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Mechanistic insights from the review and evaluation of ayurvedic herbal medicines for the prevention and management of COVID-19 patients

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ABSTRACT

Introduction: The need for specific therapeutics against infectious diseases is made very important at this moment by the COVID-19 pandemic caused by SARS-COV-2. Vaccines containing live attenuated or heat-inactivated pathogens elicit robust immune responses, but their safety is sometimes not assured. Subunit vaccines consisting of the most potent antigenic protein or carbohydrates of the pathogen are safer but often induce a weak immune response. Traditional Ayurveda medicines have a long history of safety and may act as immunomodulators or vaccine adjuvants. They can reduce the amount of vaccine booster doses required to elicit an immune response against any pathogen. The main objective of this review is a mechanistic evaluation of the antiviral potential of Ayurveda herbal compositions for their ability to increase cytokine expression and enhance NK cell activity, activate CD4/ CD8 + T cells, and increase the formation of IL-2 and IFNγ against SARS-CoV-2 infection.

Methods: Various peer-reviewed publications, books, monographs, and reputed search engines were reviewed in depth. Information available from the Ayurvedic Pharmacopoeia and in recent *in silico* analyses were compared in order to understand the mechanism of action of herbal components against SARS-CoV-2.

Results: It was found in various molecular docking and molecular dynamics studies that many bioactive natural components of Ayurvedic medicines could prevent viral entry or multiplication within a human host.

Conclusion: Ayurvedic herbal medicines can be used either independently as therapeutics or as a complement to the modern-day recombinant vaccines with immediate effect. Ayurveda-based adjuvant therapy can also efficiently manage the secondary symptoms of COVID 19 patients.

1. Introduction

All of mankind is currently challenged by an invisible enemy named Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) which is responsible for the coronavirus pandemic (Zhou et al., 2020; Chen et al., 2020). Researchers in every field are trying to find a clinically proven prophylactic or therapeutic strategy (Xu et al., 2020) to control worldwide morbidity and mortality caused by this virus. Traditional vaccines containing live attenuated pathogens or toxoids can initiate robust immune responses, yet culturing large numbers of pathogenic organisms in order to get a substantial quantity of the vaccine during the pandemic is difficult (Tandrup et al., 2016). Some vaccines used against respiratory viruses were reported to trigger undesirable responses due to the induction of cytotoxicity and enhanced antibody response (Rauch et al., 2018). Present-day subunit vaccines (de Alwis et al., 2020) consisting of the recombinant antigenic proteins of the pathogen and the virus-like particles (VLP) often induce weak immunogenic properties and sometimes fail to initiate humoral immunity (Bidokhti et al., 2019). Recently, some patients contracted a second COVID-19 infection after recovering from the initial bout, possibly due to their weak immune response. Hence, an immunoprophylactic measure offered by Indian Traditional Ayurveda medicines should be beneficial and long-lasting, functioning as natural therapeutics, immunomodulators, or as a vaccine adjuvant (Sagar and Kumar, 2020).

The Ayurveda medicine system is a treasure of single and poly-herbal formulations (Joshi et al., 2017). The immunomodulation activities and safety profiles of many of those formulations are well documented and many more resources are yet to be explored (Wu et al., 2001). A detailed description of the cause and management of the pandemic (*Janapadodhwamsa*) is documented in Ayurveda "*Rasayana Shastra*" (Jyotirmoy

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and Rekha, 2016). These classic formulations impart their effect by increased production of cytokine, IL-2 and IFNγ, enhanced natural killer cells, and $CD4^{+}/8^{+}$ T cell activities. Some of the poly-herbal formulations can be used as adjuvants with the vaccine to obtain safer and stronger immune responses (Sakure et al., 2008; Aranha and Venkatesh, 2020). The concept of immunity (*Vyadhikshamatwa*) is mentioned in the Ayurvedic system of medicine (*Rasayana*) which relies on a holistic approach for the prevention of diseases by boosting immunity through diet, lifestyle, and natural medicines (Masram et al., 2014). Using the Rasayana drugs and principles of Ayurveda, the sufferings of a large part of the population can be prevented (Nanal, 2008).

Numerous symptoms of COVID 19, which affect mostly the respiratory, immune, and other physiological systems, can be treated using adjuvant therapies. A multidisciplinary solution to enable translational research is urgently required. The authors have searched the traditional ethnopharmaceutical records, as well as recent literature, in order to understand the potential and probable mechanism of action of the Ayurvedic herbal preparations to address the issue of COVID- 19 issue. In this article, the different types of traditional Indian Ayurvedic formulations in practice have been reviewed; including their characteristics, efficiency, and mechanisms of action. The possibility of functional complementation of the Ayurvedic formulations as an adjuvant with modern era vaccine components to fight the COVID 19 pandemic were also critically evaluated.

2. Methodology

Antiviral effects of Ayurvedic herbal medicines and Ayurvedic vaccine adjuvants were searched from scientific search engines including Web of Science, Scopus, Medline, Google Scholar. The Ayurvedic Pharmacopoeia of India (Ayurvedic Pharmacopoeia of India, 2019) and the Ayurvedic Formulary of India (Ayurvedic Formulary of India, 2019) are official publications of the Ayurveda Pharmacopoeia Commission. A detailed bibliographic search from 1986 through 2020 was performed using these two ethnobotanically valuable resources. The Ministry of AYUSH (Ayurveda, Yoga and Naturopathy, Unani, Siddha, and Homoeopathy, New Delhi, India) published the guidelines to formulate the Ayurvedic herbal medicines to prevent COVID-19 (Advisory from Ministry of AYUSH, 2020). Apart from these resources, other published peer-reviewed articles, several conference proceedings, and books were also searched in order to have obtain a detailed mechanistic insight on Ayurvedic medicines used for the prevention and resolution of COVID-19 infections. Initially, the search was started at The National Center for Biotechnology Information (NCBI, 2021) or Ayurveda medicines from the "all database" category. This resulted in about 1734 distinct studies. The search was then restricted through PubMed from all databases for the keyword combination "Ayurveda" and "antivirals", which resulted in 956 pieces of literature. Another keyword was added viz, "*in silico*" study, which resulted in 250 additional published articles. However, most of them discussed general Ayurvedic compositions and their effect on the prevention of human infectious diseases. 229 published articles were thus excluded for not being specific and 217 articles were excluded because of overlapping information. Studies which described Ayurvedic herbs used for the treatment and prevention of SARS-CoV-2 viruses and their mechanism of action, relevant *in silico* studies and molecular docking were included in this study (471 articles, however most relevant ones have been discussed and cited). A flow chart describing the searching strategy and inclusion-exclusion criteria has been outlined in Fig. 1 (a) and (b). The clinical studies on prevention of COVID-19 was funded and conducted by AYUSH Research and Development Task Force, Ministry of AYUSH, Government of India, New Delhi and results were published (General Guidelines For Clinical Evaluation Of Ayurvedic Interventions, 2020). These guidelines were also consulted to prepare this review article.

3. The host immune response after SARS-CoV-2 infection

Studies have shown that a host's innate immune system can recognize the molecular pattern of the pathogen immediately after its invasion (Li et al., 2003). Viral RNA is recognized by the toll-like receptors which leads to the activation of several signaling pathways, activator protein 1 (AP-1), nuclear transcription factors, (NFk-β), and interferons. All these molecules then stimulate the inflammatory cytokines, interleukins, and chemokines (Channappanavar and Perlman, 2017). The onset of the production of interferons (IFN-α and -β) helps to suppress viral replication. For COVID-19 patients, the response by IFN type I is suppressed (de Wit et al., 2016). IgG antibodies which protect against viral N protein are detected in a patient's serum at 4–14 days and specific IgM is detected on day nine post-infection by SARS-CoV-2 (Gorse et al., 2020). CD8 + T cells are more predominant than CD4 + T cells (Tang et al., 2011). Strong T cell responses signify higher antibody production, and cytokine secretion such as interleukins 4,5, and 10 (Zhao et al., 2010). In the most severe cases, antibody concentration in patient serum remains lower. Vaccines and adjuvants should be aimed to enhance or restore the innate and humoral immune system in COVID 19 patients. Multifunctional Ayurvedic formulations could be long-lasting solutions.

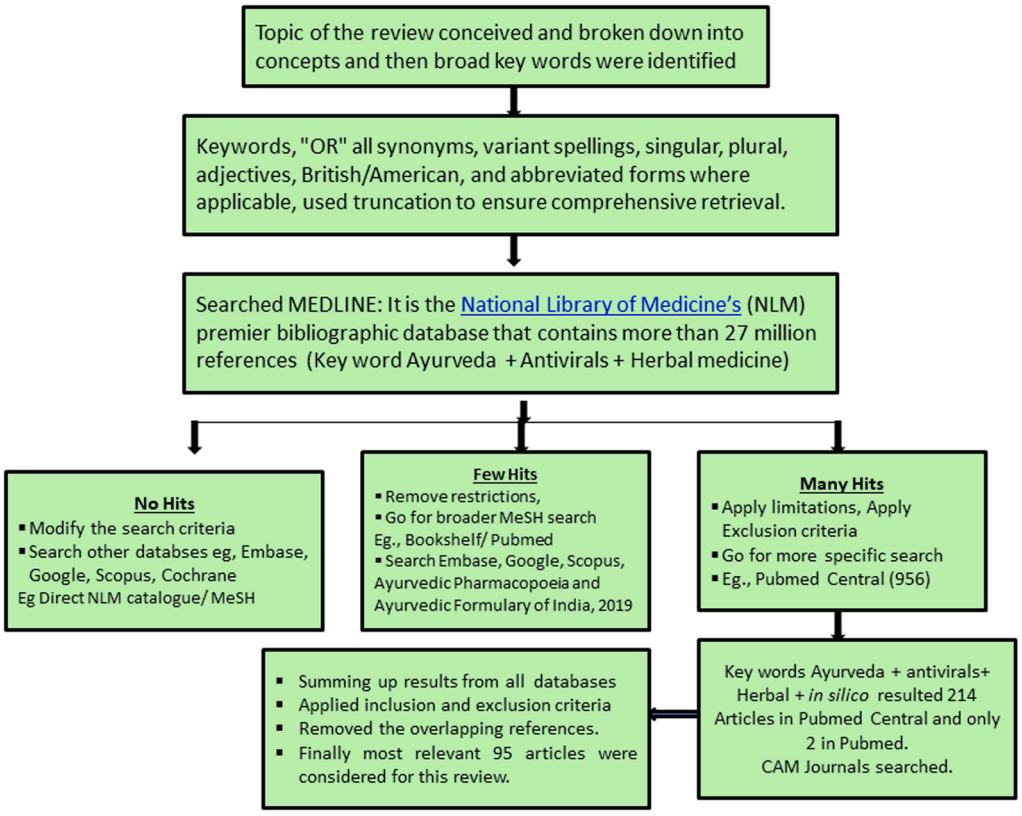
4. Vaccines and Adjuvants

The majority of conventional vaccines are either live-attenuated or inactivated forms of pathogens or toxoids (Kallerup and Foged, 2015). With the advancement of biotechnological techniques and next-generation sequencing platforms, recombinant subunit vaccines (Lu et al., 2015) and virus-like particles (VLP) composed of potent antigenic epitopes of the pathogen came onto the market (Mbewana et al., 2019; Stephen et al., 2018; Capell et al., 2020). They are safer than live vaccines but require multiple booster doses (Capell et al., 2020). Sometimes live vaccines may be higher risk and have been reported to cause liver diseases and the demyelination of neurons in experimental animals (Pedersen et al., 1981).

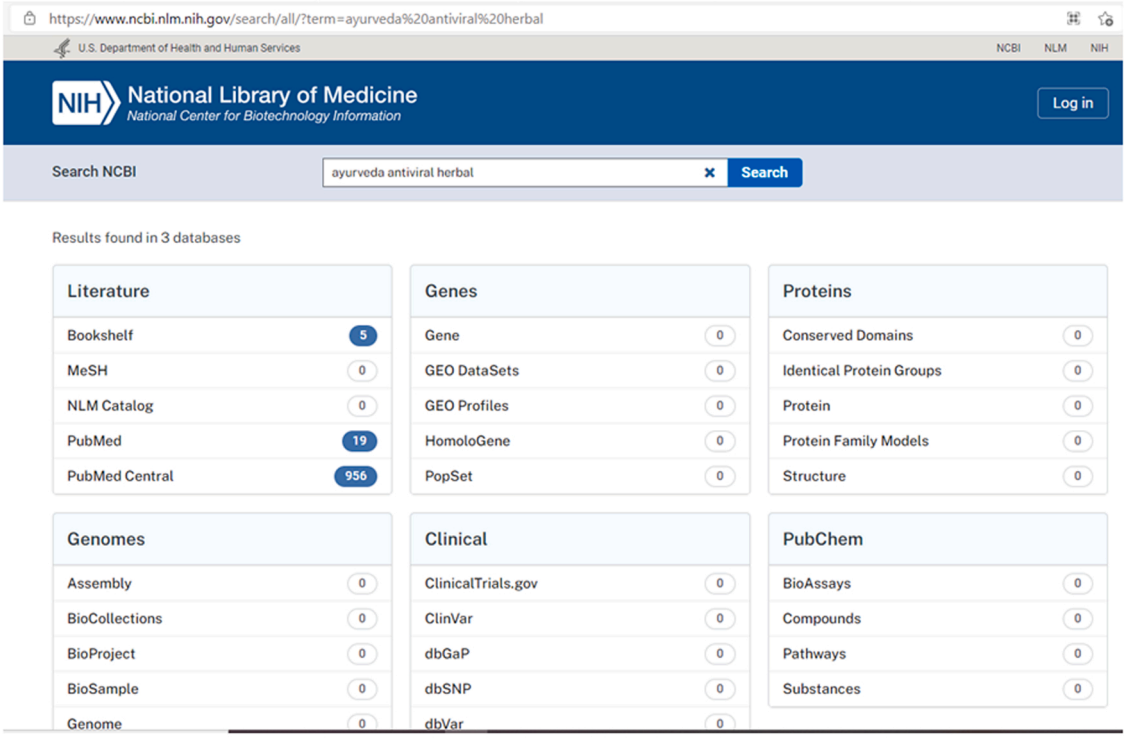
Adjuvants are single or multimolecular heterogeneous complexes that can stimulate or modulate the type of host immune response. When added to a vaccine, they increase vaccine potency while reducing the antigen amount or dose frequency and does not cause any specific effect on its own (Fig. 2) (Petrovsky and Aguilar, 2004; Di Pasquale et al., 2015). The use of bioactive Ayurvedic components as therapeutic agents and adjuvants can act as a strong immune booster without the overproduction of cytokines and chemokines.

5. Antiviral effect of Ayurvedic medicines on SARS-CoV-2

Ayurveda is an ancient medical practice which originated in India. Most modern antiviral treatments focus on the molecular level, but Ayurveda considers the whole body and mind to address disease using natural herbal extracts, Bhashmas (ashes of metals/ minerals after incineration), and some animal products. They can alleviate many side effects of allopathic medicines (Petrovsky, 2006). They are mostly immunomodulators. **Immunostimulants** (or immunopotentiators) act non-specifically as prophylactic agents in healthy individuals (Cox et al., 2006) and as an immunotherapeutic agent for immunocompromised individuals (Billiau and Matthys, 2001). Ayurvedic adjuvants turn on either cellular or humoral immune responses by activating helper T cells, dendritic cells, and cell signalling pathways. Ayurvedic **immunosuppressants** are often administered to regulate hyperimmune or autoimmune diseases or during organ transplant rejection. These may be beneficial for COVID-19 patients to avoid the activation of a cytokine storm or post-infection induction of the coagulation pathway (El-Sheikh, 2008). Ayurvedic medicines stimulate the mucosal response, induce protective non-antibody-based responses, and prevent infectious diseases such as COVID 19 (Golechha, 2020).



(a): A flowchart describing the strategy for the Literature search for this review



(b): The result showing hits and no hits for the given search queries

Fig. 1. (a) A flowchart describing the strategy for the Literature search for this review. Fig. 1(b) The result showing hits and no hits for the given search queries.

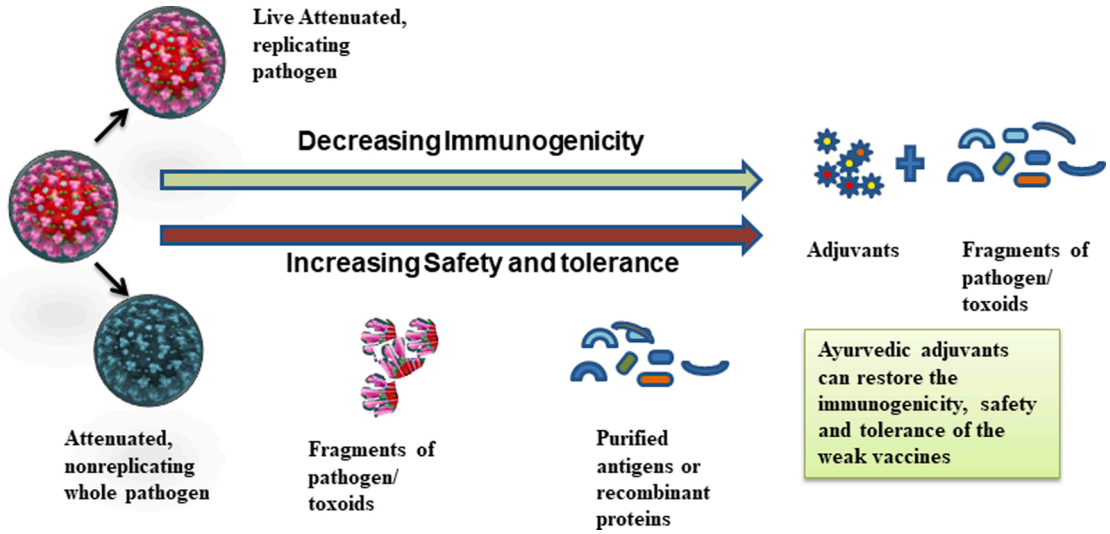


Fig. 2. Ayurvedic adjuvants increases safety, immunogenicity, and tolerance of the weak vaccines.

SARS-CoV-2 is a positive-strand RNA virus which encodes for envelop protein (E), nucleocapsid protein (N), Spike protein (S), and several ORFs (Open reading frame). The S protein binds to human ACE2 (Angiotensin converting enzyme 2) receptor through RBD (Receptor Binding Domain) followed by its priming and cleavage by cellular serine protease TMPRSS2 (Transmembrane protease serine 2) (Trujillo-Vargas et al., 2005; Li et al., 2020; Hoffmann et al., 2020). Most of the modern antiviral therapeutics used for the treatment of COVID 19 target either host cell proteases (ex. arbidol, teicoplanin, chloroquine, niclosamide, etc) or destroy viral proteins or nucleotides (ex. remdesivir, favipiravir, lopinavir/ ritonavir, and ribavirin). As revealed by molecular docking experiments, many Ayurveda formulas exert their antiviral therapeutic effects at these sites. A technical note published by Rajan et al. (2021) gives a detailed overview of the interface of Ayurveda and in silico biology. He emphasized the basic principles of ancient Ayurveda formulations and the efficacy of specific ligands to arrest COVID-19 progression. A study led by Maurya et al. (2020) evaluated the active constituents of Ayurveda formulations against the glycoprotein spike of SARS-CoV-2 and the host ACE-2 protein. Researchers used a structure-based drug design approach and considered pharmacokinetics, drug similarity, and toxicity. They also analyzed the gene regulatory network in order to understand the mechanism of COVID- 19 progression. They concluded that compounds such as eufoliatorin, amarogentin, α-amyrin, kutkin, caesalpinins, belladonnine, and β-sitosterol, have the highest affinity towards both the viral surface proteins and the host receptor protein ACE2. Other herbal compounds found in Ayurvedic formulas are discussed below.

5.1. *Shunthi (dry Zingiber officinale rhizome)*

The bioactive compounds of ginger, R-(-)– 1,2-propanediol and (6)-gingerol (Aboubakr et al., 2016; San Chang et al., 2013) were found to effectively decrease viral loads in patients (Abdel-Moneim et al., 2013). In a molecular docking (MD) study, the binding energy of the active compounds was found to be greater than hydroxychloroquine and quinine (Chakotiya and Sharma, 2020). *In silico* predictive analysis conducted on antigenic epitopes of SARS-COV-2 were found to have active antiviral properties which could induce humoral immunity. Haridas et al. (2021) provided evidence of the inhibitory effects of *Citrus medica* and *Zingiber officinale* for COVID-19 inhibition using an *in-silico* approach.

5.2. *Yastimadhu (Glycyrrhiza glabra)*

Bioactive components of Yastimadhu (*Glycyrrhiza glabra*; licorice) root were found to inhibit adsorption, penetration, and replication of SARS-CoV-2 in vitro (Cinatl et al., 2003; Pompei et al., 1979). Molecular docking experiments revealed that licorice's active constituents demonstrated a strong binding affinity with various viral proteins such as S protein, Main Protease (Mpro), and RNA-dependent RNA polymerase (RdRp) of SARS-CoV-2, as well as human furin protease and the ACE2 receptor (Maurya, 2020) (Table 1). Prediction of drug-likeness and pharmacokinetics using pkCSM server (A freely accessible web server (http://structure.bioc.cam.ac.uk/pkcsm) and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) were also analysed in *in-silico*. The antiviral components of licorice did not show any toxicity except Glabrin B (Gomaa and Abdel-Wadood, 2021).

5.3. *Ashwagandha (Withania somnifera)*

The antiviral, immunomodulatory, and anti-inflammatory profiles of *Withania somnifera* (Indian ginseng) are well documented (Pompei et al., 1979). Withanone, one of the active compounds found in *Withania somnifera*, was reported to block the entry of SARS-CoV-2 by interrupting electrostatic interactions between the viral RBD and host cell ACE2 (Balkrishna et al., 2020) and it also inhibits the virus' Mpro (Kumar et al., 2020). In silico analysis also revealed that the two protein targets of SARS-CoV-2 are RBD and NSP15 (Non-structural protein of corona virus) endoribonuclease. Withanoside, withanolide, di-hydro-withaferin A, and withanolide N were predicted to have efficient antiviral activity (Tripathi et al., 2021). A US patent was granted to the Serum Institute of India and the University of Pune (Jadhav et al., 2013) on an invention that uses withanolides from *Withania somnifera* as a vaccine adjuvant. The methanolic extract of *Withania somnifera* resulted in a dose-dependent increase in adjuvant activity when administered parenterally (Saggam et al., 2021). Animal studies and human clinical trials revealed that bioactive components of *Withania* spp. may be commercially used as an adjuvant (Nanal, 2008; Gautam et al., 2004). The work of Srivastava et al. (2020) on the *in- silico* screening of compounds derived from *Withania somnifera* and used as potential constituents effective against COVID-19 must also be noted.

5.4. *Guduchi (Tinospora cordifolia)*

Tinospora cordifolia or commonly known as Guduchi, also considered as the 'Rasayana plant" is found growing in the Indian subcontinent

Table 1
Predicted targets and binding energy of bioactive components of *Glycyrrhiza glabra*.

Bioactive components	Predicted binding energy (kcal/mol)	Target proteins of virus or host	Reference
Glycyrrhetic acid, Shinflavanone, Glycyrrhizin and Glabridin	-8.3, − 7.8, − 7.6 and − 7.2	main protease (Mpro), spike & RdRp, and host ACE2 and furin proteins	Muhseen et al. (2021),Van de Sand et al. (2021)
Apioside, Shinpterocarpin, Glycyrrhetic acid, Shinflavanone, Glabridin, and Liquiritin	-8.2, − 7.8, − 7.7, − 7.6, − 7.5 and − 7.4	Human ACE 2	Sagar and Kumar (2020)
Glycyrrhizin, Glabridin, Glabrin A and B, Liquiritin, Apioside, Shinflavanone, Shinpterocarpin, and Glycyrrhetic acid	-8.6, − 8.5, − 8.5, − 8.5, − 8.4, − 8.3, − 8.3, − 8.2	human furin protease and other host proteases	Muhseen et al. (2021), Van de Sand, 2021
Apioside, Glabridin, Glabrin B, Shinpterocarpin, Liquiritin apioside, Hispaglabridin A, Licochalcone A, Glycyrrhetic acid, A, Isoliquiritin Liquiritigenin, Shinflavanone, Prenyllicoflavone and Iso-liquiritigenin	-8.9, − 8.4, − 8.2, − 8.1, − 8.1, − 8.0, − 7.9, − 7.9, − 7.9, − 7.7, − 7.7, − 7.5 and − 7.5	Target SARS-CoV-2 Mpro and inhibit viral gene expression and replication.	Sagar and Kumar (2020), Muhseen et al. (2021)
Shinflavanone, apioside, Hispaglabridin A, Glycyrrhizin, Glycyrrhetic acid, Liquiritin, and Prenyllicoflavone	-8.8, − 8.5, − 8.4, − 8.4, − 8.3 and − 8.3	Acts on RdRp which is another key component of SARS-CoV-2 multiplication machinery	Sagar and Kumar (2020)

(Upadhay et al. (2010)). Several bioactive compounds were isolated from its root and stem bark and characterized as having immunomodulatory activity in an animal model (Nanal, 2008). The bioactive components extracted from Guduchi viz., berberine, magnoflorine, tinocordiside, and isocolumbin (Gupta and Chaphalkar, 2016) showed a high affinity to binding to surface glycoprotein (6VSB), RBD (6M0J), RdRp (6M71), and Mpro (6Y84) all found on SARS-CoV-2 (Sagar and Kumar (2020); Kalikar et al. (2008)). Different parameters related to binding efficacy like Ki (μM), IC50 (μM) and the binding affinity (Kcal/mol) of the compounds were analyzed *in silico*. The pharmacological activity of antiviral medicines viz., Favipiravir, Lopinavir/Ritonavir against 6Y84, the efficacy of Remdesivir against 6M71, and the efficacy of STGYC against 6VSB were compared to the activity of Guduchi's active constituents. The binding affinity, Ki and IC50 values of bioactive compounds from *Tinospora cordifolia* were tested by molecular docking and molecular dynamics experiments against important SARS-CoV-2 targets i.e., 1) surface glycoprotein (6VSB) and 2) Receptor binding domain (6M0J) both responsible for attachment of the virus to host cell, 3) RNA dependent RNA polymerase (6M71) and 4) main protease (6Y84) responsible for replication of the virus in the host cell. The plant constituents showed more efficacy than the allopathic drugs (Sagar and Kumar, 2020). *T. cordifolia*'s bioactive constituents have well-documented safety profiles and is well-known in the Ayurveda system, thus it may be a therapeutic option in cases of COVID-19.

5.5. Tulsi (*Ocimum sanctum*) and Neem (*Azadirachta indica*)

A crude hydroalcoholic extract containing the terpenoids and polyphenol compounds found in *O. sanctum* leaves was studied for its antiviral properties in an embryonated chicken egg model and all of them exhibited significant virucidal effects (Ghoke et al., 2018; Arora et al., 2010). Natural compounds from neem (*Azadirachta indica*) and tulsi were tested against the S protein, RdRp, and Mpro of SARS-CoV-2. Molecular docking experiments were performed using bioactive compounds found in tulsi and neem against SARS-CoV-2 protein targets (Kumar, 2020). Result showed that Ursolic acid, oleanolic acid, and methyl eugenol, the active components found in Tulsi have significant binding ability against S protein and RdRp, while Gedunin and Epoxyazadiradione, the active components found in Neem was effective against viral M protein. The binding efficacy of these natural compounds was found in *in silico* studies to be superior to those of Remdesivir and Lopinavir/Ritonavir.

5.6. Ayurvedic medicines used as adjuvants

Extracts from Vasa (*Adhatoda vasica)* were found to significantly reduce the hemagglutination of influenza virus and inhibited viral attachment and replication (Chavan, and Chowdhary, 2014), and enhanced the lymphocyte proliferation in mice (Gupta and Chaphalkar, 2016). Pneumonia caused by SARS CoV-2 may lead to acute hypoxia and fibrosis in the lungs. *Angelica sinensis* polysaccharide (ASP) extracted from garden angelica plant is used as an adjuvant. It increased the production of IFNγ and IL-2, and decreased the release of IL-4 (Maurya, 2020). IFNγ plays an important role in the clearance of a viral infection (Wohlmuth et al., 2002). An Ayurveda drug formulated from Shatavari **(***Asparagus racemosus)* was assessed for its immunoadjuvant potential successfully using an animal model (Gautam et al., 2004). A product from Katuki (*Picrorrhiza kurroa*) entitled RLJ-NE-299A was reported to have better immunostimulatory activity compared to alum and up-regulated Th1 cytokines IL-2, IL-12, IFN-γ, TNF-α, and Th2 cytokine IL-4 in lymph node cell cultures and increased IgG2a and IgG1 immunoglobulins when in serum (Khajuria et al., 2007). Curcumin, derived from turmeric (*Curcuma longa*), was reported to have antiviral activity against numerous human viruses (Praditya et al., 2019). These bioactive compounds could also be tested on SARS-CoV-2. Most of the current subunit or recombinant vaccines rely on parenteral routes of administration and predominantly elicit IgG response, while natural infection induces several antibody subtypes. Thus, the protection offered by these vaccines is short-term (Dey and Srivastava, 2011). SARS-CoV-2 enters through the nasal-oral route and produces mucosal immune responses initially. Thus, oral administration of Ayurvedic medicines and adjuvants could play an important role. Plant-based lectins (Lutsiak et al., 2006) proteins (Ford and Roach, 2010), and polysaccharides from Kiwi (*Actinidia eriantha)* and garlic (*Allium sativum*) (Hofmeyr, 2001) are good vaccine adjuvant candidates, and saponins from 'Soap bark tree' *Quillaja saponaria* and 'Gan cao' *Glycyrrhiza uralensis* are under clinical trials (Brewer and Pollock, 2004).

5.6.1. Polyherbal Ayurvedic Medicines against SARS-CoV-2

A molecular docking study using ten pharmacologically active Ayurveda medicines was conducted with SARS-COV-2 (Srivastava et. al, 2020). A mixture called Nagaradi Kashaya has been used to combat Covid 19 (*Jwara Chikitsa*, where 'Jwara' means increase in body temperature (fever) but also a feeling of malaise, unease and discomfort, and 'chikitsa' means treatment) with promising results and includes Guduchi (*Tinospora cordifolia*), Sunthi (*Zingiber officinale*), Kantakari (*Solanum virginianum*), and Pushkarmool (*Inula racemose*) (Gandhi et al., 2020). These herbs had *Tikta Rasa (bitter taste), Laghu (light),* and *Pachana Guna (digestive properties)*. These herbal extracts have bioactive components and effective to address different body ailments. Virtual screening of these drugs showed promising results against *Jwara* (fever), *Kaas* (cough), and *Shwaas* (breath), which are similar to SARS-COV-2 symptoms. An *in silico* study was conducted to predict the binding energy between bioactive constituents with TMPRSS2, ACE2, 3CLpro, PLpro, and RdRp of SARS-CoV-2 as the target proteins using autodock 4.2 (Wu

et al., 2020). Positive results emphasize that the above-mentioned active compounds can be used in antiviral therapy against COVID-19.

The Ministry of AYUSH, Government of India, has recommended an Ayurveda formulation composed of the following extracts to protect against COVID-19: *Tinospora cordifolia, Adhatoda vasica, Zingiber officinale, Piper longum, Hygrophilla auriculata, Tragia involucrata, Plectranthus amboinicus, Anacyclus pyrethrum, Terminalia chebula, Saussurea costus, Syzygium aromaticum, Andrographis paniculate, Clerodendrum serratum, Sida acuta*, and *Cyperus rotundus*. The targets of these Ayurvedic antiviral compounds (Laksmiani et al., 2020) are detailed in Table 2. However, further validation is required for this plant-based formulation.

Kumar et al. (2021) performed docking analysis using secondary metabolites mentioned in Ayurvedic text references. The group studied molecular affinities and the interactions of gingerol, curcumin, and quercetin with target proteins RdRp, furin, and ACE2 and further validated by a 100 ns molecular dynamic (MD) simulation run. The binding free energy was calculated using the Molecular Mechanics-Poisson Boltzman Surface Area (MM-PBSA) method for the calculation of binding free energies and its application in virtual screening studies to evaluate the thermodynamic stability of the conformation. The results were compared to the antiviral medication Remdesivir as a positive control. Additionally, pharmacophore features, 3D structural alignment of potent compounds, and a Bayesian machine learning model were also used to support the molecular docking and simulation studies.

Joshi et al. (2021) investigated several traditional herbal formulations for their efficacy against SARS-COV-2's viral attachment, entry and proliferation. In order to repurpose and validate the well-characterized polyherbal formulations, the team performed a molecular docking and molecular dynamics simulation. The results of the *in silico* formula comparison revealed that Sanjeevani Vati, Pathyadi kwath, Tribhuvan Keeratiras, Septillin, and Yashtimadhu were more effective compared to Samshamni Vati, AYUSH-64, and Trikatu.

6. Ayurvedic Bhasmas against SARS-CoV-2

A branch of Ayurveda called "Rasashastra" deals with the preparation and therapeutic applications of metallic and mineral compounds, together with various organic materials. They are processed after repeated heating where material is reduced into metal or mineral ashes (*Bhasmas*), which then turn into nanoparticles (Ruidas et al. (2020)). Several Ayurvedic *Bhasma* preparations are effective through sublingual and oral routes due to better bioavailability and absorption. *Swarna Bhasma* (gold nanoparticles) and *Yashada Bhasma* (zinc nanoparticles) could exhibit immunomodulation activity by stimulating macrophage functions to promote phagocytic activity and modulate the T-cell mediated immune response. Swarna Bhasma, *Rajata Bhasma* (silver nanoparticles), *Tamra Bhasma* (copper nanoparticles), and *Yashada Bhasma* have an anti-inflammatory effect in the body and can reduce cytokine production. *Swarna Bhasma, Rajata Bhasma,* and *Tamra Bhasma* have virucidal effects (Sreelakshmi et al., 2021). *Swarna Bhasma* and *Yashada Bhasma* may be effective as vaccine adjuvants alongside COVID-19 vaccines by increasing the levels of interleukins and interferons (Sarkar and Das Mukhopadhyay (2021)). They are safe and activate se)eral biochemical processes within the cells and function as nanomedicines (Ruidas et al. (2019)).

7. Existing trials, prospects, Repurposing possibilities and challenges of ayurvedic medicines

Ayurvedic medicine contains much potential and possibility for the prevention and treatment of COVID-19 and its associated symptoms. AYUSH published guidelines for improving immunity against COVID-19 by using Ayurvedic medicines (Golechha, 2020). Ayurvedic medicines have been reported to exert their effect through psycho-neuro-immune pathways. These effects may anxiety, and poor mental health, this can cause vulnerable groups to have poor immunity and thus be more prone to viral infection.

As most of the proposed herbal treatments need to be evaluated in vivo, some recent publications were also reviewed. A randomized and comparative study to assess the safety and efficacy of supplemental treatment of a herbal formulation enriched with essential oils named "Aayudh Advance" on viral load as well as recovery duration in mild symptomatic patients diagnosed with COVID-19 was carried out by Dutt et al. (2021). They used 'Covariate Adaptive Randomization' treatment technique for 14 days and reviewed the clinical signs and symptoms and compared with traditional biochemical testing. Among 74 patients the

Table 2
List of Ayurvedic immunomodulator plants *in silico* effect on SARS-CoV-2.

Name of the plant	Botanical name	Bioactive Phytochemicals	Binding energy (kcal/mol)	A possible target of action	Reference
Shatavari	*Asparagus racemosus*	Kaempferol	-6.70	Tyrosine protein kinase receptors and Interleukin-8 receptor A	Srivastava et al. (2020)
Yashtimadhu	*Glycyrrhiza glabra*	Glycyrrhizin	-8.47	Glycogen synthase kinase-3β And Tyrosine protein kinases	Srivastava et al. (2020), Wu, 2020
Parijata	*Nyctanthes arbortristis*	Nictoflorin, lupeol astragalin,	-9.18,– 8.28,– 8.68	SARS-CoV-2 protease, inhibition of viral replication.	Srivastava et al. (2020)
Kalmegh	*Andrographis paniculata*	Andrographolide	-7.62	RNA-dependent RNA polymerase	Wu, 2020, Lakshmiani, 2020
Mustaka	*Cyperus rotundus*	sugetriol-3,9-diacetate	-6.12	Papain-like proteinaseInterleukin-6 receptor subunit βSpecific protein kinases	Srivastava et al. (2020)
Guduchi	*Tinospora cordifolia*	Berberine, sitosterol, isocolumbin,	-8.67,– 8.42	SARS-CoV-2 protease, chymotrypsin-like protease Inhibitor of NFkβ kinase	Rauch, 2018, Sagar, 2020, Sakure, 2008, Wu, 2001
Ghritakumari	*Aloe barbadensis miller*	Aloenin, aloesin	-9.13, – 8.79	SARS-CoV-2 protease	Laksmiani, 2020
Adraka	*Zingiber officinale*	Gingerol, shogaol	-7.95, – 7.86	SARS-CoV-2 protease. Blocks ACE2 interactions.	Srivastava, 2020
Ashwagandha	*Withania somnifera*	Withanolide, withaferin A	-8.07–8.05	SARS-CoV-2 protease	Wu, 2020, Laksmiani, 2020
Nimba	*Azadirachta indica*	Nimbin	-8.17	SARS-CoV-2 protease	Laksmiani, 2020
Haridra	*Curcuma longa*	Demethoxycurcumin	-8.44	Inhibits ACE2 interactionsand NFkappa-β kinase, Toll-like receptor (TLR7/TLR9),Tyrosine-protein kinases	Srivastava, 2020
Sappanwood	*Caesalpinia sappan*	Brazilin	-8.32	SARS-CoV-2 protease and ACE2 inhibitor. Inhibits penetration and absorption on the host surface.	Wu, 2020
Citrus	*Citrus sp*	Naringenin	-8.31	SARS-CoV-2 protease and ACE2 inhibitor. Inhibits penetration and absorption on the host surface.	Laksmiani, 2020

recovery rate observed was 15.38% more compared to standard treatment regime. Also, no patients showed any adverse effect. The work of Bhapkar et al. (2020) on existing 197 registered trials of Ayurvedic formulations for COVID-19 deserves a special mention in this regard. The results revealed some interesting characteristics of study designs such as use of platform trial design, system specific criteria for assessment and personalized interventions, sample size, treatment arms, comparator used and study duration. This study was initiated and funded by AYUSH, India. For a conclusive outcome more datasets are required.

Hypoxia is a common symptom associated with SARS-CoV-2 infection. Gheware et al. (2021) used the whole aqueous extract of *A. vasica* to study its anti-hypoxic and anti-inflammatory effects based on the molecular hypoxia response pathway. Their preclinical studies on mouse models revealed that oral administration of aqueous *A. vasica* extract attenuated airway inflammation, levels of transforming growth factor-β1 (TGF-β1), IL-6, HIF-1α, arrested disease progression and improved the mice survival rates. From the lung transcriptomic analyses, researchers concluded that *A. vasica* could downregulate genes related to hypoxia, inflammation, TGF-β1, and angiogenesis and upregulate the genes related to adaptive immunity. These results provide scientific evidence for using Ayurvedic herbal medicine to ameliorate the hypoxia-hyperinflammation features in COVID-19 conditions.

Rajan et al. (2021) validated the Prakriti assessment scale invented by the Central Council for Research in Ayurvedic Sciences (CCRAS), an autonomous body of the Ministry of AYUSH, India, and applied it to 117 COVID 19 patients. The concept of *Prakriti* (Symptom or nature of the patient) in Ayurvedic texts is used for deciding the prophylactic and therapeutic strategy to be adopted for a patient. It is based on the systemic approach of anatomical, physiological, and psychological domains of an individual. Since COVID 19 is a new disease, the status of the susceptibility of its victim in terms of Prakriti is not known. So, rationale of this study was to determine the Prakriti of COVID 19 positive patients and to customize the treatment based on the individual reading on the Prakriti scale.

In the present review, the versatility of Ayurvedic medicines and their potential for immune response are illustrated. Since most modern vaccines are either recombinant proteins or synthetic compounds, functional complementation of adjuvants found in Ayurvedic medicine should be evaluated at the initial formulation stage. The polyherbal Ayurvedic adjuvants can conveniently complement multiple antigenic epitopes of pathogens like SARS Cov-2 and may be also able to mitigate viral mutations.

Ayurvedic medicines and adjuvants exert their effects through molecular interactions with antigen-presenting dendritic cells, B and T lymphocytes. While the safety of whole live attenuated virus vaccines may evoke harmful immune responses, Ayurveda has a high potential for the prevention of COVID-19. Most of the studies are based on computational predictions and *in-silico* analyses. More research is required towards the validation using wet laboratory experiments and analyses of bioavailability of the drugs. Evidence-based research towards the adverse effects of these preparations would be beneficial. It may be a valuable tool to healthcare services and the bio-medical system as Ayurvedic herbs offer protective immunity while supporting resiliency.

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CRediT authorship contribution statement

CDM conceived the idea and prepared the manuscript. PS and CDM together finalised the manuscript and graphical explanations, supporting tables and references.

Declaration of Competing Interest

The authors declare no conflict of interest.

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