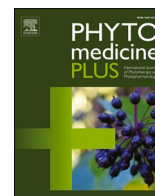




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Ayurvedic formulations: Potential COVID-19 therapeutics?

Anees Ahmed Mahaboob Ali ^a, Andrea Bugarcic ^b, Nenad Naumovski ^{c,d}, Reena Ghildyal ^{a,*}

^a Centre for Research in Therapeutic Solutions, Faculty of Science and Technology, University of Canberra, Canberra, Australia

^b National Centre for Naturopathic Medicine, Southern Cross University, Lismore, New South Wales, Australia

^c Functional Foods and Nutrition Research Laboratory, Faculty of Health, University of Canberra, Canberra, Nggunawal Country, Australia

^d Department of Nutrition-Dietetics, School of Health Science and Education, Harokopio University, Athens, Greece

ARTICLE INFO

Keywords:

COVID-19
Ayurvedic formulations
Antivirals
In silico molecular docking
Clinical trials

ABSTRACT

Background: While Molnupiravir and Paxlovid have recently been approved for use in some countries, there are no widely available treatments for COVID-19, the disease caused by SARS-CoV-2 infection. Herbal extracts have been used to treat respiratory clinical indications by Ayurvedic medicine practitioners with minimal adverse reactions and intense research efforts are currently under way to develop some of these formulations for COVID-19 treatment.

Methods: Literature search for *in silico*, *in vitro*, *in vivo*, and clinical studies on the topic of Ayurvedic formulations for potential COVID-19 treatment, in order to present the current state of current knowledge by integrating information across all systems.

Results: The search yielded 20 peer reviewed articles on *in silico* studies examining the interaction of phytoconstituents of popular Ayurvedic formulations with SARS-CoV-2 components and its receptors; five articles on preclinical investigations of the ability of selected Ayurvedic formulations to inhibit functions of SARS-CoV-2 proteins; and 51 completed clinical trials on the efficacy of using Ayurvedic formulations for treatment of mild to moderate COVID-19. Clinical data was available from 17 of the 51 trials. There was a considerable overlap between formulations used in the *in silico* studies and the clinical trials. This finding was unexpected as there is no clearly stated alignment between studies and the traditional pathway to drug discovery—basic discovery leading to *in vitro* and *in vivo* proof of concept, followed by validation in clinical trials. This was further demonstrated in the majority of the *in silico* studies where focus was on potential antiviral mechanisms, while the clinical trials were focused on patient recovery using oral treatments. In all 17 clinical trials where data was available, Ayurvedic treatments lead to a shorter period to recovery in participants with COVID-19.

Conclusion: The most commonly used Ayurvedic treatments for management of respiratory symptoms associated with SARS-CoV-2 infection appear to have prophylactic and/or therapeutic properties. It would be of particular interest to assess synergistic and concomitant systemic effects and antiviral activities of individual phytoconstituents and their combinations in the Ayurvedic treatments.

Abbreviations

3CL^{Pro} 3C like protease
M^{Pro} Main protease
ACE2 angiotensin converting enzyme 2
COVID-19 coronavirus infectious disease 2019
ERGIC endoplasmic reticulum Golgi intermediate compartment
KSK Kabasura Kudineer
(MERS)-CoV Middle Eastern Respiratory Syndrome
NSP nonstructural protein

NVK Nilavembu Kudineer
RBD receptor binding domain
SARS-CoV Severe Acute Respiratory Syndrome coronavirus
TMPRSS2 transmembrane serine protease 2
uHPLCPDA an ultra-high-performance liquid chromatographic-
photodiode array
vRNP viral ribonucleoprotein

* Corresponding author.

E-mail address: Reena.Ghildyal@canberra.edu.au (R. Ghildyal).

<https://doi.org/10.1016/j.phyplu.2022.100286>

Available online 20 April 2022

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Introduction

Human coronaviruses usually cause common cold symptoms, except for the recently emerged betacoronaviruses, including Severe Acute Respiratory Syndrome coronavirus (SARS-CoV), Middle Eastern Respiratory Syndrome (MERS)-CoV (Fung and Liu, 2019) and SARS-CoV-2 that can cause severe disease and in extreme cases, death (WHO, 2021a). The SARS-CoV-2, identified as the causative agent for COVID-19 and the current pandemic, emerged in 2019 and has rapidly spread around the world, evolving into highly transmissible strains, the delta and omicron variants and recent subvariants (WHO, 2021a). Individuals infected with COVID-19 present with a range of clinical manifestations, from asymptomatic to mild (cough, chest pain, loss of taste, back pain) to fatal (death). There have been over 464 million confirmed cases and more than 6 million deaths worldwide (WHO, 2021b) (information current as of 20 March 2022), with a concerted effort around the world to develop and deploy vaccines for protection from emerging SARS-CoV-2 strains. Although several vaccines have been approved for use in adult, adolescent and older children populations (March 2022), no vaccine has been approved for infants and young children below the age of 5 years. It is envisaged that once the majority of the global eligible population has been vaccinated, the younger, currently ineligible, population will be at a high risk of infections. Access to safe and effective therapeutics would greatly enhance our ability to manage SARS-CoV-2 infections into the future.

Currently, there are no widely available therapeutics for the management of COVID-19 (Siemieniuk et al., 2020), however, recent clinical trial data from several drug treatment options are showing promising results. Fluvoxamine (Abbott Laboratories) has shown good results in treating COVID-19 by preventing patients from developing serious illness and reducing the risk of hospitalization (Reis et al., 2022), while Novartis has declared topline data from their Phase 2 study for Ensovibep showing viral load reduction over eight days. Pfizer has announced promising outcomes from clinical trials of Paxlovid™ that targets the SARS-CoV-2 3C-like protease, while Merck has announced similar results from trials of Molnupiravir, a nucleoside analog (Drozd-zal et al., 2021; Jayk Bernal et al., 2021). The latter two experimental drugs have been authorized by the FDA and are approved for use in several countries.

The use of nutraceuticals and/or dietary supplements, including herbal medicines, is well established for the management of several respiratory indications (Chan et al., 2021; Chen et al., 2014) and the interest in their antiviral properties has also been investigated in the context of the current COVID-19 pandemic (Parisi et al., 2021; Singh et al., 2021; Steel et al., 2020). Many research groups have examined the potential use of natural products and substances already available for use for other indications, such as Ayurvedic formulations that are widely used in India and within the Indian diaspora (Ayatollahi et al., 2021). Ayurveda is an ancient Indian holistic system of medicine that utilizes plant extracts as one of the main ingredients for their formulations (National Center for Complementary and Integrative Health, 2022). Given the widespread use of Ayurvedic formulations as complementary medicine, they provide an attractive avenue for development of anti-COVID-19 treatments that would be safe and well tolerated (Singh et al., 2018; Steel et al., 2020).

In this review we aim to summarize the current literature on the efficacy of natural compounds most commonly used in Ayurvedic formulations with potential anti-SARS-CoV-2 properties, in order to gauge the current stage of knowledge in this topic and provide the stepping-stone for development of successful anti-viral treatments.

SARS-CoV-2

The main structural components of coronaviruses are the spike glycoprotein S, the transmembrane proteins M (membrane) and E (envelope), and the nucleoprotein N. The S, M and E proteins are embedded

in the virus envelope while N protein forms a viral ribonucleoprotein (vRNP) complex with the viral RNA (V'Kovski et al., 2021). The SARS-CoV-2 genome is organized similarly to other coronaviruses and the positive-stranded RNA genome has a 5'-cap and a 3'-poly-A tail, allowing its translation from the host translation machinery. A frame-shift between two open reading frames (Orf), Orf1a and Orf1b at the 5'-end of the genome allows production of two polypeptides that are then proteolytically processed to produce 16 non-structural proteins (Nsp1–16) that are involved in various processes of the virus infection cycle. The structural S, N, M and E proteins are encoded at the 3'-end. The life cycle of SARS-CoV-2 involves the initial infection of human cells through the host cell receptor angiotensin-converting enzyme 2 (ACE2), together with the cell surface serine protease TMPRSS2 that acts as a coreceptor mediated by the S glycoprotein via its receptor binding domain (RBD). Following entry via endocytosis, the positive-sense RNA genome is translated into a polyprotein with RNA polymerase function; this produces negative-sense sub-genomic mRNA before making positive-sense sub-genomic mRNA ready for translation. Following translation, the components associate in the endoplasmic reticulum Golgi intermediate compartment (ERGIC) before being released by exocytosis. We would like to direct the reader to excellent articles on the structure and life cycle of SARS-CoV-2 and its relationship to other known coronaviruses (V'Kovski et al., 2021; Yang and Rao, 2021) as these aspects will not be discussed in detail in this manuscript. Most of the antiviral development efforts against SARS-CoV-2 have targeted the glycoprotein S which is the major receptor binding protein. This glycoprotein is also the focus of the vaccine development efforts as it is the main target of our immune response. As the coronavirus 3C-like protease 3CL^{PRO} (also termed M^{PRO}) is critical for virus polyprotein processing and virus replication this enzyme poses as another viable anti-viral target.

Search strategy

The main motivation for this review was to understand how Ayurvedic formulations can support the clinical management of individuals infected with COVID-19. Our search strategy involved five electronic databases: Scopus, PubMed, AYUSH Research Portal, Clinical Trial Registry-India (CTRI), and ClinicalTrials.gov since the journals and websites inception until 22 October 2021. We have also performed updated searches for any additional articles that may be of interest just prior to re-submission of this manuscript (20 March 2022). The keywords used included “SARS-CoV-2” AND/OR “COVID-19” AND “Ayurveda”. The resulting database of research literature and clinical trials included *in silico* studies, pre-clinical laboratory studies and clinical trials published in English. Clinical trials where no trial data was available were excluded. The various Ayurvedic formulations mentioned in this review and their herbal constituents are listed in Table 1.

In silico investigations

The searches have identified 20 peer reviewed publications of studies utilizing *in silico* techniques to determine the possible direct antiviral effect of known phytoconstituents of Ayurvedic formulations. The Ayurvedic formulations were composed of natural extracts from multiple plant sources, therefore, it is essential to understand the possible antiviral effect of the phytoconstituents and their metabolites within the extracts. Among various antiviral activities exhibited by plant metabolites, angiotensin converting enzyme 2 (ACE2) inhibitors have been explored in detail and shown to be a possible way to manage SARS-CoV-2 infection (V'Kovski et al., 2021). Ayurvedic formulations have also been studied for their potential inhibition of M^{PRO} activity (Gurung et al., 2020; Havranek and Islam, 2021). Although there are relatively limited studies examining the potential of Ayurvedic formulations to inhibit RNA polymerase activity, these formulations may present excellent

Table 1
Herbal constituents of Ayurvedic formulations referred to in the text.

Ayurvedic name	Constituents
Adathodai Manapagu	<i>Adhatoda vasica</i>
Agnikumara Rasa	<i>Piper nigrum</i>
Amalaki Churna	<i>Phyllanthus emblica</i>
Amruth	<i>Tinospora cordifolia</i>
Amrutha Sanjeevini	<i>Tinospora cordifolia</i> , <i>Phyllanthus emblica</i> , <i>Ocimum tenuiflorum</i> , <i>Andrographis paniculata</i> , <i>Glycyrrhiza glabra</i> , <i>Withania somnifera</i>
Amukkara	<i>Withania somnifera</i> , <i>Zingiber officinale</i> , <i>Piper nigrum</i> , <i>Piper longum</i> , <i>Elettaria cardamomum</i> , <i>Cinnamomum verum</i> , <i>Syzygium aromaticum</i> , <i>Saccharum officinarum</i> , <i>Messua ferrea</i>
Anu Taila	Herbal oil with >20 ingredients*, including <i>Ocimum tenuiflorum</i> , <i>Elettaria cardamomum</i> , <i>Cinnamomum zeylanicum</i>
Arogya Kashayam-20	<i>Tinospora cordifolia</i> , <i>Zingiber officinale</i> , <i>Phyllanthus niruri</i> , <i>Glycyrrhiza glabra</i> , <i>Terminalia chebula</i> , <i>Piper longum</i> , <i>Piper nigrum</i>
AyurCoro-3	Seven ingredients, including <i>Curcuma longa</i>
AYUSH 64	<i>Alstonia cholaris</i> , <i>Picrorhiza kurroa</i> , <i>Swertia chirata</i> , <i>Caesalpinia crista</i>
Bilvadi	Seven herbs and spices, including <i>Zingiber officinale</i> , <i>Coriandrum sativum</i> , <i>Piper longum</i> , <i>Piper nigrum</i> , <i>Aegle marmelos</i>
Brahmananda Bhairavam	<i>Zingiber officinale</i>
Bresol	11 ingredients, including <i>Curcuma longa</i> , <i>Ocimum Sativum</i> , <i>Adathoda vasica</i> , <i>Zingiber Officinale</i>
CIM-MEG19	<i>Andrographis paniculata</i>
CLEVIRA	ten ingredients, including <i>Tinospora cordifolia</i> and <i>Andrographis paniculata</i>
CONTAZAP	<i>Andrographis paniculata</i> , <i>Rheum emodi</i> , <i>Embelica officinalis</i>
Dashamula Kwatha	<i>Aegle marmelos</i> , <i>Premna mucronata</i> , <i>Oroxylum indicum</i> , <i>Stereospermum suaveolens</i> , <i>Gmelina afborea</i> , <i>Solanum indicum</i> , <i>Solanum xanthocarpum</i> , <i>Tribulus terrestris</i> , <i>Desmodium gangeticum</i> , <i>Uraria picta</i>
Giloy	<i>Tinospora cordifolia</i>
Golden Milk, Ojovardhini	<i>Curcuma longa</i>
Immunofree	14 ingredients, including <i>Andrographis paniculata</i> , <i>Phyllanthus niruri</i> , <i>Glycyrrhiza glabra</i> , <i>Ocimum tenuiflorum</i> , <i>Tinospora cordifolia</i> , <i>Curcuma longa</i>
Immuzan	<i>Ocimum tenuiflorum</i> , <i>Curcuma longa</i> , <i>Embelica officinalis</i> , <i>Zingiber officinale</i>
Jeevaneeyam	15 ingredients, including <i>Tinospora cordifolia</i> , <i>Phyllanthus emblica</i> , <i>Ocimum tenuiflorum</i> , <i>Glycyrrhiza glabra</i>
Kalmegh	<i>Andrographis paniculata</i>
Kantakaryavaleha	14 ingredients, including <i>Tinospora cordifolia</i> , <i>Piper longum</i> , <i>Piper nigrum</i> , <i>Zingiber officinale</i>
Mahasudarsan Chooranam	Polyherbal with >50 herbal ingredients.
Maldevi Chendooram	not a herbal
Nagaradi kwath	<i>Zingiber officinale</i> , <i>Terminalia chebula</i> , <i>Tinospora cordifolia</i>
Nochi Kudineer Chooranam	<i>Vitex negundo</i> , <i>Artemisia pallens</i> , <i>Piper nigrum</i> , <i>Allium sativum</i> , <i>Piper betle</i>
NOQ19	19 ingredients, including <i>Withania somnifera</i> , <i>Aegle marmelos</i> , <i>Glycyrrhiza glabra</i> , <i>Pluchea lanceolata</i> , <i>Adhatoda vasica</i> , <i>Piper longum</i> , <i>Curcuma longa</i> , <i>Cissampelos pareira</i> , <i>Phyllanthus fraternus</i> , <i>Andrographis paniculata</i> , <i>Alstonia scholaris</i> , <i>Ocimum tenuiflorum</i> , <i>Tinospora cordifolia</i>
Oma theeneer	<i>Trachispermum roxburghianum</i>
Pathyadi Kwatha	<i>Terminalia chebula</i> , <i>Terminalia bellirica</i> , <i>Embelica officinalis</i> , <i>Andrographis paniculata</i> , <i>Curcuma longa</i> , <i>Azadirachta indica</i> , <i>Tinospora cordifolia</i>
PINAK	<i>Erythrina indica</i> , <i>Magnefera indica</i> , <i>Eugenia jambolana</i> , <i>Jusminum sambac</i>
Pipli	<i>Piper longum</i>
Rasona	<i>Allium sativum</i>
Reginmune	<i>Echinacea purpurea</i> , <i>Aloe vera</i> , <i>Uncaria tomentosa</i>
Sanshamani Vati	<i>Tinospora cordifolia</i>
Septilin	

Table 1 (continued)

	~35 ingredients, including <i>Commiphora wightii</i> , <i>Tinospora cordifolia</i> , <i>Phyllanthus emblica</i> , <i>Glycyrrhiza glabra</i>
Shadangodak	<i>Cyperus rotundus</i> , <i>Fumaria indica</i> , <i>Vetiveria zizanioides</i> , <i>Santalum album</i> , <i>Andropogon vetiveria</i> , <i>Zingiber officinale</i>
Shakthi	<i>Embelica officinalis</i> , <i>Withania somnifera</i> , <i>monnieri</i> , <i>Eclipta alba</i> , <i>Tinospora cordifolia</i> , <i>Asparagus racemosus</i> , <i>Convolvulus pluricaulis</i> , <i>Glycyrrhiza glabra</i> . <i>Bacopa Zingiber officinale</i>
Shunthi	<i>Curcuma longa</i> and other ingredients
SSV Formulation	<i>Ocimum tenuiflorum</i> , <i>Solanum virginianum</i> , <i>Tinospora cordifolia</i> , <i>Zingiber officinale</i> , <i>Curcuma longa</i> , <i>Adathoda vasica</i> , <i>Elettaria cardamomum</i> , <i>Piper nigrum</i> , <i>Piper longum</i> , <i>Acorus calamus</i>
Surasadi Kadha	10 ingredients, including <i>Glycyrrhiza glabra</i> , <i>Syzygium aromaticum</i> , <i>Cinnamomum zeylanicum</i> , <i>Pistacia chinensis</i> , <i>Capparis noonii</i> , <i>Zingiber officinale</i> , <i>Piper longum</i>
Swasari Ras	11 ingredients, including <i>Terminalia chebula</i> , <i>Zingiber officinale</i> , <i>Punica granatum</i> , <i>Asparagus racemosus</i> , <i>Piper longum</i> , <i>Myristica fragrans</i> , <i>Leptadina reticulata</i> and <i>Tinospora cordifolia</i>
T-AYU-HM Premium	14 ingredients, including <i>Piper nigrum</i> , <i>Piper longum</i> , <i>Zingiber officinale</i> , <i>Elettaria cardamomum</i>
Thaleesadhivadagam	<i>Zingiber officinale</i> , <i>Piper longum</i> , <i>Piper nigrum</i>
Trikatu Churna	Proprietary formulation with >50 ingredients.
Trishun	<i>Tinospora cordifolia</i> , <i>Ocimum tenuiflorum</i> , <i>Zingiber officinale</i> , <i>Curcuma longa</i> , <i>Piper nigrum</i> , <i>Phyllanthus emblica</i> including others.
ViroNil	<i>Solanum xanthocarpum</i> , <i>Zingiber officinalis</i> , <i>Tinospora cordifolia</i> , <i>Piper longum</i>
Vyaghryadi Kashaya	<i>Glycyrrhiza glabra</i>
Yastimadhu Ghanavati	

*other constituents are non-herbal and may include minerals.

candidates for development as RNA polymerase inhibitors as they are usually less toxic than other antiviral strategies (Antonio et al., 2020). The most common targets of the phytoconstituents studied are the S protein, ACE2 receptor, TMPRSS2 (SARS-CoV-2 co-receptor) and M^{pro} (Fig. 1).

One of the most popular Ayurvedic therapies currently used for treatment of COVID-19 (asymptomatic, mild to moderate) in India, particularly in the state of Tamil Nadu, is the “Kabasura Kudineer” (KSK), a polyherbal formulation containing over 32 plant metabolites that is generally used to treat other respiratory symptoms (Jeyanthi and Kumar, 2020; Kiran et al., 2020; Rao et al., 2020). The KSK constituents include Magnoflorine, scutellarein, chrysoeriol, 5-hydroxy-7,8 dimethoxyflavanone, tinosponone, circimaritin, chrysoeriol, 6-methoxy-ygenkwanin, vasicinone, quercetin and luteolin, obtained from *Sida acuta*, *Andrographis paniculata*, *Tinospora cordifolia*, *Plectranthus amboinicus*, *Justicia adhatoda*, and *Costus speciosus*. All of these were found to have excellent predicted binding affinity with the SARS-CoV-2 S protein (Kiran et al., 2020). The molecular dynamics simulation showed that scutellarein, chrysoeriol, luteolin, circimaritin, and magnoflorine have strong affinity towards the spike protein via hydrogen bond contact with amino acids Arg 346, Asn343, Asp364, Cys336, Gly339, Ser373, Thr345, and Val341. The same research group have also found that phytoconstituents vasicine (quinazoline alkaloid), andrographolide (labdane diterpenoid), ursolic acid (pentacyclic triterpenoid acid), quercetin (flavonol) and meliacine (peptide) were predicted to inhibit the ACE2 receptor (Kiran et al., 2020). “Nilavembu Kudineer” (NVK), an Ayurvedic formulation containing nine herbal ingredients, is commonly used to treat respiratory illnesses due to its anti-inflammatory, anti-analgesic, and anti-pyretic effects. One of the most predominant phytoconstituents of NVK is andrographolide, derived from the herb *A. paniculata* that is used for supporting the immune function and relieve symptoms of the common cold. The findings of *in silico* studies have indicated that andrographolide can dock successfully in the binding site of SARS-CoV-2 M^{pro} (free energy –3.094 kJ/mol) and shows further promise in potential therapeutic use due to its relatively high solubility and favourable pharmacodynamics characteristics (Enmozhi et al., 2021). The

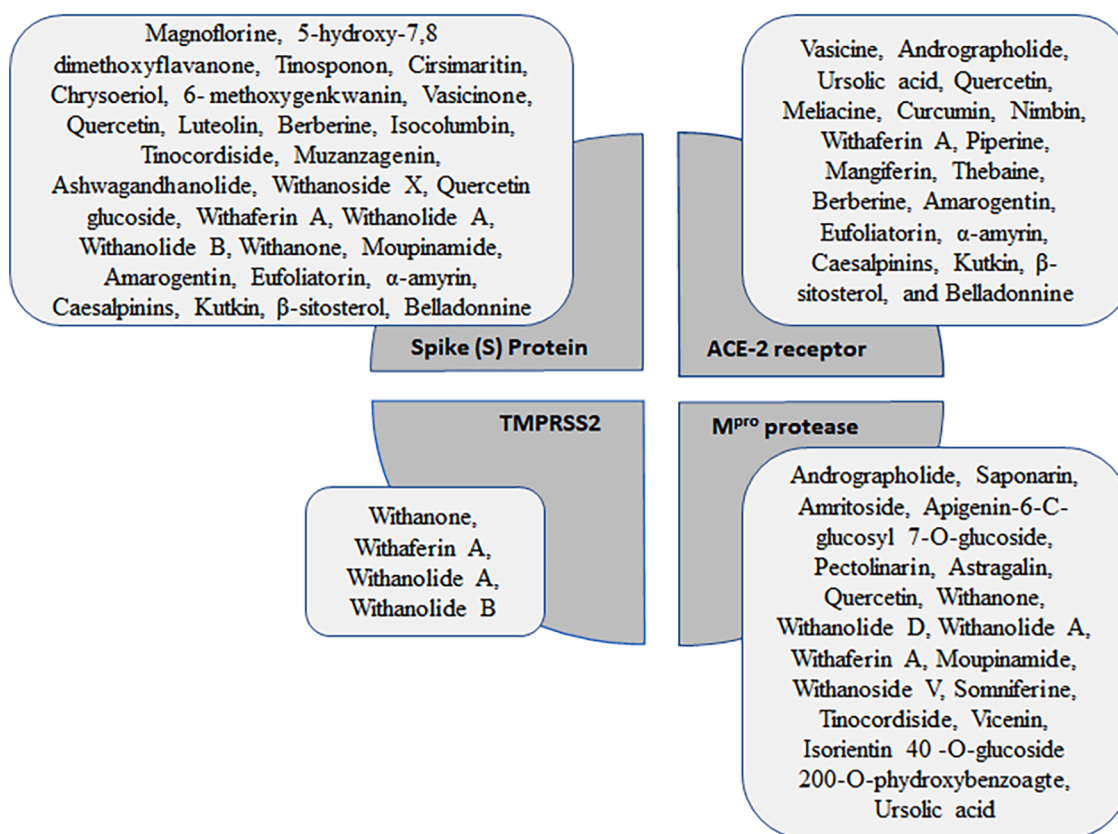


Fig. 1. Common phytoconstituents of Ayurvedic formulations and their targets.

A summary of the results of the *in silico* studies reviewed is provided. The most common targets of the phytoconstituents studied are shown in the central quadrants, while the phytoconstituents are listed in the associated rectangles. The size of the rectangles is relative to the number of compounds.

relevant chemical structures are shown in Fig. 2.

T. cordifolia, a common constituent of several different Ayurvedic formulations, including KSK and NVK, has received considerable attention for its potential anti-SARS-CoV-2 properties. The aqueous extract of *T. cordifolia*, known as “Guduchi Ghan Vati” or “Shilajatu Rasayana”, is used widely in Ayurvedic medicine management of several health conditions (Upadhyay et al., 2010). An *in silico* molecular docking study found that its phytoconstituents including berberine (alkaloid), isocolumbin (diterpenoid), magnoflorine (quaternary benzyloquinoline), and tinocordiside (sesquiterpene glycoside) could potentially bind with high efficiency to the S glycoprotein in its prefusion state and to the receptor binding-domain of the mature S glycoprotein (Sagar and Kumar, 2021). These phytoconstituents may also provide preventative measures for COVID-19 by inhibiting the attachment of the virus to its host cells as evidenced by the low IC₅₀ (<1 μ M) of tinocordiside and isocolumbin. A study by Mulpuru and Mishra (2021) found that of all *T. cordiflora* phytoconstituents investigated, saponarin (flavone glucoside) showed the highest affinity for docking with SARS-CoV-2 M^{pro}, with affinity (– 8.75 kCal/ml) exceeding that of a nucleotide prodrug of an adenosine analog, Remdesivir (Mulpuru and Mishra, 2021). Remdesivir has been shown to shorten the time of recovery of individuals hospitalized with COVID-19 and evidence of lower respiratory tract infections (Beigel et al., 2020). Additionally, molecular dynamics simulation showed that saponarin-M^{pro} interaction was stable, without any significant fluctuations. In another *in silico* molecular docking and dynamics study, two bioactive constituents of *T. cordiflora*, amritoside (tannin) and apigenin-6-C-glucosyl-7-O-glucoside, were predicted to bind to the M^{pro} with high affinity, – 60.35 and – 50.50 kCal/mol respectively (Murugesan et al., 2021). In the same study, pectolarin (cirsium isolate), a bioactive compound found in extracts of *Phyllanthus niruri*, as well as astragalin (3-O-glucoside of kaempferol) and quercetin,

bioactive constituents of *Emblca officinalis* extracts, were also found to efficiently occupy the substrate binding cleft of M^{pro} *in silico*. *P. niruri* and *E. officinalis* are used in Ayurvedic medicine as components of several formulations and on their own as extracts for various conditions.

The “Ayurvedic Rasayana” describes a group of herbs used as dietary supplements that are proposed to exhibit immunomodulatory and antioxidant activities with extracts of *T. cordifolia*, *Withania somnifera*, and *Asparagus racemosus* major constituents of Rasayana therapy. A study by Borse et al. (2021) used an ultra-high-performance liquid chromatographic-photodiode array (uHPLC-PDA) and identified 31 phytoconstituents in the combined extract (Borse et al., 2021). All 31 phytoconstituents were tested for their immunomodulatory potential using an *in silico* network pharmacology model, followed by *in silico* molecular docking investigations with the SARS-CoV-2 S, M^{pro} and polymerase proteins. Network analysis showed that 53 protein targets in 28 of the 31 phytoconstituents were involved in 20 immune pathways, suggesting that the extract could concomitantly modulate multiple immune pathways. Two phytoconstituents, ashwagandhanolide (dimeric withanolide; Fig. 2A) and withacoagin, had high docking scores of – 10 and – 7.6 kCal/mol respectively, for SARS-CoV-2 S protein, forming strong hydrogen bond interactions with Arg403, Asn501, Ser494, Thr500, and Tyr495. However, the authors raised concerns about their drug-likeness as determined by the Lipinski’s rule of five (Borse et al., 2021).

A literature review on the use of withanolides (phytoconstituents of *W. somnifera*) in COVID-19 (Dhawan et al., 2021) indicated that withanolides inhibit the ACE2 mediated entry of SARS-CoV-2 into host cells and subsequent M^{pro} activity. There was no direct evidence provided of either action with most of the molecular docking studies included. The authors proposed that withanone, withanolide D, withanolide A, and withaferin A have the potential to alter M^{pro} activity, while withanoside

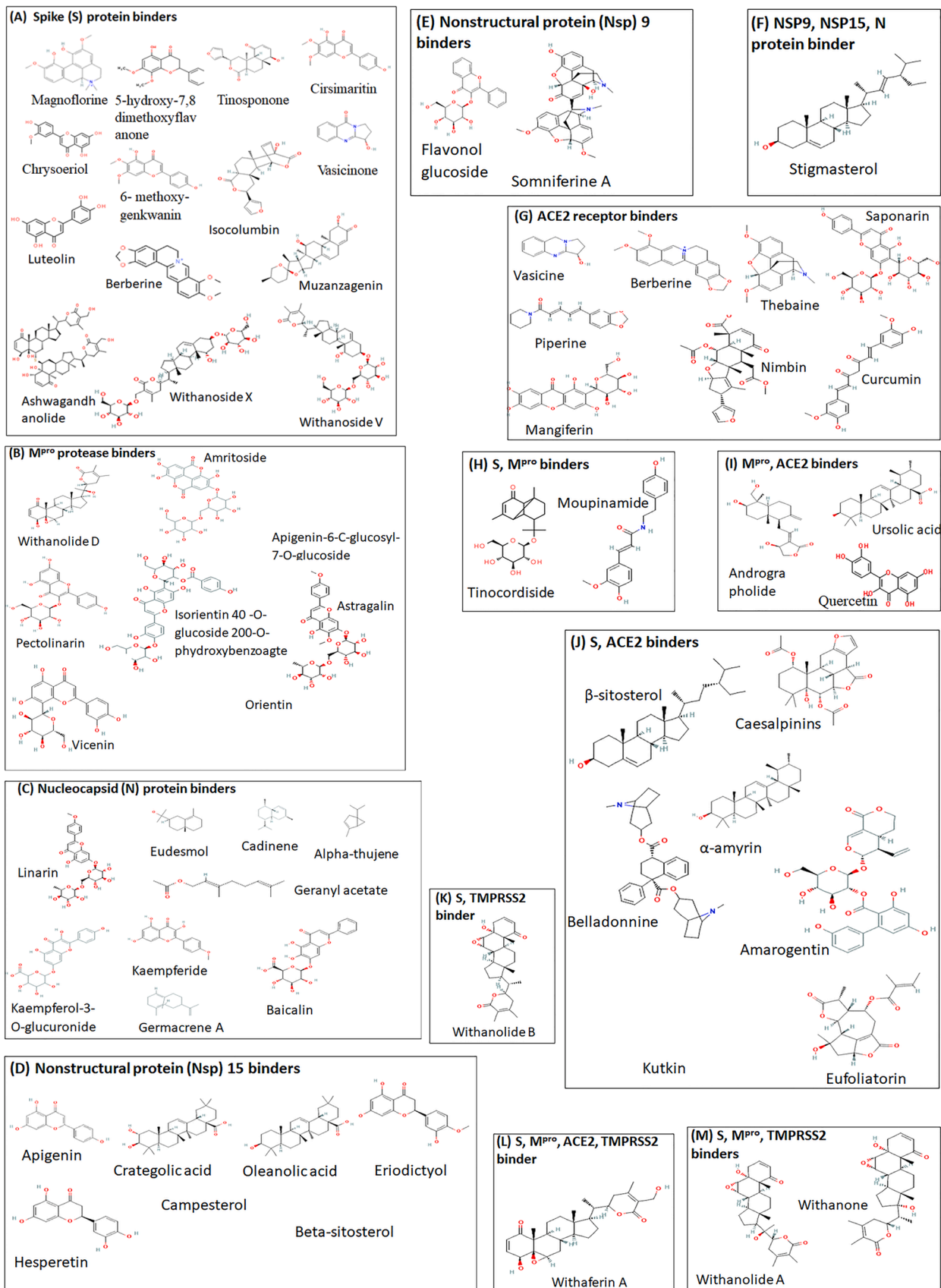


Fig. 2. Structure of common phytoconstituents of Ayurvedic formulations. Chemical structures of compounds, arranged by the target binding protein, are shown. Compounds that bind to SARS-CoV-2 spike (S) protein (A), large protease M^{pro} (B), nucleocapsid (N) protein (C), Nonstructural protein Nsp15 (D), Nsp9 (E) are shown. Compounds that bind to ACE2 receptor are shown in (G). Compounds that bind to more than one relevant target are shown in F, H, I, J, K, L, M. TM^{PRSS2} is a co-receptor for SARS-CoV-2.

II, withanoside IV, withanoside V, sitoindoside IX, withanolide R and 2, 3-dihydrowithaferin A interfere with M^{PRO} action (Dhawan et al., 2021; Kumar et al., 2020c; Parida et al., 2020; Tripathi et al., 2021). Interestingly, withanone and withaferin A (Fig. 2K–M) were also predicted to bind and stably interact at the catalytic site of TMPRSS2, the SARS-CoV-2 co-receptor, inducing changes at the allosteric site, which would potentially inhibit virus entry into host cells (Kumar et al., 2020c). Withanoside X and quercetin glucoside (Fig. 2D) may bind to the active site of NSP15 endoribonuclease and S protein RBD (Chikhale et al., 2021). A 100 ns molecular dynamics simulation suggests that withanoside X has high binding affinity with free energy value of -89.42 kCal/mol. Withaferin A, withanolide A, withanolide B, and withanone also have excellent predicted binding affinity to S protein, ACE2 receptor, and M^{PRO} (Srivastava et al., 2020). Additionally, molecular dynamics simulation suggested that withanolides A and B bind stably to the S protein and the papain-like protease (PLpro) of SARS-CoV-2.

A survey of 17 bioactive phytoconstituents of commonly used herbs in Ayurveda for management of fever, inflammation and immune disorders, found that curcumin, nimbin, withaferin A, piperine, mangiferin, thebaine, berberine, and andrographolide have favourable drug-likeness characteristics and may effectively inhibit the interaction of SARS-CoV-2 S protein with its receptor (Maurya et al., 2020b). Nimbin and curcumin had very high docking scores of -148.621 kCal/mol and -141.36 kCal/mol respectively, comparable to the results for the antiviral nafamostat and hydroxychloroquine. Both nimbin and curcumin were predicted to interact via hydrogen bonding with S protein amino acid residues Arg 765, Asn317, Gln314, Lys304, Thr768, and Ser316. In a study by Maurya et al. (2020a), 20 phytoconstituents from herbs commonly used in Ayurveda, *Allium cepa*, *Allium sativum*, *Alstonia scholaris*, *Artemisia vulgaris*, *Atropa belladonna*, *Caesalpinia crista*, *Glycyrrhiza glabra*, *Nigella sativa*, *Picrorhiza kurroa*, *Piper nigrum*, *Swertia chirata*, *T. cordifolia* and *Zingiber officinale* were investigated for their ability to bind to the SARS-CoV-2 S protein and its ACE2 receptor (Maurya et al., 2020a). Computational structure-based drug design revealed that the compounds amarogentin, eufoliorin, α -amyrin, caesalpinins, kutkin, β -sitosterol, and belladonnine have potentially high affinity towards both the S protein and the ACE2 receptor (Fig. 2). Amarogentin had the highest docking score of -149.76 kCal/mol against the S protein. Molecular dynamics simulation revealed that it interacts with the S protein via hydrogen bonding with amino acids Arg765, Cys738, Gln314, Ser735, Thr739, Thr768, and Val736, and with ACE2 receptor via hydrogen bonding with Asp350, Asp382, Asn394, and Tyr385.

An important Ayurvedic method for enrichment of active pharmacological agents from herbs involves preparation of Kadha (decoction) for oral consumption. In a molecular docking study by Maurya and Sharma (2020), the potential for phytoconstituents of common Kadha components to bind to SARS-CoV-2 proteins, ACE2 receptor or pro-inflammatory mediators were investigated (Maurya and Sharma, 2020). The authors reported the findings of 108 phytoconstituents from *Withania somnifera*, *Ocimum tenuiflorum*, *T. cordifolia*, *Z. officinale*, *C. longa*, *P. nigrum*, *Syzygium aromaticum*, *Elettaria cardamomum* and *Citrus limon*. Many of the phytochemicals tested were found to have a strong binding affinity with SARS-CoV-2 proteins. Orientin, withanolide, withanolide B, crategolic acid, ursolic acid, withaferin A, apigenin, eriodictyol, hesperetin, oleanolic acid, stigmasterol and withanone had a high binding affinity with NSP15. Whereas, withanone, withanolide B, withaferin A, withanolide, beta-sitosterol, campesterol, stigmasterol, flavonol glucoside and somniferine A had a high binding affinity to the Nsp9 protein (Fig. 2E). Several phytochemicals, including quercetin, withanone, withanolide and stigmasterol (Fig. 2F), also had high binding affinity to the N protein. Importantly, withaferin A, withanolide B, withanolide, withanone, campesterol, cyclocurcumin, somniferine A, stigmasterol, eriodictyol, isopiperine, oleanolic acid, rhamnetin, orientin, quercetin, piperine and vicenin had high binding affinity for

inflammatory mediators cyclooxygenase-2 (COX2), phospholipase A2 (PLA2), NF- κ B-Inducing Kinase (NIK), and interleukin-1 receptor associated kinase 4 (IRAK-4) (Maurya and Sharma, 2020). Molecular dynamics simulations were performed for M^{PRO} and ACE2 receptors with the above-selected phytoconstituents, and root mean square deviations (RMSD) and root mean square fluctuations (RMSF) plot analysis demonstrated that their interactions are stable with very low variability. As COVID-19 is characterized by high inflammatory response (Mishra et al., 2020), the anti-inflammatory action of the phytoconstituents may be very important for potentially reducing disease symptoms. Moupinamide (Fig. 2), one of the phytoconstituents of Kadha used to relieve symptoms of respiratory influenza like illness, was found to have strong potential to bind stably to both the S protein and the M^{PRO} (Shukla et al., 2021).

Based on the previous findings (discussed above), *W. somnifera*, *T. cordifolia*, and *O. tenuiflorum* are commonly used components for various Ayurvedic formulations. Over one hundred different active phytoconstituents of these herbs were identified as potential M^{PRO} binders and further analyzed for drug-likeness. This has resulted in the identification of six potential phytochemical inhibitors with high stability as evidenced by the RMSD values (withanoside V, somniferine, tinocordiside, vicenin, isorientin 4'-O-glucoside 2''-Op-hydroxybenzoate and ursolic acid) (Shree et al., 2020). Similarly, nine compounds (linarin, eudesmol, cadinene, geranyl acetate, alpha-thujene, germacrene A, kaempferol-3-O-glucuronide, kaempferide, and baicalin) out of 127 phytoconstituents from *Mentha arvensis*, *Coriandrum sativum*, and *O. tenuiflorum* were found to have potentially high binding affinity towards the catalytic pocket of N protein, with high stability and few fluctuations, as well as favourable drug-likeness (Fig. 2C) (Muthumanickam et al., 2021).

A major limitation of all *in silico* studies that have thus far been included in this review (Banerjee et al., 2021; Borse et al., 2021; Chikhale et al., 2021; Dhawan et al., 2021; Enmozhi et al., 2021; Kiran et al., 2020; Kumar et al., 2020c; Maurya and Sharma, 2020; Maurya et al., 2020a, 2020b; Mulpuru and Mishra, 2021; Murugesan et al., 2021; Muthumanickam et al., 2021; Parida et al., 2020; Sagar and Kumar, 2021; Shree et al., 2020; Shukla et al., 2021; Srivastava et al., 2020; Tripathi et al., 2021; Upadhyay et al., 2020) is that none have investigated the potential synergistic effects. Given that whole herbs are used for Ayurvedic formulations, it is not clear whether the activities attributed to individual phytoconstituents would be functional in practice. Therefore, further laboratory studies using whole herb extracts, and observational clinical studies in cohorts using Ayurvedic formulations are needed to investigate the *in silico* predictive studies for potential therapeutic properties.

To the best of our knowledge, only one study included in the present review used a disease association model and attempted to predict synergy among component molecules to contribute to the immunomodulatory potential of the whole extract (Banerjee et al., 2021). The 12 phytoconstituents identified in an ethanolic extract of *A. paniculata* were further analysed *in silico* by network pharmacology methods. The gene-disease network analysis identified several targets that were associated with viral infections and immunodeficiency. Andrographolide and andrographodin A were predicted to interact with important immune markers (toll like receptors) and to modulate the PI3K-AKT pathway that is implicated in coronavirus infection (Kindrachuk et al., 2015). The authors conclude that there is a synergistic action between phytoconstituents that may affect an anti-inflammatory response.

In summary, *in silico* studies clearly show that phytoconstituents of commonly used Ayurvedic herbal formulations have potential to inhibit SARS-CoV-2 infection and COVID-19 disease. As most formulations are commonly delivered orally, it is not clear if, and how, the bioactive compounds reach the respiratory system, thus the question whether some of the interactions with host factors take place in circulation and hence reduce systemic disease markers.

Pre-clinical investigations

Based on our searches, only five studies (Balkrishna et al., 2021b, 2021c, 2020; Gheware et al., 2021a; Upadhyay et al., 2020) have tested the ability of selected bioactive phytoconstituents of Ayurvedic herbs to directly bind and/or inhibit the function of SARS-CoV-2 proteins. *Terminalia chebula* is the major constituent of the Ayurvedic formulation known as Triphala and it has been reported to be beneficial in disease caused by herpes simplex virus-2 (Kesharwani et al., 2017) and Influenza A virus (Li et al., 2020). The aqueous extract of *T. chebula* was shown to be effective in inhibiting the proteolytic activity of SARS-CoV-2 3CL^{pro} in an *in vitro* protease assay based on cleavage of casein (Upadhyay et al., 2020).

Withanone, a steroidal lactone, highlighted repeatedly as a potential inhibitor of ACE2-S protein interaction in molecular docking studies, was found to inhibit the interaction of the ACE2 receptor with the RBD of S protein *in vitro*, in a dose dependent fashion (Balkrishna et al., 2021c). Importantly, a herbal extract of *W. somnifera* enriched with withanone was able to ameliorate pathological symptoms in a zebrafish xenotransplant model that used the S protein as a marker for SARS-CoV-2 infection. The same group also investigated *T. cordifolia* (Balkrishna et al., 2021b) and Swasari Vati (Balkrishna et al., 2020) as potential treatment options for COVID-19 using the zebrafish xenotransplant model. Swasari Vati is a calcium rich Ayurvedic mixture of 16 herbs or their extracts, processed into a tablet containing three excipients and is commonly prescribed for chronic cough, common cold and asthma. Treatment with Swasari Vati led to the complete recovery of the zebrafish as measured by renal degeneration, necrosis, and survival. Importantly, Swasari Vati treatment showed progressive recovery of immune cell infiltration with very few pro-inflammatory infiltrates, suggesting its effectiveness in reducing the inflammatory damage caused by SARS-CoV-2 spike protein (Balkrishna et al., 2020). In a similar study, *T. cordifolia* extract in the form of orally delivered powdered tablet, was also found to be effective in reversing the pathological symptoms in the zebrafish model (Balkrishna et al., 2021b).

Only one group investigated the effect of an Ayurvedic herbal extract in a mouse model of COVID-19 disease (Gheware et al., 2021a). The authors have previously shown that an extract of *Justicia adhatoda* (one of the component herbs in KSK, see above) has an anti-hypoxic property and can reduce severe airway inflammation induced by an augmented hypoxic response in a mouse model of treatment-resistant asthma (Gheware et al., 2021b). The group extended their investigations to show that *J. adhatoda* extract can inhibit SARS-CoV-2 replication in cell culture and reverse the pulmonary fibrosis in a bleomycin mice model where treatment with the extract resulted in reduced pro-inflammatory mediators and markers of hypoxia. The authors concluded that *J. adhatoda* extract may be a potential candidate for development of anti-SARS-CoV-2 treatment.

Clinical trials

A number of clinical trials are currently ongoing or have been completed on the use of Ayurvedic formulations in mild and moderate COVID-19 disease. The clinical trials are mostly observational studies on cohorts of patients who are using Ayurvedic formulations as part of their treatment regime. In this context, the Indian Government has set up guidelines for the clinical management of COVID-19 patients based on Yoga and Ayurveda (Government of India, 2020) that includes preventative measures for general use in the community.

There were 51 completed clinical trials on the use of Ayurvedic formulations to prevent or treat COVID-19 registered at ClinicalTrials.gov (<https://clinicaltrials.gov>) and Clinical Trial Registry-India (<http://ctri.nic.in/Clinicaltrials/login.php>) as of 20th March 2022. The protocol details of all completed clinical trials on different Ayurvedic formulations are provided in Table 2. The majority are small, single-center trials undertaken in different jurisdictions in India in SARS-CoV-2 positive

subjects with asymptomatic or mild disease, comparing the Ayurvedic treatments with standard care. The outcomes of these studies varied considerably between the studies; however, trial outcome data was only available for 17 completed trials and these are discussed below.

Various formulations containing *T. cordifolia*, *A. paniculata*, *W. somnifera*, *O. tenuiflorum*, *Z. officinale*, *T. chebula*, *C. longa*, and *E. officinalis* have shown positive results in prophylaxis and treatment of COVID-19. Several clinical studies have been reported on the use of KSK, with reduction in SARS-CoV-2 viral load and faster recovery of COVID-19 patients (CTRI2020/05/025,215, CTRI/2020/08/027,286), (Natarajan et al., 2020, 2021; Srivastava et al., 2021a, 2021b). Patients in the placebo group had a significantly longer stay in the hospital (mean \pm SD = 8.4 ± 2.0 days) as compared to patients receiving KSK (mean \pm SD = 4.2 ± 1.5 days; Kruskal-Wallis test, $P = 0.0001$) (Srivastava et al., 2021b). Further study showed that patients given KSK as a decoction had a larger reduction in viral load as compared to the control group of patients who received vitamin C and zinc tablets (no statistical analysis provided) (Natarajan et al., 2021).

In one clinical trial (CTRI/2020/06/025,856), several Ayurvedic formulations were evaluated in combination with a 'standard treatment' which consisted of five pharmaceuticals and 2 nutraceuticals. It should be noted that none of the seven drugs have been shown to have any direct effect on SARS-CoV-2 or COVID-19 although some have generalised effects that may be beneficial (Siemieniuk et al., 2020). The pharmaceuticals included were Hydroxychloroquine, Ivermectin, azithromycin, paracetamol, Omez as well as Vitamin C and zinc. The Ayurvedic add on treatment included KSK, Adathodai manapagu, Vasantha kusumakaram mathirai and Thippili rasayanam; all of which are used in the community for relief of respiratory symptoms. The authors reported improved recovery of those given the Ayurvedic formulations as add-on to the 'standard treatment' (Chitra et al., 2021), with a reduction in the average number of days taken for symptoms to improve following treatment (3.21 with add-on vs 5.13 with only standard treatment ($P < 0.001$)). This was not surprising as most of the components in the 'standard treatment' have not been shown to have a direct effect on COVID-19 clinical progression (Bartoszek et al., 2021; Siemieniuk et al., 2021).

A trial by Thakar et al. (CTRI/2020/07/026,433) compared the effect of 'standard treatment' (Vitamin C, azithromycin, and paracetamol) with an Ayurvedic add-on treatment (Thakar et al., 2021). The Ayurvedic add-on treatment consisted of the State Government recommended combination of Dashamula and Pathyadi kwatha, Trikatu churna, Guduchi ghan vati, AYUSH-64 and Yastimadhu ghanvati, all of which are polyherbals used primarily in respiratory and influenza like illness. Dashamula, alone and in combination with aspirin has previously shown to have anti-inflammatory, analgesic, and anti-platelet effects comparable to aspirin alone (Parekar et al., 2015). Trikatu powder contains black and long pepper and probably augments the metabolic process by improved absorption of nutrients through the action of the phytoconstituent piperine (Zhang et al., 2021). Given its predicted ability to bind to the S protein (Maurya et al., 2020b), piperine may also have a role in inhibiting SARS-CoV-2 infection. Yastimadhu (*G. glabra*) or licorice is used widely in both Ayurveda and Traditional Chinese Medicine and shown to have antimicrobial activity (Wang et al., 2015). The AYUSH-64 is a polyherbal formulation with its basis in Ayurvedic herbs and has previously been shown to be effective in malaria (Willcox, 2011) and in influenza like illness (Gundeti et al., 2020). In this study participants in the 'add-on treatment' group showed similar outcomes to CTRI/2020/06/025,856 clinical trial with shortened duration of symptomatic phase of the infection compared to those on standard treatment alone (mean \pm SD = 3.66 ± 1.55 compared to 5.34 ± 3.35 days, $p < 0.001$); no adverse reactions or drug interactions were observed. There was no difference in progression to symptomatic disease (27.6% compared to 24.6%).

A retrospective, open label clinical study by Balkrishna et al. (2021a) found that Ayurvedic treatment alone led to shorter time to recovery of

Table 2
List of completed clinical studies with Ayurvedic formulations.

Formulation/ Compound	Study design	clinical phase	Intervention details	Outcome measures	Sample size	clinical study identifier
Dabur™ Chyawanprash (DCP)	Randomised, Parallel-Group Trial	N/A	Adults (13 –70 years): One teaspoonful (approx. 12 gm of Chyawanprash) twice daily, followed by milk Children (5–12 years): Half teaspoonful (approx. 6 gm of Chyawanprash) twice daily, followed by milk	Comparative assessment of the incidence of COVID-19 in subjects who take DCP and those who do not take it for three months (90 days)	600	CTRI/2020/05/024,981
AYUSH 64	Randomised, Parallel Group, Active Controlled Trial	Phase 2/ Phase 3	Capsules (500 mg each) thrice a day after food	Early recovery from COVID-19 infection	80	CTRI/2020/05/025,214
Kabasura Kudineer	Randomised, Parallel-Group Trial	Phase 1/ 2	Kabasura Kudineer 60 ml twice a day for 14 days	Reduction in Viral load, clinical recovery	50	CTRI2020/05/025,215
Ashwagandha, Giloy, Tulsi	Randomised, Parallel-Group, Placebo-Controlled Trial	N/A	500 mg twice a day after Breakfast/ Dinner 1000 mg twice a day after Breakfast/ Dinner 500 mg twice a day after Breakfast/Dinner 4 drops twice a day 2 gm twice a day before Breakfast/ Dinner	Reduction in viral load	120	CTRI/2020/05/025,273
Anu Taila Swasari Ras						
Chyawanprash	Randomised, Parallel-Group Trial	Phase 3	Twice a day - on an empty stomach in the morning at least 1 h before breakfast and in the evening two hours after dinner	Percentage of participants with SARS-Cov-2 positivity	200	CTRI/2020/05/025,275
Sanshamani Vati, Nagaradi kwath, Amalaki Churna,	Single Arm Trial	Phase 3	1 gm twice a day with 50 ml kwath decoction 3 gm with water once a day at 5 pm, for 14 days Once daily, 9 pm, for 14 days	Time and number of patients transitioning from an asymptomatic to a symptomatic state	50	CTRI/2020/05/025,276
Golden Milk						
Tab PINAK	Single Arm Trial	Phase 2	Moderate to severe illness: 4 times a day for five days; Mild: 3 times a day for five days.	Early recovery and reduced mortality	30	CTRI/2020/05/025,326
AYUSH-64	Single Arm Trial	Phase 3	2 Tablets (500 mg) thrice daily	Clinical recovery	40	CTRI/2020/05/025,335
AYUSH –64	Single Arm Trial	Phase 2/ Phase 3	2 Tablets (500 mg each) thrice a day after food	Early recovery and changes in liver enzymes, Renal functions	40	CTRI/2020/05/025,338
Guduchi Ghan Vati	Non-randomised, Multiple Arm Trial	N/A	500 mg two times a day for 30 days.	Comparative assessment of incidence of infection in clinically stable participants in the community who have already identified at least one confirmed case with the standard prophylaxis control arm	30,000	CTRI/2020/05/025,385
Curcumin with black pepper	Randomised, Parallel-Group Trial	N/A	525 mg. Two to four tablets a day.	Variations in D-Dimer, CRP, LDH, CBC, Ferritin, Troponin, cardiac myoglobin, PT INR, clinical improvement.	50	CTRI/2020/05/025,482
CLEVIRA	Randomised, Parallel-Group Trial	Phase 3 / Phase 4	Tablet twice daily for 14 days	Recovery and reduction of viral load	100	CTRI/2020/05/025,483
Ashwagandha (Shakti)	Single Arm Trial	Phase 3/ Phase 4	5 drops three times a day 1 tablet twice a day 1 tablet twice a day 10 drops thrice a day	Early clinical recovery	50	CTRI/2020/06/025,592
Amruth, Turmeric, Tulsi						
Shunthi	Randomised, Parallel Group, Active Controlled Trial	Phase 2	10 day treatment 2 gm twice daily 1 gm once daily 50 ml twice daily 500 mg twice daily 125 mg twice daily	To minimize the likelihood of disease progression in terms of severity. Reduction of viral load and clinical recovery	120	CTRI/2020/06/025,800
Rasona Vyaghryadi Kashaya Samshamani Vati Agnikumara Rasa Septilin and Bresol	Randomised, Parallel-Group, Active Controlled Trial	Phase 2/ Phase 3	One tablet twice daily from day one upto 14 days.	Early recovery and changes in liver enzymes, Renal functions To minimize the likelihood of disease progression in terms of severity.	40	CTRI/2020/06/025,801

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Table 2 (continued)

AYUSH-64	Randomised, Parallel-Group Trial	Phase 2	2 Capsules of 500 mg thrice daily for 14 days	Recovery.	200	CTRI/2020/06/025,855
Kabasura Kudineer	Non-randomised, Multiple Arm Trial	Phase 2	60 ml twice a day, before food.	Resolution of Symptoms and recovery	200	CTRI/2020/06/025,856
Vasanthakumaram			1 tablet twice a day, after food.			
Thippili Rasayanam			2 g twice a day, after food.			
Adathodai Manapagu			15 ml twice a day with 30 ml lukewarm water, after food for 14 days			
Arogya Kashayam-20	Randomised, Parallel Group, Active Controlled Trial	Phase 2	100 ml Twice a day for ten days	Check the progression of the disease	100	CTRI/2020/06/026,221
Dashamula Kwatha, Pathyadi Kwatha, Trikatu Churna	Randomised, Parallel Group, Active Controlled Trial	N/A	Decoction of 20 ml each of the Kwathas and 2 gm Trikatu Churna twice a day on an empty stomach	Clinical recovery	25	CTRI/2020/07/026,433
Sansamani Vati,			500 mg two tablets twice a day after meal,			
AYUSH-64,			Two tablets twice a day after a meal,			
Yastimadhu Ghanavati			One chewable tablet (500 mg) six times a day every two hours in a daytime			
Immusante and Guduchi	Randomised, Parallel Group, Active Controlled Trial	Phase 3	Each tablet: 1 tablet twice daily for 30 days	Improvement in the immune status based on the adapted Immune status	100	CTRI/2020/07/026,579
Surasadi Kadha,	Single Arm Trial	Phase 2/ Phase 3	50 ml twice a day for seven days	Clinical recovery	70	CTRI/2020/07/026,601
ViroNil,			Capsule (500 mg) daily for seven days			
Bilvadi			Capsule twice a day for seven days			
SSV Formulation	Randomised, Parallel Group, Active Controlled Trial	Phase 3	Tablet of (500 mg), twice a day immediately after meals for ten days	Reduction of clinical symptoms and disease severity	200	CTRI/2020/07/026,839
Guduchi Ghan vati	Randomised, Parallel Group, Active Controlled Trial	N/A	Two capsules/tablet (250 mg each) twice a day after food	Early recovery.	30	CTRI/2020/07/026,840
Kabasura Kudineer, Nilavembu Kudineer	Double Blinded, Three arm, Single center, Placebo Controlled, Randomized Controlled Trial	N/A	Placebo or NVK or KSK, 60 ml Morning and Night after Food, along with standard Allopathy Treatment for 10 days.	Reduction in viral load	125	CTRI/2020/08/027,286
Immunofree	Randomised, Parallel-Group Trial	Phase 3/ Phase 4	Two tablets 500 mg thrice a day for ten days	Early recovery.	100	CTRI/2020/08/026,957
Reginmune			One capsule 750 mg twice a day for ten days			
Nochi Kudineer Chooranam,	Randomised, Parallel Group, Active Controlled Trial	Phase 2	Decoction – Two times daily before 30 min of meals for seven days	Reduction of viral load and clinical recovery	100	CTRI/2020/08/026,999
Mahasudarsan Chooranam,			5 gm – Two times daily after meals for seven days with warm water/ Honey.			
Maldevi Chendooram,			100 mg. Two Times daily, after meals for seven days with honey.			
Adathodai Manapagu,			10 ml- Morning and Evening for seven days.			
Omatheeneer			10 ml –Twice daily with water after meals for seven days.			
Shakthi,	N/A	Phase 3/ Phase 4	5 drops, three times a day	Changes in anti-oxidant biomarkers (Superoxide dismutase, Catalase,	100	CTRI/2020/08/027,009

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Table 2 (continued)

Amruth, Turmeric Plus, Tulsi Arka			1 tablet, two times a day 1 tablet, two times a day 10 drops, three times a day	MalondialdehydeGlutathione) and Immune biomarkers (IFN- α , IFN- β)		
Ashwagandha Tablet, Shunti Capsule	Randomised, Parallel Group, Active Controlled Trial	Phase 2/ Phase 3	2 Tablets 250 mg each, twice a day, 2 hrs after food 2 capsules 500 mg each, twice a day at least 30 min after food	Early recovery	60	CTRI/2020/08/027,224
T-AYU-HM Premium	Single Arm Trial	Phase 2	600 mg twice a day for 21 days	Changes in clinical symptoms	30	CTRI/2020/08/027,477
Kabasura Kudineer, Amukkara, Thalesadhivadagam, Brammanandhabairavam, Adathodaimanapagu	Single Arm Trial		Decoction – Two times daily before 30 min of meals for seven days 2 tablets - Three times daily after meals for seven days 2 Chewable tablets – Three times daily after meals for seven days 1 pill - Two times daily after meals for seven days	Reduction in viral load and clinical symptoms	20	CTRI/2020/08/027,490
Kabasura Kudineer	Single Arm Trial	Phase 2	Decoction – Twice daily 30 min before meals for 7 days	Reduction in viral load and clinical symptoms	20	CTRI/2020/08/027,491
Amukkara Chooranam Mathirai Thalesadhivadagam Brammanandhabairavam Adathodaimanapagu			Two tablets - Thrice daily after meals for 7 days Two tablets – Thrice daily after meals for 7 days One pill - Twice daily after meals for 7 days 10 ml –Twice daily with warm water after meals for 7 days			
<i>Elaeocarpus sylvestris</i> var. ellipticus extract	Randomised, Parallel-Group, Placebo-Controlled Trial	Phase 2	480 mg per day orally for ten days	Early, changes in mental health, and changes in levels of prostaglandin E2, TNF α , and IL-6	60	CTRI/2020/09/027,775
Tulsi, Giloy ki Ghanvati, Kalmegh, Dabur™ Chyawanprash	Randomised, Parallel-Group Trial	Phase 2/ Phase 3	1 tablet two times a day for 28 days, 1 tablet two times a day for 28 days 1 tablet two times a day for 28 days 1 teaspoonful (Approx 10–12 gs) two times a day for 28 days	Early recovery	72	CTRI/2020/09/027,914
AyurCoro-3	Single Arm Trial	N/A	10 ml of liquid, three times a day for one day, repeat every 15 days for three months	Clinical recovery	120	CTRI/2020/10/028,324
AyurCoro-3	Randomised, Parallel-Group Trial	Phase 3/ Phase 4	10 ml three times a day for three days. Medication for a day	Clinical recovery	500	CTRI/2020/10/028,333
Bilwadi Yog, Kantakaryavaleha	Randomised, Parallel Group, Active Controlled Trial	Phase 3	1 gm twice a day 3 gm twice a day	Early recovery from COVID-19 infection and reduction in viral load	380	CTRI/2020/10/028,437
*Shadangodak NIFAy.C-19 (CONTAZAP)	Randomised, Parallel-Group Trial	Phase 3/ Phase 4	40 ml twice a day 640 mg- 2 Capsules, twice daily after meal.	Clinical recovery	120	CTRI/2021/01/030,169
Jeevaneeyam, Ojovardhini, Amrutha Sanjeevini	Non-randomised, Active Controlled Trial	Phase 3/ Phase 4	Twice a day after meal for 14 days	Reduction in clinical symptoms and early recovery	60	CTRI/2021/02/031,353
Kabasura Kudineer	Randomised Parallel	Phase 4	Two tablets a day thrice before food	Early clinical recovery	200	CTRI/2021/04/032,755

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Table 2 (continued)

AEV01 (Kutki/ <i>Picrorhizakurroa</i>)	Randomised, Parallel-Group, Placebo-Controlled Trial	Phase 3	Thrice daily orally after food for 30 days	Alleviation of COVID-19 symptoms.	70	CTRI/2021/04/032,804
Zandu™ Chyawanprash, Zandu™ Pure Honey, *Trishun, Immuzan	Randomised, Parallel-Group Trial	Phase 2/ Phase 3	10–12 gm twice daily for 8 weeks 1 teaspoonful twice daily for 8 weeks 1 tablet twice daily for 2 weeks 2 tablets twice daily for 8 weeks	Clinical recovery. Comparative assessment of post-clinical recovery based on signs and symptoms.	60	CTRI/2021/01/030,733
CIM-MEG19	Non-randomised, Active Controlled Trial	Phase 2	2 Tablets twice daily after meal for 21 days	Recovery and improvement in WHO-QOL	80	CTRI/2021/05/033,472
CIM-MEG19	Randomised, Parallel Group, Active Controlled Trial	Phase 2	Two tablets two times a day after meal	Changes in time to 2-point improvement (from time of enrolment) on the WHO ordinal scale	80	CTRI/2021/05/033,543
NOQ19	Randomised, Parallel Group- Placebo Controlled Trial	Phase 4	Two tablets– Thrice daily after meals for 3 months	Prevention of COVID-19 and symptom severity in infected cases	5000	CTRI/2021/07/034,606
NOQ19	Randomised, Parallel Group- Placebo Controlled Trial	NA	Two tablets– Thrice daily for 14 days	Duration of hospital stay, resolution of symptoms, and early clinical recovery	100	CTRI/2022/01/039,370
Amukkura chooranam	NA	Phase 2	5 gs, twice daily for 15 days	Prevention or reduction of post COVID-19 complications	1409	CTRI/2021/07/035,028
NOQ19	Randomised, Parallel Group- Placebo Controlled Trial	Phase 2	Two tablets– Thrice daily for 10 days	Early clinical recovery and symptoms	155	CTRI/2021/08/036,025
NOQ19	Randomised, Parallel Group- Placebo Controlled Trial	Phase 3/ 4	Two tablets- Three times a day for 14 days	Reduction in Viral load, clinical recovery	40	CTRI2021/10/037,423
Guduchi Ghan Vati	Retrospective study	N/A	Two tablets (500 mg each) twice daily were given orally after meal for 28 days	Early recovery and reduction in viral load	91	NCT04480398
Guduchi Ghan Vati	Single-arm study	N/A	Two tablets (1000 mg) twice daily for two weeks	Early recovery and reduction in viral load	46	NCT04542876
Shanshamani Vati plus (Guduchi and Pipli)	Single Group Assignment	N/A	Shanshamani Vati Plus was given twice daily	Alleviation of symptoms	26	NCT04621903
Ashwagandha,	Community-Based Participatory Research	N/A	250 mg – 5 g based on age, weight and severity of symptoms	Early recovery	28	NCT04716647
Giloy			500 mg – 1 g based on age, weight and severity of symptoms			
Tulsi			500 mg–1 g based on age, weight and severity of symptoms			
Guduchi Ghanvati	Non-Randomized	N/A	500 mg of Samshamani vati or Giloy Ghanavati twice daily	Clinical recovery	216	NCT04920773

COVID-19 patients, when compared to patients receiving the Ayurvedic treatment as an add-on with standard drug treatment (azithromycin with Vitamin-C and anti-histamines (not specified) and acetaminophen as prescribed) (Balkrishna et al., 2021a). The Ayurvedic treatment consisted of the polyherbal formulations Swasari Ras, Tulsi Ghanvati and Anu Taila, and herbal extracts of *T. cordifolia* and *W. somnifera*. A trial by Devpura et al. (2021) in a geographically distant region of India (CTRI/2020/05/025,273) compared the anti-COVID-19 efficacy of the same Ayurvedic treatment with placebo (Devpura et al., 2021) and

found that treatment was better than placebo in reducing time to a negative diagnostic test. Several phytoconstituents of both *T. cordifolia* and *W. somnifera* are predicted to bind to SARS-CoV-2 proteins S and M^{pro} and the ACE2 receptor in molecular docking studies (refer to section above) and oral *T. cordifolia* treatment reversed pathology in a zebrafish xenotransplant model of COVID-19 (Balkrishna et al., 2021b). Interestingly, totality of evidence from *in silico* studies, animal models and clinical trials strongly suggest a multipronged mechanism underlying the observed effects of *T. cordifolia* on COVID-19.

A trial by Rais et al. (CTRI/2020/06/025,800) evaluated the efficacy of Vyaghryadi kashaya and Samshamani vati or Shunthi churna and Rasona kalka relative to 'standard treatment' of paracetamol with Vitamin C (Rais et al., 2021) in SARS-CoV-2 positive subjects with very mild or no clinical symptoms. Participants on either Ayurvedic treatment had a shorter period to recovery. 98–100% of participants on the Ayurvedic 'add-on' treatments tested negative for COVID-19 on day 7, compared to 68% in the 'standard treatment' group ($p < 0.001$). There was some variation in the efficacy of the two Ayurvedic treatments in relation to specific clinical symptoms of fever, sore throat, cough, and loss of taste.

A treatment regime of *T. cordifolia*, *O. tenuiflorum*, *A. paniculata* tablets and Chyawanprash, an Ayurvedic supplement, as an add-on to (unspecified) 'standard of care' was evaluated for efficacy in mild to moderate COVID-19 hospitalized patients (CTRI/2020/09/027,914). The authors found that participants given the add-on treatment with the 'standard of care' had a shorter period to recovery (mean \pm SD = 10.77 \pm 3.24 days), compared to those on 'standard of care' alone (16.30 \pm 5.93; $p < 0.001$) (Gupta et al., 2021).

Two separate retrospective clinical trials studied the efficacy of *T. cordifolia* extract in confirmed asymptomatic COVID-19 patients (NCT04480398) compared to 'standard of care' (isolation and clinical monitoring). Although no results have been posted on the Clinical Trials site, preliminary findings are included in a preprint article (Kumar et al., 2020b). The treatment group remained asymptomatic and cleared the virus in a shorter period relative to the group on 'standard of care'. A companion single arm study of the efficacy of *T. cordifolia* extract with no control group was also conducted by the same group (NCT04542876) (Kumar et al., 2020a) on a cohort of asymptomatic SARS-CoV-2 positive subjects. Of the subjects who completed the study, none developed any symptoms and 95% returned a negative diagnostic test on day 7.

A pilot community-based participatory research study (Back to Roots, NCT04716647) investigated the safety and efficacy of *T. cordifolia* and *P. longum* extracts in patients in Leicester, UK, with mild to moderate COVID-19. The primary measures were clinical improvement and deterioration in clinical status to severe/critical. The study outcomes are available as a MedRxiv preprint and showed no participants were hospitalised for medical or oxygen requirements and none developed breathing difficulties with average time to recovery being 4.85 (\pm 1.8) days compared to 13.5 \pm 6.4 days in the control group ($p < 0.0001$) (Kulkarni et al., 2021).

Curcumin, and its bioactive formulations have gained significant attention in recent years for the treatment of inflammatory diseases. One randomized clinical trial (CTRI/2020/05/025,482) investigated the usefulness of Bioperine®, containing curcumin with piperine, as adjuvant therapy for COVID-19 patients (Pawar et al., 2021). In line with the other trials discussed in this review, the authors found that Ayurvedic treatment resulted in early symptomatic recovery compared to the control group who received probiotics. This is the only trial we have found that included patients with mild, moderate and severe disease.

A randomized, controlled, multi-center clinical trial compared *W. somnifera* extract with hydroxychloroquine for efficacy in pre-exposure prophylaxis (CTRI/2020/08/027,163). The interim results (Chopra et al., 2021) showed that *W. somnifera* extract was no worse than hydroxychloroquine; the latter had been recommended for such use by the Indian Council for Medical Research. One participant in the *W. somnifera* group and three in the hydroxychloroquine group contracted COVID-19 during the initial eight weeks.

A recent randomized, placebo controlled, single center clinical trial (CTRI/2021/10/037,423) investigated the efficacy of the polyherbal NOQ19 on the rate of recovery and clinical improvement among moderate to severe COVID-19 patients who were not on a ventilator or in intensive care unit. The outcomes of the trial are available as a preprint on ResearchSquare (Arun et al., 2021). Participants enrolled in the study received either NOQ19 or placebo, along with the 'standard of care' which included Doxycycline, Azithromycin, Ivermectin, Vitamin C, Zinc

and paracetamol. Hospitalised participants also received supportive care, e.g. intravenous fluids and oxygen therapy, as determined by the overseeing physician. There were more participants with 'severe' disease in the NOQ19 arm (59%) compared to the placebo arm (22%) and all enrolled participants were monitored for 14 days. There was no statistically significant difference in recovery times between the two arms as a whole. However, a significant increase in the number of participants returning a negative test for COVID-19 on day 7 was observed in the NOQ19 arm when 'moderate' and 'severe' participants were considered separately.

The discussion of the limited trials data available is strongly suggestive of some advantage in the use of specific Ayurvedic formulations in the clinical management of COVID-19, with no adverse events associated with such treatment. All treatments used in clinical trials were those used commonly for respiratory indications, and hence did not present a significant risk. As Ayurvedic treatments are well accepted in the global Indian community, and the Government of India has recommended specific Ayurvedic treatments in its COVID-19 management guidelines, the trials have been conducted primarily within that population group.

Conclusions

The emergence of SARS-CoV-2 and the resulting COVID-19 global pandemic has seen a resurgence of research into the antiviral efficacy of Ayurvedic herbal formulations. This is primarily due to the current lack of effective, safe, widely available antiviral treatments in addition to the herbal treatments being relatively cheap and easy to transport without requirement for specific storage conditions. Furthermore, the widespread use of Ayurvedic formulations in India presented an opportunity for these treatments to be examined using well designed and controlled clinical trials.

In the current review article, the evaluation of *in silico*, *in vitro*, *in vivo* and clinical evidence for use of Ayurvedic formulations and their herbal constituents is presented:

- In silico* techniques were reported in 20 peer review publications. It appears that a variety of different Ayurvedic formulations exhibit antiviral activities as ACE2 and/or 3CL^{pro} inhibitors. Despite relatively limited literature describing the RNA polymerase inhibitions, these formulations may be considered as a platform for development of future antiviral treatments.
- The findings of pre-clinical animal models were reported in only five studies where Ayurvedic herbs were found to inhibit the proteolytic activity of 3CL^{pro} of SARS-CoV-2 proteins.
- Over 50 clinical trials were completed where Ayurvedic formulations were used to prevent or treat COVID-19 symptoms. A majority of the trials were relatively small in size (less than 30 participants), single-centered, and in participants who were asymptomatic or had mild self-reported symptoms.

Many of the studies in this review have focussed on *in silico* investigations and the potential of individual phytoconstituents to bind to SARS-CoV-2 proteins. As these formulations are almost always taken as herbal extracts, these studies, although useful in themselves, provide relatively limited understanding of the clinical mechanisms that result in reduced period to recovery observed in clinical trials. Clearly, use of Ayurvedic formulations is warranted in all levels of COVID-19 disease as it is, at least, as effective as some of the other currently available treatments and may be rather beneficial in shortening period to recovery. Furthermore, use of Ayurvedic formulations as an "add-on" treatment to the already established medical interventions can also produce beneficial outcomes in individuals at different levels of SARS-CoV-2 infection (asymptomatic and mildly symptomatic).

Limitations of this literature review include its narrative design and that the studies included are reporting on observational findings with

relatively limited descriptions of mechanisms of action. Nevertheless, the findings of this literature review also highlight the possibility of applying knowledge gained from traditional medicine with modern medicine approaches across patient populations in addition to those with COVID-19.

Funding

None

CRedit authorship contribution statement

Anees Ahmed Mahaboob Ali: Investigation, Writing – original draft, Writing – review & editing, Formal analysis. **Andrea Bugarcic:** Writing – review & editing. **Nenad Naumovski:** Writing – review & editing, Visualization. **Reena Ghildyal:** Conceptualization, Writing – original draft, Writing – review & editing, Validation, Visualization.

Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgements

The authors acknowledge the help of Mrs Amanda Bulman in proofreading the final manuscript for English language.

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