of system biology studies was conducted to investigate the binding affinity of andrographolide and its derivative (14-deoxy-11,12-didehydroandrographolide) with targets related to COVID-19 and their probable role in regulating multiple pathways in COVID-19 infection[88]. It was concluded that andrographolide could possess higher importance than 14-deoxy-11,12-didehydroandrographolide as it scored higher interaction with the targeted proteins of COVID-19.

Another molecular docking analysis showed that andrographolide could inhibit NFκB1 and TNF, thus blocking the pathways responsible for COVID-19 and repurposing andrographolide as a potential drug molecule to reduce cytokine storm[89]. An *in silico* study has confirmed that 5,4'-dihydroxy-7-O-β-D-pyran-glycuronate butyl ester and andrographolide glycoside 3-O-β-D-glucopyranosyl-andrographolide compounds from *A. paniculata* have lower free binding energy and highest similarity in types of interaction with amino acid residues against COVID-19 M^{pro}. Overall, the toxicity prediction of these compounds from *A. paniculata* shows their safety, and they could be potential lead molecules as COVID-19 M^{pro} inhibitors. However, further investigation into its use for COVID-19 treatment is strongly needed to assess its safety and effectiveness in treating COVID-19 patients[90]. Another study reveals that dihydroxy dimethoxy flavone from *A. paniculata* is significantly bound with the active site of SARS-CoV-2 M^{pro} and may be helpful in further development[91]. Moreover, it was revealed that there is a strong affinity of andrographolide to receptor binding domain and NSP12 of the SARS-CoV-2 virus, indicating andrographolide acts as an inhibitor of receptor binding domain and NSP12.

Another *in silico* docking analysis shows that *A. paniculata* interacts with SARS-CoV-2 proteins (open & closed state S protein, 3CL^{pro}, PL^{pro}, and RdRp). Among 14 phytochemicals from *A. paniculata*, isoandrographolide showed the highest binding energy affinity/dock score compared to remdesivir. This meets the minimum threshold for any compound used for drug development against SARS-CoV-2 and *A. paniculata* is found to be non-carcinogenic, water-soluble, and biologically safe[92]. The molecular docking study of andrographolide shows strong binding energy with the best and most stable binding of ACE receptor macromolecules. The data from this study suggests that andrographolide can be a suitable inhibitor for the human ACE2 complex interface, inhibiting the S protein of SARS-CoV-2 to the ACE2 receptor without showing any side effects. It was concluded that andrographolide is the best lead molecule, which shows good interaction with the ACE2 receptor. It has been suggested that targeting SARS-CoV-2 nsp16 could be a potential candidate for its antiviral activity to inhibit SARS-CoV-2 nsp16[93,94]. Another recent *in silico* study has also demonstrated that andrographolide showed a good binding affinity with nsp16 at its active site with a good docking score, and could be explored as a potential antiviral agent[95].

The most recent finding of COVID-19's M^{pro} structure opens the possibility of detecting potential pharmacological targets in successful COVID-19 therapy. From the recent *in silico* studies, it is clearly shown that andrographolide had good binding energy and could inhibit the protease activity of the M^{pro} of COVID-19. Furthermore, as compared to other bioactive andrographolides (andrograpanin, neoandrographolide, 14-deoxyandrographolide, and 14-deoxy-11,12-didehydroandrographolide), andrographolide has a higher binding energy towards M^{pro} of COVID-19. The binding energy value of −6 kcal/mol is considered the upper threshold value for ligand binding study; if the binding energy value is > −6 kcal/mol, then the ligand is deemed to have a good binding affinity with the receptor. Further, ADMET studies revealed that selected bioactive compounds of andrographolides have no ADMET violation, and they could be useful therapeutic molecules against SARS-CoV-2 for controlling viral attachment to host cells by inhibiting M^{pro} of SARS-CoV-2[96].

In *A. paniculata*, a total of 18 compounds were investigated. Andrographolactone, andrographolide, and andrographidine-A exhibited the most robust binding affinity to the SARS-CoV-2 RdRp protein in comparison to other compounds and positive controls like remdesivir and favipiravir. The interaction between these compounds and the RdRp protein involves active amino acids, forming strong hydrogen bonds. Notably, amino acids such as TYR 619, LYS 621, ASP 760, and ASP 623 play a pivotal role in this interaction. Among these compounds, andrographolactone emerged with the highest potential as an inhibitor of SARS-CoV-2 RdRp, positioning it as a promising and novel therapeutic candidate for further exploration[97].

The SARS-CoV-2 M^{pro} is considered a potential drug target due to its role in processing proteins translated from viral RNA. Molecular dynamics simulations and MMGBSA studies indicate that the previously reported N3 inhibitor and andrograpanin exhibit effective binding interactions involving identical amino acid residues and the same binding pockets of the SARS-CoV-2 M^{pro}. This theoretical experiment suggests that andrograpanin could be considered a promising inhibitor against SARS-CoV-2 M^{pro}[98–103]. In another *in silico* study on the potency of five compounds against COVID-19 M^{pro} receptors and IL-2 proteins, andrographolide showed a docking score comparable to the promising drug. Therefore, the presence of a heterocyclic atom, along with more polar nitrogen and oxygen analogs in its structure, suggests the potential of andrographolide in advancing the development of a novel drug course for CoVs infectious diseases[104]. A review by Shavira *et al.* highlighted recent *in silico* study results, indicating that 50 out of 107 *A. paniculata* phytocompounds (46.73%) exhibited activity against SARS-CoV-2. Notably, certain key phytocompounds from *A. paniculata*, including andrographolide, neoandrographolide, isoandrographolide, 14-deoxyandrographolide, 14-deoxy-11,12-didehydroandrographolide, and andrographidin C, were identified as potential antiviral agents. This is attributed to their strong binding affinities and stable conformations towards SARS-CoV-2 proteins,

specifically targeting M^{pro}, PL^{pro}, RdRp, Nsp15, and spike protein. Andrographolides from *A. paniculata* exhibit a significant affinity for major key proteins of SARS-CoV-2, including the M^{pro}, PL^{pro}, RdRp, Nsp15, and the spike protein[105].

In silico studies using computational models suggest that *A. paniculata* extract and its key compound andrographolide, along with related molecules, may be powerful natural inhibitors against the SARS-CoV-2 virus. These studies predict that they can interfere with various proteins the virus needs to function and Supplementary Table 1 provides more details on these findings[106–117]. Based on the available literature from the above studies, it is suggested that certain phytochemicals in *A. paniculata* may possess potential as antiviral agents, which is attributed to their robust binding affinities and stable conformations towards SARS-CoV-2 proteins.

In another *in silico* study, researchers performed a target-based virtual ligand screening using andrographolides against RdRp and 3CL^{pro}. The findings unveiled a notable binding affinity, indicating the potential efficacy of these compounds in the treatment of SARS-CoV-2. Consequently, formulations that feature these compounds as primary constituents in herbal remedies may have significant implications for the treatment of SARS-CoV-2 infections. *In silico* studies have consistently predicted that andrographolide possesses potent anti-SARS-CoV-2 activity by specifically targeting both the host ACE2 receptor and various viral factors. These include RdRp, M^{pro}, 3CL^{pro}, PL^{pro}, and spike proteins. Computational analyses suggest that andrographolide may interfere with multiple crucial elements involved in the viral replication and infection process, making it a promising candidate for further exploration in the context of COVID-19[118].

In silico studies on the potential efficacy of andrographolides against COVID-19 proteins have yielded promising insights into the molecular interactions and binding affinities. These virtual experiments, based on computational models, suggest that andrographolides may possess inhibitory properties against specific viral proteins associated with the SARS-CoV-2 virus. The computational analysis has provided a foundation for further *in vitro* and *in vivo* experiments to validate the observed effects and assess the translational potential of andrographolides as a therapeutic option against COVID-19. While *in silico* studies offer valuable predictions, it is crucial to acknowledge the need for rigorous experimental validation to confirm the actual antiviral activity of andrographolides. Future research should focus on comprehensive pharmacological investigations, including cell-based assays and animal studies, to assess the safety, efficacy, and potential mechanisms of action of andrographolides in combating COVID-19. These findings contribute to the growing body of evidence exploring natural compounds as potential candidates for antiviral interventions. However, it is essential to exercise caution and await further experimental verification before considering andrographolides as a definitive solution for COVID-19 treatment. The collaborative efforts of computational and experimental research will play a

pivotal role in advancing our understanding of potential therapeutic interventions against emerging viral threats.

8. Safety profile

According to a recent systematic search, *A. paniculata* should be carefully advised and continuously monitored for any common adverse events associated with gastrointestinal disorders and skin disorder systems due to increasing utilization[119]. The genotoxic potential of a standardized extract of *A. paniculata* (KalmCold) was tested, and the results showed that KalmCold is genotoxically safe[120]. In sub-chronic testicular toxicity, it was found that *A. paniculata* dried extract did not produce any structural changes in Leydig cells, and testosterone levels remained normal[121]. Studies of acute and subacute toxicity have shown that the LD₅₀ of andrographolide is more than 5 g/kg when administered orally[122–124]. Out of 14 reports, six reports stated ($n = 579$) that they did not observe any adverse effects. Considering these data, *A. paniculata* and its formulations did not produce any major type of adverse events in treating subjective symptoms of common cold or URTIs. It might be safe for adults and children (Tables 2 and 3). While recent clinical trials involving patients with COVID-19 did not report any major adverse events associated with *A. paniculata* and its formulations, it is important to note that long-term investigations and comprehensive clinical studies with enough patients are crucial. Such studies are essential to establish *A. paniculata*-based alternate therapies and to provide a more thorough understanding of its safety profile and potential benefits in the context of COVID-19 treatment.

9. Conclusion

This review summarizes the benefits of *A. paniculata* in respiratory viral infections due to its immunomodulatory effects, highlighting its potential role in the treatment or prevention of viral infections. While numerous *in silico* studies have explored the anti-SARS-CoV-2 activity of individual small molecules derived from *A. paniculata*, it is essential to acknowledge certain limitations in translating these findings to *in vitro* and *in vivo* contexts. *In silico* studies provide valuable insights into potential interactions at a molecular level, but they are computational predictions and do not replace the need for experimental validation. In addition, preclinical and clinical trial evaluations of this herb for COVID-19 have not specifically been conducted, so further investigations related to this are warranted. A suggestion for the clinician is that *A. paniculata* and its formulations must be administered carefully to patients, even if they are healthy. Nevertheless, high-quality RCTs are needed to validate the effectiveness and adverse events of *A. paniculata* further in the treatment of viral infections and COVID-19.

Conflict of interest statement

There is no potential conflict of interest during the review.

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Data availability statement

The data supporting the findings of this study are available from the corresponding author.

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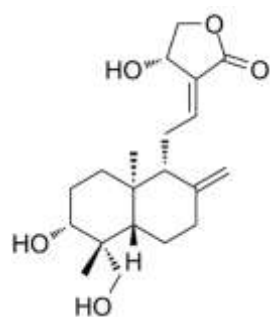
Antiviral efficacy of *Andrographis paniculata* and andrographolides: A narrative review

Running title: Review on antiviral potential of *Andrographis paniculata*

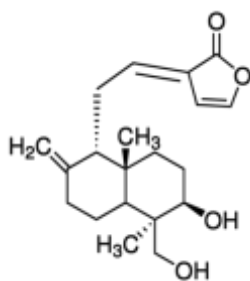
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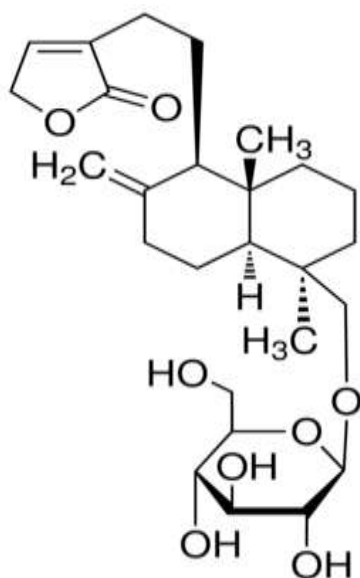
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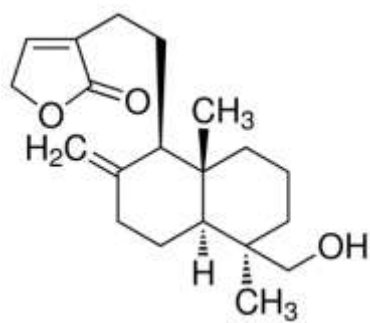
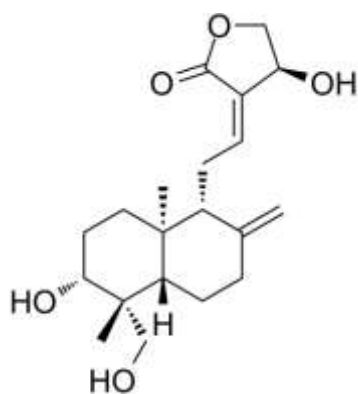
Andrographolide (AGP1)



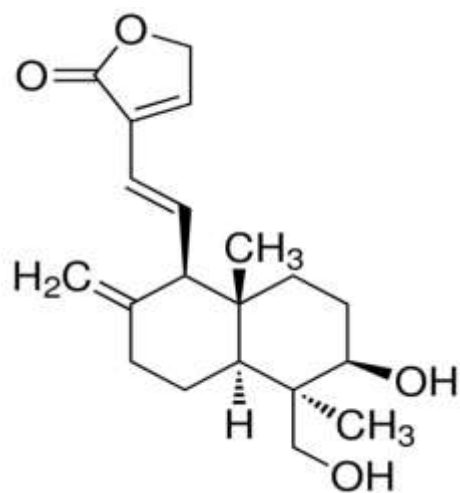
Dehydroandrographolide



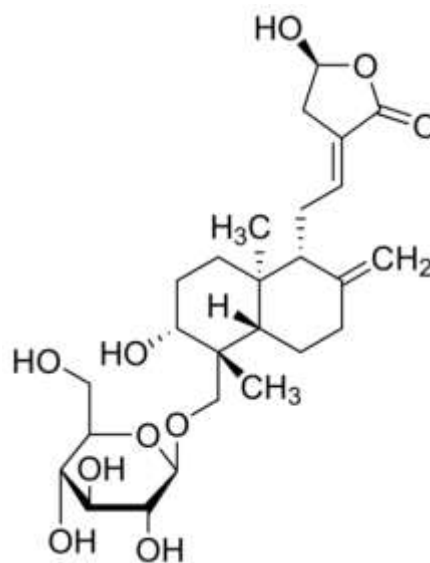
Neoandrographolide (AGP 3)



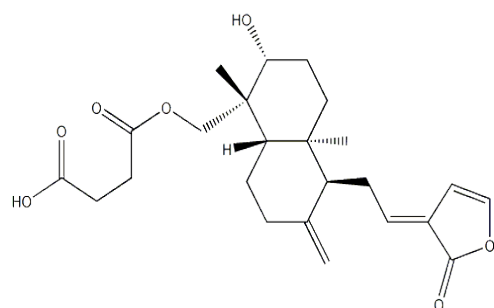
Andropanolide



Andrograpanin

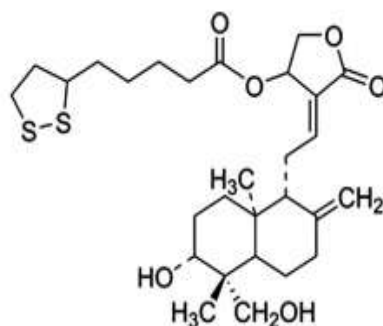


14-Deoxy-11,12-didehydroandrographolide (NCTU 048)

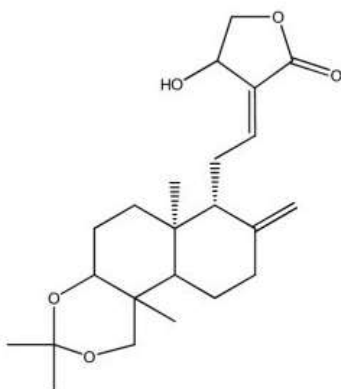


Andrographiside

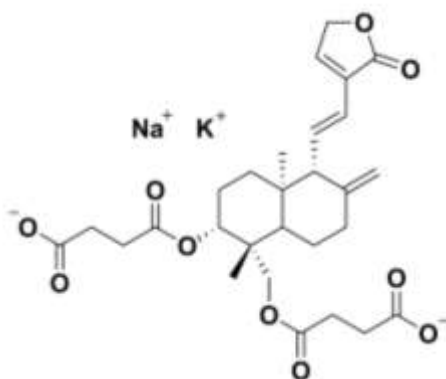
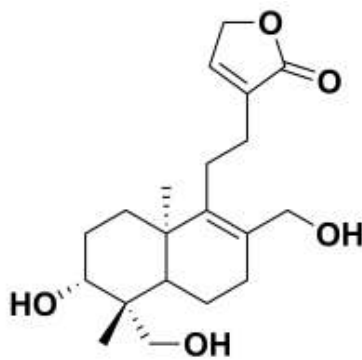
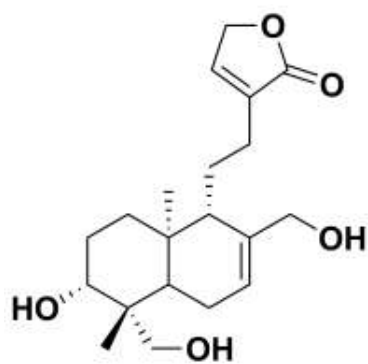
Dehydroandrographolide succinic acid monoester



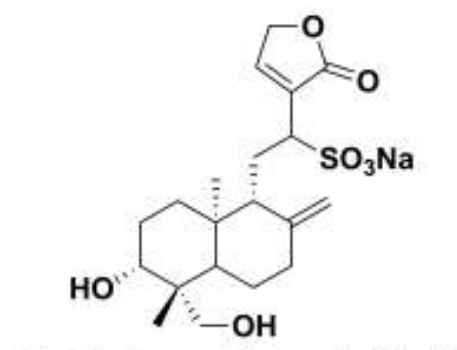
14-alpha-lipoly andrographolide (AL-1)



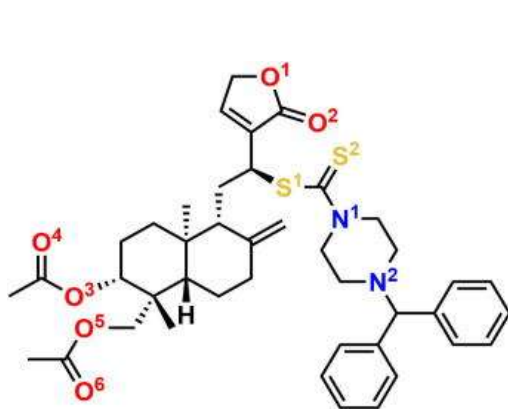
3, 19-isopropylideneandrographolide



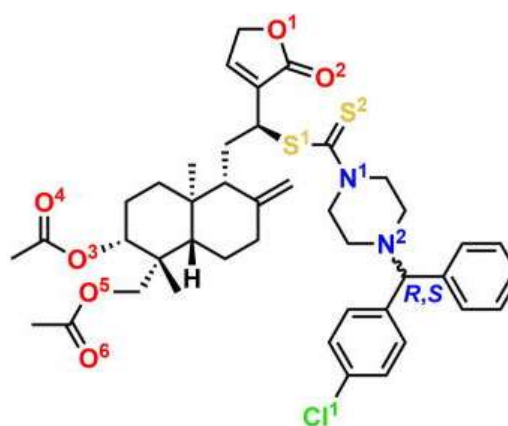
14-dehydroxy-11, 12-didehydroandrographolide-3, and 19-bis (Succinic acid) potassium sodium salt (Yanhuning injection)



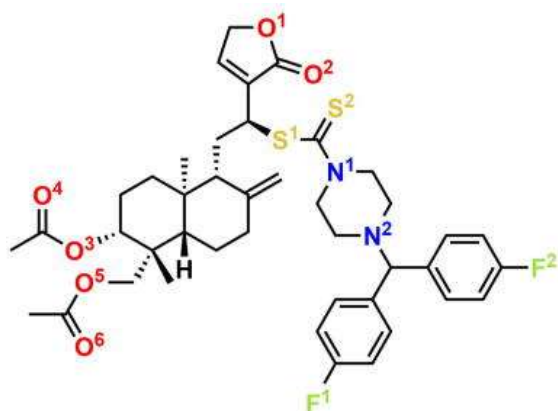
14-dihydroxyandrographolide-12-sulfonic acid sodium salt (Lianbizhi Injection)



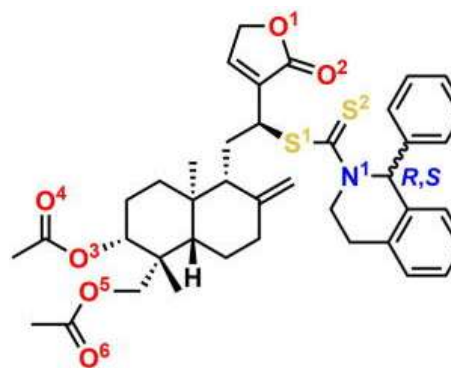
3l



3m

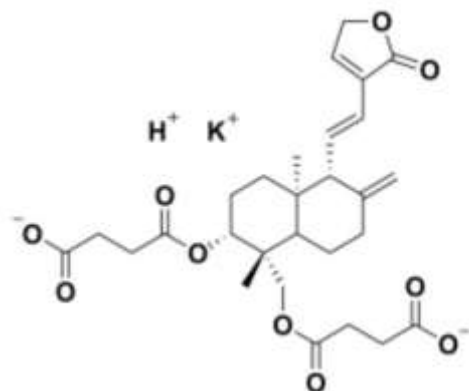


(R,S)-3l

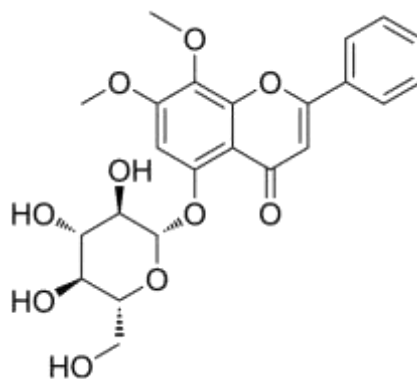


(R,S)-3t

Chemical structures of 12-dithiocarbamate-14-deoxyandrographolide analogues (3l, 3m, (R,S)-3l and (R,S)-3t)



14-dehydroxy-11, 12-didehydroandrographolide-3, 19-bis (Succinic acid) potassium salt
(Chuanhuning injection)



Andrographidine C

Supplementary Figure 1. Chemical structure of antiviral and immunomodulatory andrographolide derivatives.

Antiviral efficacy of *Andrographis paniculata* and andrographolides: A narrative review

Running title: Review on antiviral potential of *Andrographis paniculata*

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Supplementary Table 1. Energy binding affinity (or docking scores) of various synthetic and natural bioactive compounds of *Andrographis paniculata* with different target proteins of SARS-CoV-2 and 2019-nCoV.

Compounds/Extracts of <i>Andrographis paniculata</i>	Target SARS-CoV-2 proteins	IC₅₀ (µg/mL; µM/mL) /Dock score or Binding affinity (kcal/mol;)	Positive control Dock score/ Binding affinity Positive Control (kcal/ mol; µg/mL; µM/mL)	Reference
Andrographolide Andro-NBD fluorescent andrographolide derivative (ANDRO-NBD), 14-Deoxy-11,14-didehydroandrographolide; AP 10; NCTU 048	NF-κB-p50 pathway, SARS-CoV-2 Mpro	Not Available	-7.85 kcal/mol	[72]
12-dithiocarbamate-14-deoxyandrographolide (3k, (R)-3l, (S)3l, 3m, (R)-3t and (S)-3t)	Mpro	-13.03, -12.81, -26.57, -11.16, -17.00 and -11.01	Not Available	[73]
Andrographolide 14β-Andrographolide (9) Andrographolide 1,4 a-dimethyl-6-	KEAP1/NRF2	-26.2	Not Available	[75]

methylene- N-propyl-decalin-1-carboxamide Derivative Andrographolide 1,4 a-dimethyl-6- methylene- N-(2-phenylethyl)decalin-1 carboxamide Derivative		-29.4 - 27.3 -36.4	Not Available	
AGP3	3CLPro	– 31.4	Remdesivir – 44.4	[85]
Andrographolide	TNF	-7.52	Not Available	[89]
DGE GAD	3CL-protease (6LU7)	–8.37 –8.30	Remdesivir –8.23 Indinavir –8.23	[90]
Andrographolide	Spike glycoprotein (Spike RBD) RdRP (NSP12)	–10.346 0 –10.731 3	Not Available	[91]
Andrographolide* Dihydroxydimethoxyflavone* 14deoxy12hydroxyandrographolide* 14deoxyandrographolide*	Main protease (5R82)	– 6.26 – 6.23 – 5.11 – 3.88	Remdesivir – 6.38) Hydroxychloroqui ne – 5.47 Nelfinavir – 5.93	[92]
Isoandrographolide 14-deoxy-14,15- didehydroandrographolide 14-Deoxyandrographolide	S-Protein	– 9.10 – 8.00 – 8.00	Remdesivir – 8.00	[93]

19-O-acetyl-14-deoxy-11,12-didehydroandrographolide Neoandrographolide		– 8.40 – 8.20		
Andrographolide	ACE2	– 9.1	Hydroxychloroquine – 7.1	[94]
Andrographolide Andrograpanin	NSP16	–7.9 –7.1	Not Available	[95]
Andrographolide	CoV-2 6LU7 (Mpro)	–7.69	Native ligand Inhibitor N3 – 8.09	[96]
Andrographolactone Andrographolide Andrographidine A Andrographanine Neoandrographolide 14-deoxy-11-oxoandrographolide 12S-hydroxyandrographolide 14-deoxyandrographolide Dehydroandrographolide Deoxyandrographolide Paniculide C Paniculide B	SARS-CoV-2 RdRp	-8.86 -7.74 -7.68 -7.58 -7.55 -7.54 -7.08 -7.04 -6.98 -6.95 -6.42 -6.15	Remdesivir -5.73 kcal/mol Favipiravir -5.20 kcal/mol	[97]
14-deoxy-11,12-	Mpro	-6.96, IC ₅₀ -		[97]

didehydroandrographolide Andrograpanin 14-deoxy-11,12- didehydroandrographolide Andrograpanin		15.8 μ M -7.02, IC ₅₀ - 14.42 μ M -5.83 -4.97	Not available	
Andrographidine C	RdRp	-8.12 mg/mol	Not available	[98]
Andrographolide Neoandrographolide Neoandrographolide 14-deoxy-11, 12-didehydro andrographolide 14-deoxy andrographolide	SGpro Npro MGpro PLpro Nsp9 RdRp Epro Nsp15 3CLpro	-86.939 -99.409 -78.741 -108.23 -99.366 -130.312 -121.731 -121.725 -125.849	Hydroxychloroqui ne -86.794 Nelfinavir - 133.822 Nelfinavir -96.610 Nelfinavir - 140.903 Nelfinavir - 133.978 Nelfinavir - 149.479 Nelfinavir - 128.736 Nelfinavir - 145.663 Nelfinavir -165.7	[99]
Andrographolide 3,19-isopropylidene andrographolide	Spike protein Nsp15 Main protease	-5.7, -6.55, - 8.62 -6.41, -8.25 - 10.08	Remdesivir -3.3, -5.51, -7.62 Hydroxychloroqui ne	[100]

14-acetyl andrographolide 14-acetyl-3,19- Isopropylideneandrographolide 14-deoxy- 15-isopropylidene-11,12- Didehydroandrographolide 19-o-acetyl anhydroandrographolide		-5.48, -7.14 - 9.03 -6.54, -7.91, -10.36 -5.88, -7.61, -8.69 -5.78, -7.20, -8.49	-4.17, -5.35, -8.28	
Andrographolide	Mpro IL-2	-9.44 -9.47	Remdesivir -9.19 (Mpro) IL-2 inhibitor 8.62 (Mpro)	[101]
14-deoxy-11,12- didehydroandrographolide 14-deoxy-11- oxoandrographolide Andrographolide	Mpro/3CLpro	-7.1 -7.1 -6.9	Not available	[102]
Andrographolide	Spike protein	-7.85	Hydroxychloroquine -8.35	[103]
Andrographolide Hydroandrographolide Isoandrographolide Neoandrographolide Oxoandrographolide	RNA polymerase Main Protease/3 CLpro	-6.3, -6.5 -6.3, -6.1 -6.3, -6.1 -7.0, -6.5 -7.1, - 6.9	Remdesivir -7.5, -5.1	[104]
Andrographolide	ACE2	-4.63	Oseltamivir -3.14	[106]

Ergosterol peroxide	6LU7 main protease	-9.6	Remdesivir -7.5	[107]
Andrographolides	Toll-like receptor pathway, PI3/AKT pathway and MAP kinase pathways, SARS-CoV-2 Mpro	Not available	Not available	[108]
Andrographolide	SARS-CoV-2 spike glycoprotein ACE2	- 98.80 ^{\$} - 99.354 ^{\$}	Hydroxychloroquine - 105.28 - 105.05	[109]
14-deoxy-11,12-didehydroandrographolide 14-deoxy-14,15-didehydroandrographolide 14-deoxy-14,15-didehydroandrographolide Andrograpanin Andrograpanin	Spike glycoprotein Mpro PLpro ACE2 Importin subunit α -5 Importin	-7.76 -7.26 -7.67 -6.91 -6.11	Arbidol -5.19 Lopinavir -5.21 Disulfiram -3.11 Hydroxychloroquine -6.98 Ivermectin -7.97 Ivermectin -8.18	[110]

Andrograpanin	subunit β -1	-7.34		
Andrographolide	Furin TMPRSS-2 ACE-2 Cathepsin L	-10.54 -9.50 -8.99 -8.98	Not available	[111]
Andrographolide Neo-andrographolide	PLpro 3CLpro	Docking score -79.17, - 97.24 -75.97, 93.74	Remdesivir -103.82, -86.18	[112]
Andrographolide	ACE2 TMPRSS2 RdRp 3CLpro PLpro	-7.67, -7.92,- 3.18, -7.85, -7.79	Remdesivir -4.21 (RdRp) Chloroquine -7.69 (ACE)	[113]
Andrographolide	Mpro, Main peptidase	-9.4, -9.8	Not available	[114]
Bis-andrographolide Andrographolide	Mpro Spike protein ACE2	-90.00, -61.30, -68.00	Not available	[115]
Andrographolide	3CLpro/Mpro Spike protein ACE2	-7.1 -7.8 -7.0	Not available	[116]
Andrographidine C	RNA polymerase Main Protease/3 CLpro	-101.8	Not available	[117]

*Glide Score; [#]Recommended value >80% is high; [@]Desired value (Lipinski rule of 5) <5; DGE-5,4'-Dihydroxy-7-O- β -D-pyran-glycuronate butyl ester; AGP3-Neoandrographolide; GAD-3-O- β -D-glucopyranosyl-andrographolide; RBD- Receptor binding domain; NSP- Nonstructural Proteins; RdRP – RNA-dependent RNA-Polymerase; 6LU7- The crystal structure of COVID-19 main protease; ACE2 - Angiotensin-converting enzyme 2; Mpro - The main protease; 2019-nCoV- Novel Coronavirus; NSP16 - The nonstructural protein 16; LD₅₀ - The median lethal dose; 3CLPro - 3C-like protease (3CL) or main protease (M); TNF-Tumor Necrosis Factor; ^{\$}-Molecular Docking Score, KEAP1- Kelch-like ECH-associated protein 1; NRF2- Nuclear factor erythroid 2-related factor 2; S-Protein: Spike glycoprotein; SARS-CoV-2 RdRp - Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-RNA-dependent-RNA-polymerase.