Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Phytomedicine Plus 3 (2023) 100398

Contents lists available at ScienceDirect

Phytomedicine Plus

journal homepage: www.sciencedirect.com/journal/phytomedicine-plus

A randomized controlled pilot study of add-on therapy of CIM-MEG19 (standardized *Andrographis paniculata* formulation) in mild to moderate COVID-19

Karuna Shanker [a,*], Hrishikesh Rangnekar [b,*], Asmita Wele [c,*], Pravin Soni [d], Pranesh Gaikwad [e], Anirban Pal [f], Dnyaneshwar U. Bawankule [a,f], Debabrata Chanda [f]

[a] *Analytical Chemistry, Phytochemistry Division, CSIR-Central Institute of Medicinal and Aromatic Plants (CSIR-CIMAP), Lucknow-226015, India*
[b] *Questt Clinicals and Ayurceuticals, Pune, India*
[c] *Department of Rasashastra and Bhaishajyakalpana, College of Ayurved, Bharati Vidyapeeth (Deemed to be University), Pune-411043, India*
[d] *Pimpri Chinchwad Municipal Corporation's Postgraduate Institute, Yashwantrao Chavan Memorial Hospital, Pimpri, Pune-411018, India*
[e] *Panchakarma Department, Dr. D. Y. Patil College of Ayurved and Research Centre, Dr. D. Y. Patil Vidyapeeth Pune (Deemed to be University), Pimpri, Pune-411018, India*
[f] *Bio-Prospection and Product Development Division, CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow-226015, India*

ARTICLE INFO

Keywords:
Antiviral
SARS-CoV-2
Inflammation
Kalmegh
Ayurvedic pharmacopeia of India
Immunomodulation

ABSTRACT

Background: Traditional knowledge and scientific shreds of evidence strongly support the repurpose of Kalmegh (*Andrographis paniculata*, CIM-MEG19) as an alternate therapy for prophylactic management and treatment of severe acute respiratory syndrome coronavirus (SARS-CoV) and associated health disorders.
Purpose: The study aimed to assess the efficacy and safety of the CIM-MEG19 (standardized *A. paniculata* extract formulation), a proprietary *Ayurvedic* medicine in the COVID-19 management, clinical recovery, and outcomes in terms of hospitalization days as well as any sign of severity due to drug-drug interaction between CIM-MEG19 ™ and standard of care (SoC).
Methods: A randomized, parallel-group, active-controlled interventional pilot clinical study was conducted. The Group-A subjects were assigned to CIM-MEG19 add-on to SoC treatment using modern medicine without antiviral drug whereas Group-B patients with SoC treatment using modern medicine and recommended antiviral drug for COVID-19 management. Eighty RTPCR (real-time polymerase chain reaction) positive and eligible COVID-19 patients of age >18 years, having mild or moderate severity, were enrolled.
Results: Clinical improvement in reduction of symptoms showed significant ($p<0.0001$) results in the average days in subjects of group-A (Investigational intervention arm) compared to Group B (SoC). The RT-PCR investigation exhibited COVID negative for 50 % in CIM-MEG19 add-on and 47% in SoC treatment after 8-11 days. Similarly, biochemical investigations showed that CIM-MEG19 group-A had a significant ($p \leq 0.05$) effect on C-Reactive Protein (CRP) and Interleukin-6 (IL-6) after 14 days of treatment. Additionally, improvement in D-Dimer, ESR, and LDH in CIM-MEG19 add-on therapy was also observed.
Conclusions: The study demonstrated an excellent safety profile, declining the severity of the infection and halting the disease advancement/progression. CIM-Meg19 might be used as a potential natural drug for treating COVID-19.

Introduction

The world is experiencing a pandemic due to the dreadful and fatal severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), resulting in pneumonia and respiratory complications termed COVID-19. Since its declaration in March 2020 by the World Health Organization, and due to the non-availability of any proven drug, various therapies, many vaccines, several antivirals, immunomodulatory, and repurposed drugs such as anti-malarial, anti-HIV were used to treat the

* Corresponding authors.
E-mail addresses: k.shanker@cimap.res.in, kspklko@yahoo.com (K. Shanker), dr.rangnekar@gmail.com (H. Rangnekar), asmita.wele@bharatividyapeeth.edu (A. Wele), drpravinsoni18@gmail.com (P. Soni), pranesh.gaikwad@dpu.edu.in (P. Gaikwad), a.pal@cimap.res.in (A. Pal), du.bawankule@cimap.res.in (D.U. Bawankule), d.chanda@cimap.res.in (D. Chanda).

https://doi.org/10.1016/j.phyplu.2022.100398

Available online 20 December 2022

Abbreviations

ASU	Ayurveda Siddha & Unani
COVID	Coronavrus Disease 2019
CRP	C-reactive protein
ESR	Erythrocyte sedimentation rate
IL-6-	Interleukin-6
LDH-	lactate dehydrogenase
MoHFW	Ministry of Health and Family Welfare
SGOT	Serum Glutamic-Oxalo Acetic Transaminase;
SGPT	Serum Glutamic Pyruvic Transaminase
SoC	Standard of Care
ICH	International Conference on Harmonization
WHO	World Health Organization
APPI	Active Phytopharmaceutical Ingredient

same. Unfortunately, no specific antiviral agent or drug therapy against COVID-19 disease has been confirmed as the gold standard till now (Kim et al., 2021). Except for vaccines, most antiviral drugs are have demonstrated not to be useful due to adverse or limited efficacy in managing COVID-19. Although the reported cases and deaths have declined significantly, the SARS-CoV-2 virus is undergoing mutations and still circulating worldwide. Even in highly vaccinated populations, immunity might not be absolute (United-Nations, 2022). Therefore, the research is still ongoing to develop new or alternate therapies to be available in future. Respiratory system disorder is defined as 'Raktasthivi Sanniat' according to the *Ayurvedic* description, severely affecting the respiratory system.

Based on the experiential leads, prior knowledge, and clinical evidence as anti-malarial, a polyherbal *Ayurvedic* formulation (AYUSH-64) was also repurposed to treat COVID-19 (Valecha et al., 2000). In terms of natural product intervention, several other ASU (*Ayurveda, Siddha, and Unani*) and TCM medicines have been employed to alleviate the clinical manifestation and severity of the disease and have shown some efficacy (Wanjarkhedkar et al., 2022). Various preclinical and clinical studies revealed that *A. paniculata* (Burm.f.) Nees (Acanthaceae) possesses antiviral potential in addition to anti-inflammatory, anti-cancer, anti-obesity, and anti-diabetes properties. It has shown antiviral activity for viral infections like chikungunya, herpes simplex virus type-1, and dengue (Kaushik et al., 2021). Analytical, computational modeling has documented its potency against COVID-19 and its probable mechanism. Due to these supportive antiviral activities, *A. paniculata* was chosen as a drug for COVID-19 management. It is used in more than 26 classical *Ayurvedic* formulations. It is used to treat a broad spectra of diseases like *Ajirna* (dyspepsia); *Arsa* (piles); *Atisira* (diarrhea); *Jvara* (fever); *Kanadu* (itching); *Kamala* (jaundice); *Kustha* (diseases of the skin); *Prameha* (increased frequency and turbidity of urine); *Pravahika* (dysentery); *Tvakvikara* (skin disorders); *Vrana* (wound); *Yakrutvikara* (disorders of the liver) (API, 2010).

The antiviral potential of A. paniculata and its key phytochemical andrographolide against hepatitis-B, C (Chen et al., 2014), influenza–A (Calabrese et al., 2000), and studies on upper respiratory diseases (Saxena et al., 2010) have supported that it could be a safer and effective choice in suppressing the severity of COVID-19 infection. Diterpene lactones of A. paniculata, particularly andrographolide, have been positioned as one of the promising phytochemicals for controlling COVID-19 through entry into host cells and interaction with its key targets (Srikanth and Sarma, 2021). A. paniculata is also one of the plants identified by the Task Force on Repurposing of Drugs for COVID-19 S&T Core Group on COVID-19 constituted by Principal Scientific Advisor) (PSA) to the Government of India as one of the potential herbs for repurposing for COVID-19 (Premnath, 2020). Considering the challenge posed to the robust efficacy of crude powder-based polyherbal formulations, Council of Scientific and Industrial Research (CSIR)-Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow has developed a single herb-based standardized extract of single herb base formulation (CIM-MEG19) with defined antiviral phytochemical ingredient for clinical translational research.

The present trial evaluated the add-on therapeutic effect to conventional standard of care (SoC) treatment of COVID-19 in terms of the number of hospitalization days required for a 2-point improvement on the WHO ordinal scale. The secondary objective was to assess the effect on oxygen saturation, inflammatory markers, an embargo period of COVID-19 by RT-PCR negatives test, and occurrence of possible disease severity. During the pandemic, the standard of care was constantly evolving, and treatment guidelines kept changing. There was a constant need for a concrete supportive drug from a safe herbal background, which is intended to be provided by this trial. For ethical reasons, we had to conduct an 'add-on' trial, no standalone treatment permitted during pandemic. Otherwise a standalone treatment with head-on with the standard of care (SoC) could also have conducted.

Materials and methods

Investigational drug

The repurposing of the CIM-MEG19 for COVID-19 management was proposed and executed by CSIR-CIMAP, Lucknow. The product is based on the product technology developed at CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, India. The manufacturing of the investigation drug (CIM-MEG19 ^{TM}) was approved in the GMP facility with due permission from the authorities (A-1811/90/2015 vide 888I/D-2685/89 dated 26/03/2021) by M/s Meghdoot Gramodyog Sewa Sansthan, Lucknow according to the *Ayurvedic Pharmacopeia of India*. The quality control by chemical standardization based on active phytopharmaceutical ingredients of (APPI) CIM-MEG19 is defined below.

Quality assurance and chemical standardization of CIM-MEG19

The raw plant material (*A. paniculata*, aerial part), extract, and finished product (CIM-MEG19) were chemically standardized using HPLC-PDA chemical profiling. The presence of bioactive phytochemicals is warranted to ensure uniform quality, robust efficacy, and shelflife. Reverse-phase HPLC conditions were optimized, and the method was validated for the quantitative analysis of four diterpene lactones viz., andrographolide (**1**), neo-andrographolide (**2**), 14-deoxy-11,12-didehydroandrographolide (**3**), and andrograpnin (**4**). A representative HPLC profile of the *Kalmegh (A. paniculata)* aerial part, its extract and standardized medication (CIM-MEG19), and the method parameters are presented in the Fig. 1.

Trial design, medication, and location

The present trial was conducted by adopting the International Conference on Harmonization (ICH) Guidelines for Good Clinical Practice in compliance with the Declaration of Helsinki. Additionally, the prevailing guidelines issued by the ICMR-AYUSH Department Government of India for the COVID-19 management were also rigorously followed. The Group-A of mild to moderate patients received CIM-MEG19 as an *add-on* treatment to the SoC. The SoC is the treatment that otherwise the patient would have received if she/he was not a study participant. It included NSAIDs, antibiotics, antacids, and supplements. The Group B of mild and moderate patients received SoC alone. The only difference is that group B antivirals (like Favipiravir and Remdesivir) were allowed at baseline.

Before initiating the study, the protocol was reviewed and approved by the Institutional Ethics Committee approval from Yashwantrao Chavan Memorial Hospital (YCMH/IEC/KAVI/i/45/2021 dated 28/04/2021) and Dr. D. Y. Patil Medical College, Hospital & Research Centre (AY/IEC/341/2021 dated 20/05/2021), Pimpri, Pune India. The study

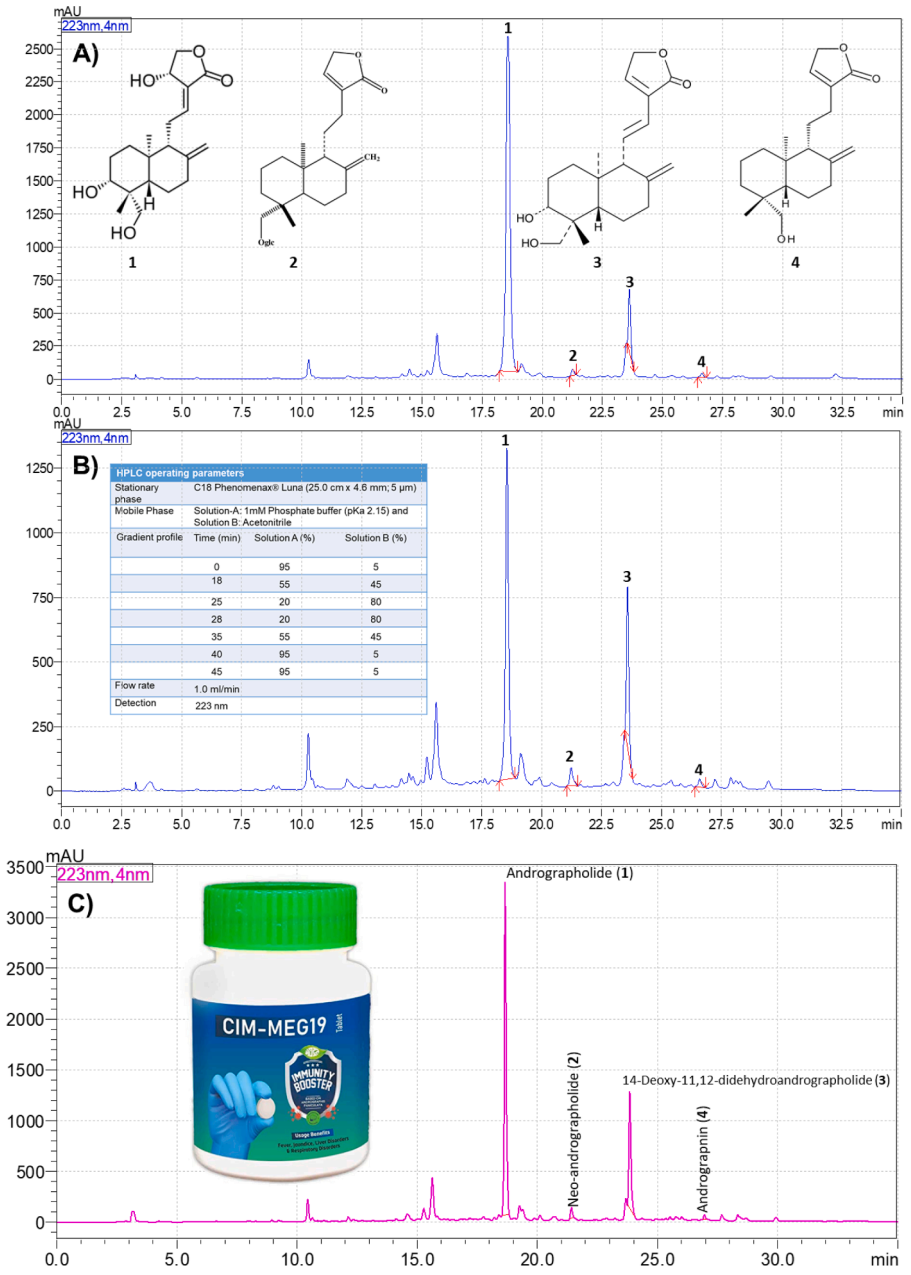

Fig. 1. Representative HPLC chromatogram of – A) *Andrographis paniculata* aerial part, B) standardized extract, and C) CIM-MEG19 showing the presence of key labdane diterpenes. HPLC method parameters are summarized in the Chromatogram-B.

protocol was registered (*CTRI number: CTRI/2021/05/033543*) on 11/05/2021 at the Central Trial Registry of India. Written informed consent from all the subjects before the study's enrolment and the data will be published.

Inclusion criteria

COVID-19 RT-PCR positive patients; Male or Female of age 18-70 years; Subject ready to give written Informed consent; Can take oral medicines; Mild to moderate grade of the disease. Mild- Upper respiratory tract symptoms of fever with or without shortness of breath or hypoxia; Moderate- Any one of- 1. Respiratory rate more than 24/min, breathlessness 2. SpO_2: 90-93% on room air. 3. PaO_2/FiO_2: 200-300/g as per AIIMS/ICMR-COVID-19 National Task Force/Joint Monitoring Group recommendations not participating in any other interventional drug study and agreeing to follow all study procedures (AIIMS, 2021).

Exclusion and withdrawal criteria

Patients do not fall within the age criteria of inclusion. Patients with known sensitivity to the ingredients of the investigational drug, bleeding haemorrhoids, pre-existing gastrointestinal symptoms like nausea or vomiting, acute hypoxic respiratory failure, e.g., ICU stay, need mechanical ventilation, falling in the severity or critical category, and pregnancy or lactation was excluded. Before trial initiation, an informed consent form duly approved by IEC was obtained from the patients after explaining the study in Hindi/English for those unfamiliar with the local language Marathi. The study adheres to CONSORT guidelines.

Sample size and patient recruitment

The sample size was derived using the ratio of sample size, unexposed/exposed, percentage of unexposed with the outcome, percentage

of exposed with the outcome, OR, risk/prevalence ratio, and risk/prevalence. The sample size was calculated to be 80 based on power 80, a two-sided significance level of 95, and allowing for a 35% dropout rate, i.e., 40 in each group. Considering dropouts, up to 40 in each arm were considered for enrolment. The study adheres to the CONSORT (CONsolidated Standards of Reporting Trials) flow diagram as shown in Fig. 2.

Forty patients in each group, corresponding to an odds ratio of 4 (Fleiss et al., 2013) for the cross-sectional cohort (Dean et al., 2019), were followed in the present study. It was a two-arm trial, and after enrolment into the study, patients were assigned either to the intervention arm or the control arm (1:1 ratio). The study arm received CIM-MEG19 (0.2 g b.id.) as an *add-on* therapy to the Standard of Care (SoC) with or without antiviral therapy, depending on the clinical symptoms and severity condition of the subjects. Twenty-four patients received Remdesivir, 13 Favipiravir antiviral therapy, and 3 only with SoC. Forty patients were treated with CIM-MEG19 plus SoC. The SoC of the COVID-19 patients involved administering medication depending on the clinical symptoms (Fig. 2). The WHO ordinal clinical severity scale was assessed every day (WHO, 2021). The scale has five patient stages: 0-uninfected, 1-ambulatory, 2-hospitalized with mild disease, 4-hospitalized with severe disease, and 5-death.

Randomization and concealment

The admitted patients were screened for the study and randomized to Arm-A (intervention arm: SoC *w/o* antiviral + CIM-MEG19) or Arm B (control arm: SoC treatment *w* or *w/o* antiviral) after enrolment. A total of 80 patients were randomized with an allocation ratio of 1:1. Concealment was achieved because the principal investigator provided the number of group allocations. At the same time, the research associate examined the patient for eligibility, and the staff nurse gave medications *per* allocation. However, the otherwise eligible patients who had been prescribed/administered antiviral treatment were considered for Arm B only, and the corresponding randomization number was marked booked for Arm B.

Outcomes

The time required for a 2-point reduction in the WHO ordinal scale was considered the primary outcome for efficacy. Clinical outcome was considered a primary endpoint, as this mattered most to the patients admitted to the hospital. Other outcome measures included the occurrence of adverse and severe adverse events; disease progression; and investigations like repeat RT-PCR, Hemogram, CRP, LDH, and D-Dimer on a few patients.

Adverse events and withdrawal criteria

The withdrawal/stopping criteria were disease progression by symptoms, intolerance to an investigational drug, or any adverse reactions during the trial were the withdrawal/ stopping criteria. The withdrawal was recorded in the predesigned form.

Statistical analysis

The analysis was performed using Microsoft-Excel and SPSS version-21 (IBM, Statistics software). A non-parametric (Wilcoxon signed-rank) test was applied to evaluate the effect of therapy on vital organs. Parameters of improvements in the clinical symptoms, days of hospitalization, and the number of patients diagnosed as COVID-19 positive fully recovered are presented in this study. One-way analysis of variance with post hoc Tukey's and Levene's tests were performed to analyze the homogeneity of variance in clinical improvement on the WHO scale. The level of significance was set at $p < 0.05$.

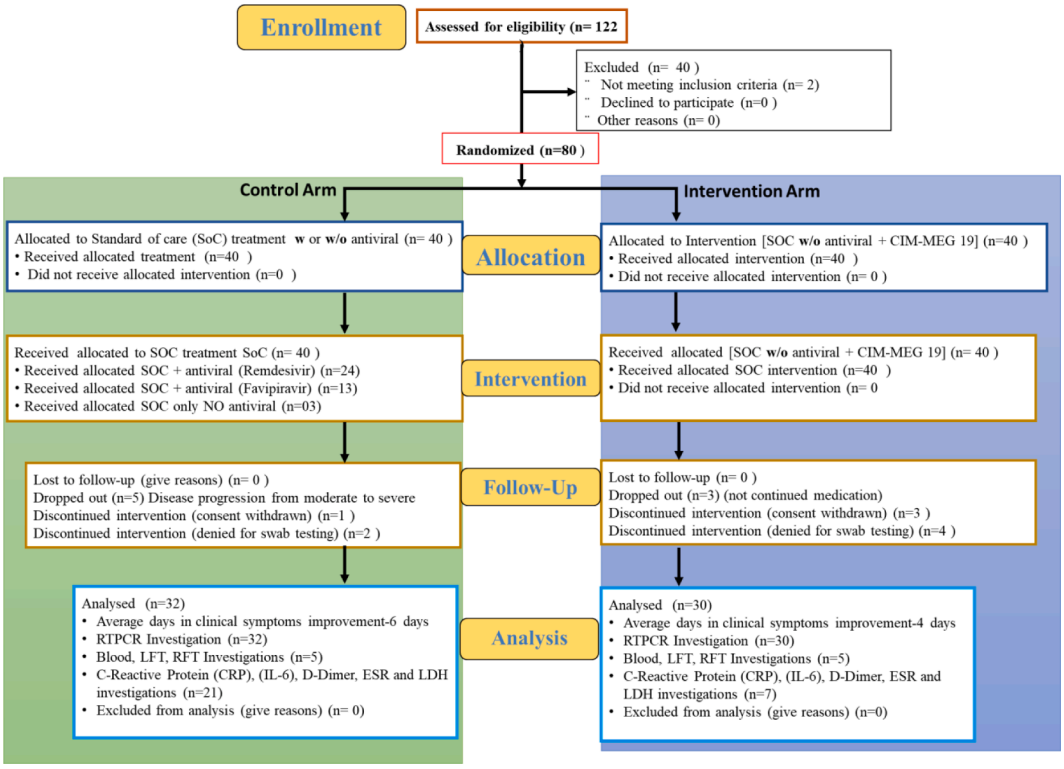

Fig. 2. CONSORT flow diagram of the current study.

Results

Quality standardization of CIM-MEG19

M/s Meghdoot Gramodyog Sewa Santhan, Lucknow, has manufactured the CIM-MEG19- a methylcellulose coated tablet) containing 150 mg *A. paniculata* standardized extract (CIM-Megh^{CIMAP}@150 mg) and permitted dehydrating agents, binders, and excipients. The quality assurance of the raw material, its extract, and the CIM-MEG19 was performed by bioactive labdane diterpenoids (andrographolide, neoandrographolide, 14-deoxy-11,12-didehydroandrographolide, and andrograpnin) based standardization. One tablet was dissolved in 10 mL HPLC methanol under sonication for 30 mins. The solution was centrifuged and filtered with a 0.45 μm nylon filter before HPLC analysis. The sum of all four labdane diterpenoids in CIM-MEG19 was 45-55 mg/g with a major bioactive compound, andrographolide 35-40 mg/g. Other physicochemical and control parameters, viz. heavy metals, pesticide residue, microbial load, and aflatoxins, were also evaluated and complied with the requirement of the *Ayurvedic* Pharmacopeia of India. A representative HPLC chromatogram of CIM-MEG19 depicting the presence of antiviral compound andrographolide and other key labdane diterpenoids are presented in Fig. 1.

Demographic and COVID-19 -associated clinical symptoms

The demographic details of the subjects under study showed that the patients in the age group 18-30 years fell in the intervention group while subjects of 41-60 years to SoC (Table 1). The disease severity ratio of mild to moderate on the WHO scale was 92:8 % in the CIM-MEG19 treated group while 30:70% in the SoC-treated group (Table 2). It is worth mentioning that during the second wave, the population below 50 was most affected by SARS-Cov-19 viral infection. At the time of admission, patients had significant clinical symptoms like feverishness or measured fever (≥98.6°F), cough, shortness of breath (dyspnoea/tachypnoea), sore throat, runny nose, general weakness, headache, irritability/confusion, nausea/vomiting, and diarrhea (Table 3). The change in these symptoms was assessed for 21 days at the interval of 04 days as per the study protocol (Fig 2).

Clinical improvement

The study's primary endpoint was to assess the time required for a 2-point improvement on the WHO ordinal scale. The clinical improvement in the WHO ordinal scale is summarised in Table 4. It was observed that the mean time required for 2-point improvement in interventions and control treatment was 4.17 (0.56) days and 6.23 (1.95) days, respectively. The high significant ($p<0.001$) obtained by the independent one-way ANOVA test, followed by Levene's test, showed the equality of variances. The difference between interventions and control treatment is

Table 1
Distribution of patients by gender and age.

Treatment/distribution	**Number and distribution**				
	Age in years	**Male**	**%**	**Female**	**%**
Control arm: SoC treatment *w* or *w/o* antiviral	18-30	4	10	2	5
	31-40	7	17.5	4	10
	41-60	14	35	2	5
	> 61	1	2.5	6	15
Intervention arm: SoC treatment *w/o* antiviral + CIM-MEG19	18-30	12	30	5	12.5
	31-40	7	17.5	2	5
	41-60	9	22.5	2	5
	> 61	1	2.5	2	5

SoC- Standard of care of COVID patients as per hospital guidelines.

Table 2
The category of illness as per WHO criteria adopted and recommended by MoHFW, Govt. of India for COVID-treatment guidelines the time of admission.

Category of COVID Infection	Manifestation of clinical symptoms			
	Intervention arm [SOC treatment w/o antiviral+CIM-MEG 19] (n=40)		Control arm [SoC treatment w or w/o antiviral] (n=40)	
	Number	%	Number	%
Asymptomatic or Presymptomatic Infection[a]	0		0	
Mild illness[b]	37	92%	12	30%
Moderate illness[c]	3	8%	28	70%
Severe illness[d]	-		-	
Critical Illness[e]	-		-	

WHO- World Health Organization
MoHFW – Ministry of Health & Family Welfare.
SoC- Standard of Care of COVID patients as per hospital guidelines.
[a] positive for SARS-CoV-2 using a virologic test but have no symptoms consistent with COVID-19.
[b] positive for SARS-CoV-2 using a virologic test but have signs and symptoms of COVID-19 (e.g., fever, cough, headache, muscle pain, sore throat, malaise, nausea, diarrhea, vomiting, loss of taste and smell)
[c] positive for SARS-CoV-2 using a virologic test with the sign of evidence of lower respiratory disease and oxygen saturation (SpO_2) ≥94%
[d] positive for SARS-CoV-2 using a virologic test and oxygen saturation (SpO_2)<94%, or (PaO_2/FiO_2) <300 mm Hg, or respiratory rate >30 breaths/min, or lung infiltrates >50% erespiratory failure, septic shock, and/or multiple organ dysfunction
[a,d.e]patients of these category were not part of the study.

Table 3
Major symptoms/complaints observed at the time of admission in present study.

Symptoms/complaints	Control arm: Standard of care (SoC) treatment w or w/o antiviral	Intervention arm: SoC w/o antiviral + CIM-MEG 19
Feverishness or measured fever ≥37°C or 98.6°F	21	15
Cough	27	23
Shortness of breath (Dyspnoea) OR Tachypnoea	28	3
Sore throat	5	4
Runny nose	5	12
General weakness	15	8
Headache	6	5
Irritability/confusion	0	0
Nausea/Vomiting	1	1
Diarrhea	1	0
Others:	7	5

Table 4
Effect of treatment on two-point clinical improvement as per WHO ordinal scale.

Treatments	No. of days required for 2 points improvement in the WHO score			Levene's Test for Equality of Variances
	Number of subjects	baseline	treatment	
Intervention arm: SoC treatment *w/o* antiviral + CIM-MEG 19	38	3.02 ±0.30	4.17 ±0.56	F= 31.710 p<0.001
Control arm: SoC treatment *with* antiviral (remdesivir/favipiravir)	40	3.17 ±0.30	6.23 ±1.95	

SoC- standard of care of COVID patients as per hospital guidelines.
Values are presented as mean±sd

statistically significant. The early recovery of COVID-19 symptoms than SoC on 2-point improvement on the WHO ordinal scale in the intervention CIM-MEG19 group can be observed (Table 4).

Repeat RT-PCR test

Out of eighty, 62 swabs were tested on follow-up (8±3 days). From overall sixty-two, 32 were positive, and 30 were negative. In the intervention arm, 50% (15 of 30) were negative, whereas 47% (15 of 32) were negative in the control arm receiving SoC treatment *w* and *w /o* antiviral (remdesivir/ favipiravir) (Table 5).

Blood investigations

Based on symptoms and observations of the clinicians, 28 patients from both groups (21 from control and 7 from the CIM-MEG19) were advised for key blood biomarkers. Data of baseline and follow-up (day 4 ±2) was analyzed using F-Test. The drug group showed a statistically significant ($p < 0.05$) decline in C-Reactive Protein (CRP) and Interleukin-6 (IL-6). At the same time, a marginal improvement in other parameters such as D-Dimer, ESR, and LDH can be observed. The results of drug cases on baseline and follow-up at day four are summarized in Table 6.

Safety evaluation

No deaths were reported in the study, while five severe adverse events (SAEs) in the form of prolonged hospitalisation were noted, all from the control group. The blood biochemical parameters were also analyzed before and after CIM-MEG19 add-on treatment to evaluate any adverse reaction on the vital organs, viz. renal and liver. The values of Total Protein, Albumin, Globulin, Bilirubin, SGOT, SGPT, Alkaline Phosphatase, Urea, and Creatinine were analyzed by Wilcoxon signed-rank (a non-parametric test) due to their non-normal distribution. The non-significance ($p \geq 0.4$) values indicate no adverse effect of the CIM-MEG19 add-on therapy on the liver and renal function of the COVID-19 RT-PCR positive patients. Furthermore, all the cases in whom liver and renal function tests were done were within normal limits (Table 7).

Discussion

Ayurvedic drugs, Traditional Chinese Medicines (TCM) other components of complementary and alternative medicine from the global perspective, are also being studied to manage COVID-19 because there is no specific remedy for the same (Seifert et al., 2020). Various studies have shown that *Ayurvedic* interventions have helped patients in early recovery and symptomatic relief (Wanjarkhedkar et al., 2022). Most clinical studies based on natural product interventions, registered in the Clinical Trial Registry of India (CTRI), comprise either polyherbal or herbo-mineral formulations in different dosage forms, e.g., tablets, powders, decoctions, and A*sava- Arishta* (fermented hydroalcoholic extract of herbs). Common herbs are Alstonia scholaris, Glycyrrhiza glabra, *A. paniculata,* Picrorhiza kurroa, Swertia chirayita, Caesalpinia crista, *Tinospora cordifolia,* Gingiber officinalis, and *Piper longum* among others. Also common metallic viz. *Abhraka bhasma* (incinerated mica), Tamra *bhasma* (incinerated copper), and mercurial preparation, e.g., cinnabar and *Kajjali*, were also used with many herbs. Although these drugs comply with the Indian regulation, the robust quality is still challenging due to non-uniform raw material collected from forests not produced through standardized captive cultivation. Additionally, none of these was used singularly. Unlike these reported studies, in the present clinical study, the bioactive marker-based chemically standardized and pre-clinically tested extract of *A. paniculata* (CIM -MEG19) has

Table 6
Effect of CIM-MEG-19 add-on therapy on the blood inflammatory markers.

Parameter	Unit	Baseline values (mean±sd)	Values after Follow-up* (mean ± sd)	Significance difference (baseline vs follow-up) ANOVA One way (df=6)
ESR	mm at the end of 1hr	21.14 ± 19.12	13.85 ± 11.65	$F_{cal}<F_{cri}$ = 2.69<4.28, p=0.12
LDH	IU/L	242.14 ± 98.86	215.85 ± 58.72	$F_{cal}<F_{cri}$= 2.83<4.28, p=0.01
CRP	mg/L	21.5 ± 41.5	9.98 ± 14.5	$F_{cal}>F_{cri}$= 8.13>4.28, p=0.01
D-DIMER	ng/ml (FEU)	188 ± 51.8	146.57 ± 88.94	$F_{cal}>F_{cri}$= 0.33>0.23,p=0.10
IL-6	pg/ml	7.13 ± 2.33	8.69 ± 6.35	$F_{cal}<F_{cri}$= 0.13<0.23, p=0.01

*Follow-up after 4 day of treatment, ESR: Erythrocyte Sedimentation Rate, LDH: Lactate Dehydrogenase, CRP: C-Reactive Protein; IL-6: Interleukin-6

Table 7
Effect of CIM-MEG-19 add-on therapy on the renal and liver parameters.

Parameter	Unit	Range (Adult)	Baseline values (mean±sd)	Values after Follow-up*† (mean ± sd)
Total Protein	gm/dl	5.5 to 8.0	7.44 ± 0.44	7.52 ± 0.41
Albumin	gm/dl	3.5-5.2	4.14 ± 0.23	4.48 ± 0.33
Globulin	gm/dl	2.0 to 3.5	3.30 ± 0.46	3.04 ± 0.62
Bilirubin ±Total	mg/dl	0.1 to 1.2	0.40 ± 0.14	0.51 ± 0.34
Bilirubin Direct	mg/dl	0.00 to 0.30	0.15 ± 0.06	0.18 ± 0.07
Bilirubin Indirect	mg/dl	0.20 to 1.20	0.24 ± 0.07	0.34 ± 0.27
SGOT	IU/L	1 to 35	24.61 ± 7.52	23.05 ± 6.67
SGPT	IU/L	1 to 45	23.00 ± 8.83	29.04 ± 13.63
Alkaline Phosphatase	IU/L	20 to 130	62.22 ± 18.8	67.2 ± 16.53
Urea	mg/dl	17 to 49	23.63 ± 7.51	19.4 ± 1.62
Creatinine	mg/dl	0.70 to1.30	0.77 ± 0.06	0.7 ± 0.11

SGOT – Serum glutamic-oxalo acetic transaminase; SGPT – Serum glutamic pyruvic transaminase; *Follow-up after 04 day of treatment; †Wilcoxon Signed Rank test ($p \geq 0.05$, n=38).

Table 5
Interpretation based on RT-PCR investigational analysis performed at follow-up time 8(+3) days.

Treatments/RTPCR- score	Total investigations performed	Positive		Negative		Chi- square test	Relative Risk for standard treatment:RR (95%CI)
		Number	%	Number	%		
Intervention arm: SoC treatment *w/o* antiviral + CIM-MEG 19	30	15	50	15	50	$\chi^2 = 0.125$ p = 0.7236	1.133 (0.48-3.072
Control arm: SoC treatment *w* and *w/o* antiviral (remdesivir/ favipiravir)	32	17	53	15	47		

SoC- standard of care of COVID patients as per hospital guidelines.

better control on the quality of the product and robust therapeutic efficacy than non-standardized poly-herbal formulations.

The chemical profile of raw material, its extract, and formulation has ensured the presence of all four labdane diterpenoids, viz., andrographolide, neoandrographolide 14-deoxy-11,12-didehydroandrographolide, and andrograpanin (Fig. 1). Among these four compounds, andrographolides have been studied extensively for various antiviral actions, including SARS-CoV-2. The antiviral action of andrographolide seems to be due to different mechanisms, e.g., regulating viral entry-stage via blocking the ACE2 receptor, gene replication, synthesizing of mature functional proteins, and inhibiting the non-structural proteins like main protease (M^{pro}), 3C-like proteinases ($3CL^{pro}$), and papain-like protease (PL^{pro}) of SARS-CoV-2 (Enmozhi et al., 2021).

A. paniculata is also superior to the placebo in controlling uncomplicated upper respiratory tract infection (Coon and Ernst, 2004). It is also reported to shortened cough, improved sore throat, and sick leave compared to regular treatment and care (Hu et al., 2017). It has also been used to combat respiratory disorders and seasonal allergies and enhance the functioning of immune system. A clinical case study of a fixed ayurvedic regimen (tablets of *O. sanctum, T. cardifolia,* and *A paniculata*) as an *add-on* to the SoC showed a significant reduction in recovery time in mild to moderate COVID-19 patients (Gupta et al., 2021).

Based on the *Ayurvedic* clinical practice and new knowledge about its antiviral and immune-strengthening aspects, drug CIM-MEG19 was formulated and tested in patients. The study aimed to arrest the disease progression and to help alleviate symptoms in COVID-19 patients having mild to moderate severity. All the patients were hospitalized in the COVID ward of the hospital, and hence it was easier to maintain the rigor of the study. In the first wave, the SARS-CoV-2 infected the population predominantly older than 60 and was at high risk of death if associated with comorbid conditions. Nevertheless, this was not the same during the second wave; surprisingly, younger adults between 25 and 50 years were also infected, even though some died at a young age (Jain et al., 2021). Despite a large vaccination drive, during the third wave, a large population was again re-infected and still vulnerable to many known-unknown mutants/variants of SARS-CoV-2, which is not apparent and yet to be scientifically explored. In this situation, A. paniculata based alternative therapy could provide better protection and treatment in the case of re-infection due to its antiviral, immune-modulatory, and anti-inflammatory actions.

The present trial indicates that the CIM-MEG19 may be beneficial as *add-on* therapy in mild to moderate COVID-19 patients to reduce the severity of the disease as assessed by the ordinal scale. The probability value was significantly lower in the intervention arm (SoC *w/o* antiviral + CIM-MEG19), pointing out the clinical efficacy of CIM-MEG19. This clinical improvement was supported by statistically significant values favoring CIM-MEG19 for the decline in CRP and IL-6. Cytokines are signaling peptides, proteins, or glycoproteins secreted by many cell types, such as immune, epithelial, endothelial, and smooth muscle cells. The "cytokine storm" occurs when the interactions leading to cytokine production become 'abnormal'. This results in uncontrolled inflammation within tissues and critical organs (Yiu et al., 2012). Cytokine storm is also witnessed in many conditions like sepsis, septic shock, acute respiratory distress, and a toxic response to medication (Gupta et al., 2020). The trials in COVID-19 patients have reported a decrease in peripheral blood lymphocyte count and an increase in serum inflammatory cytokine (Huang et al., 2020). Cytokine storms can be extremely severe and can be life-threatening by causing organ failure(s). SARS-CoV-2 infection can quickly activate pathogenic T cells and produce granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-6. It further activates inflammatory monocytes and inflammatory factors resulting in a cascade of interactions and cytokine storm. This can cause severe immune damage to the lungs and other organs (Zhou et al., 2020). CRP and IL6 were amongst those parameters that were closely related to the disease severity. It was also confirmed that an increase in IL6 indicated disease exacerbation, whereas a decrease indicated the treatment effectiveness (Liu et al., 2020). CRP levels are reported to be elevated in hospitalized COVID-19 patients, and there is a correlation between the severity of the disease and mortality. In one retrospective study, CRP and IL-6 values on admission were independent predictors of disease severity. In a small prospective study IL-6 and CRP levels correlated with the development of respiratory failure (Herold et al., 2020). One study has explicitly highlighted CRP's importance in hospitalized COVID-19 patients for its prognostic value (Sharifpour et al., 2020). The reduction of CRP and IL6 in the intervention arm ($p = 0.01$) indicates the efficacy of the drug CIM-MEG19. There is no significant difference for the RTPCR parameter in the follow-up duration, even with the clinical improvement in the CIM-MEG19 treated group.

The present pilot study was a randomized controlled trial on CIM-MEG19 based on the standardized extract of *A. paniculata* aerial part. The intervention arm received the CIM-MEG19 in addition to the SoC, whereas the control arm received SoC treatment *w* or *w/o* antiviral. The patients receiving an antiviral regimen were *not* enrolled in the intervention arm but in the control arm. This eligibility criterion allowed the head-on comparison between CIM-MEG19 and antivirals like Favipiravir and Remdesivir to a limited extent. No adverse or severe adverse events (SAE) occurred in the intervention arm, whereas five SAEs occurred in the control arm. The primary endpoint of the study was met. CIM-MEG19 could reduce the scores on an ordinal scale with statistical significance. The clinical improvement in the intervention arm was supported by laboratory assessment. Both CRP and IL6 were reduced in the intervention arm with statistical significance. This reduction in biomarkers might have halted the disease progression and early recovery. Additionally, no patient had to be shifted to the intensive care unit (ICU). CIM-MEG19 *is* proved to be dependable support, though used as an adjuvant.

Although the study was an 'add-on' intervention, the eligibility criteria facilitated a comparison between CIM-MEG19 and the *antivirals* like Favipiravir and Remdesivir. Hence, the study could have a head-on trial by following ethical principles. Also, the endpoints were multiple, engaging both clinical and laboratory domains. These were the strengths of the study. The weakness of this study lies in the inability to avoid some bias at enrolment and the use of antiviral like remdesivir/ favipiravir in the control arm. Due to ethical reasons, critical patients could not be enrolled. Therefore, a multi-centric study with more COVID-19 patients with severity considering the other critical inflammatory cytokines and other blood biomarkers is needed to validate the preliminary outcome of the present study.

The present pilot trial of CIM-MEG19 as an add-on to SoC has demonstrated the safety and efficacy in COVID-19 with mild to moderate severity. Therefore, a detailed clinical study with an adequate number of patients would be essential, encompassing severe and critical patients with COVID-19 infection to establish a natural product-based alternate therapy.

Conclusion

The present pilot clinical study has shown the efficacy of CIM-MEG19 in arresting the disease progression of COVID-19 by alleviating the inflammatory markers. The study highlighted a 2-day benefit in recovery for those who received CIM-MEG19. The bioactive labdane diterpene lactones (LDDs) based standardized *A. paniculata* single-herb extract based CIM-MEG19 therapy is safe and effective in mild-to-moderate COVID-19 patients as an *add-on* regimen.

Source of funding

The authors acknowledge the funding received from the primary sponsor (M/s Meghdoot Gramodyog Sewa Sansthan, Lucknow, India) for the test drug and secondary sponsor [Council of Scientific and Industrial Research, Government of India] for funding under Herb-Natural

Product-COVID-19 Research Program (MLP-010). Both primary and secondary sponsors had no direct or indirect role in the study design, execution, data collection, reporting, or decision to publish the manuscript.

Ethical statement

The clinical research protocol was reviewed and approved by the Institutional Ethics Committee approval from Yashwantrao Chavan Memorial Hospital (YCMH/IEC/KAVI/i/45/2021 dated 28/04/2021) and Dr. D. Y. Patil Medical College, Hospital & Research Centre (AY/IEC/341/2021 dated 20/05/2021), Pimpri, Pune India. Written informed consent from all the subjects before the study's enrolment and the data will be published.

Data availability

The data supporting this study's findings are available within the article and its supplementary material.

CRediT authorship contribution statement

Karuna Shanker: Conceptualization, Supervision, Project administration, Funding acquisition. **Hrishikesh Rangnekar:** Conceptualization, Formal analysis, Investigation, Writing – review & editing. **Asmita Wele:** Supervision, Visualization, Data curation, Writing – review & editing. **Pravin Soni:** Conceptualization, Investigation, Writing – review & editing. **Pranesh Gaikwad:** Methodology, Investigation, Data curation. **Anirban Pal:** Resources, Writing – review & editing. **Dnyaneshwar U. Bawankule:** Resources, Writing – review & editing. **Debabrata Chanda:** Resources, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Acknowledgments

The authors are thankful to Director CSIR-CIMAP, Lucknow, and the learned members of the Institutional Ethics Committee of YCM and DPU, Pune, for providing the necessary facilities and support for conducting the present study. The financial assistance for the clinical studies from M/s Meghdoot Gramoudyog Sewa Santhan, Lucknow, is also acknowledged.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.phyplu.2022.100398.

References

AIIMS, 2021. Clinical guidance for sydromic management of suspected COVID-19 cases. AIIMS/ICMR-COVID-19 National task force/joint monitoring group. (Dte.GHS) ministry of health & family welfare, government of India. https://dghs.gov.in/WriteReadData/News/2021042902541793132 92AlgorithmSyndromicManagement.pdf updated on 29 April 2021.

API, 2010. The Ayurvedic Pharmacopoeia of India, Part-I, Vol-VIII. Ministry of Health and Family Welfare, Government of India, New Delhi, pp. 101-113.

Calabrese, C., Berman, S.H., Babish, J.G., Ma, X., Shinto, L., Dorr, M., Wells, K., Wenner, C.A., Standish, L.J., 2000. A phase I trial of andrographolide in HIV positive patients and normal volunteers. Phytother. Res. 14, 333–338. https://doi.org/10.1002/1099-1573(200008)14. :5<333::AID-PTR584>3.0.CO;2-D.

Chen, H., Ma, Y.-B., Huang, X.-Y., Geng, C.-A., Zhao, Y., Wang, L.-J., Guo, R.-H., Liang, W.-J., Zhang, X.-M., Chen, J.-J., 2014. Synthesis, structure–activity relationships and biological evaluation of dehydroandrographolide and andrographolide derivatives as novel anti-Hepatitis B virus agents. Bioorg. Med. Chem. Lett. 24, 2353–2359. https://doi.org/10.1016/j.bmcl.2014.03.060.

Coon, J.T., Ernst, E., 2004. *Andrographis paniculata* in the treatment of upper respiratory tract infections: a systematic review of safety and efficacy. Planta Med. 70, 293–298. https://doi.org/10.1055/s-2004-818938.

Dean, A., Sullivan, K., Soe, M., 2019. OpenEpi: open source epidemiologic statistics for public health, version 3.01. Updated April 6, 2013 https://www.openepi.com/SampleSize/SSCohort.htm.

Enmozhi, S.K., Raja, K., Sebastine, I., Joseph, J., 2021. Andrographolide as a potential inhibitor of SARS-CoV-2 main protease: an *in-silico* approach. J. Biomol. Struct. Dyn. 39, 3092–3098. https://doi.org/10.1080/07391102.2020.1760136.

Fleiss, J.L., Levin, B., Paik, M.C., 2013. Statistical Methods For Rates And Proportions. Formulas 3.18 and 3.19, 3rd ed. John Wiley & Sons Inc Publication, USA.

Gupta, A., Vedula, S., Srivastava, R., Tamoli, S., Mundhe, N., Wagh, D., Batra, S., Patil, M., Pawar, H.B., Rai, R.K., 2021. Prospective, randomized, open-label, blinded end point, two-arm, comparative clinical study to evaluate the efficacy and safety of a fixed *Ayurvedic* regimen (FAR) as add-on to conventional treatment in the management of mild and moderate COVID-19 patients. J. Pharm. Bioallied Sci. 13, 256. https://doi.org/10.4103/jpbs.jpbs_242_21.

Gupta, K.K., Khan, M.A., Singh, S.K., 2020. Constitutive inflammatory cytokine storm: a major threat to human health. J. Interferon Cytokine Res. 40, 19–23.

Herold, T., Jurinovic, V., Arnreich, C., Lipworth, B.J., Hellmuth, J.C., von Bergwelt-Baildon, M., Klein, M., Weinberger, T., 2020. Elevated levels of IL-6 and CRP predict the need for mechanical ventilation in COVID-19. J. Allergy Clin. Immunol. 146, 128–136. https://doi.org/10.1016/j.jaci.2020.05.008 e124.

Hu, X.-Y., Wu, R.-H., Logue, M., Blondel, C., Lai, L.Y.W., Stuart, B., Flower, A., Fei, Y.-T., Moore, M., Shepherd, J., 2017. *Andrographis paniculata* (Chuān Xīn Lián) for symptomatic relief of acute respiratory tract infections in adults and children: A systematic review and meta-analysis. PloS One 12, e0181780. https://doi.org/10.1371/journal.pone.0181780.

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., Zhang, L., Fan, G., Xu, J., Gu, X., 2020. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 395, 497–506. https://doi.org/10.1016/S0140-6736(20)30183-5.

Jain, V.K., Iyengar, K.P., Vaishya, R., 2021. Differences between first wave and second wave of COVID-19 in India. Diabetol. Metab. Syndr. 15, 1047–1048. https://doi.org/10.1016/j.dsx.2021.05.009.

Kaushik, S., Dar, L., Kaushik, S., Yadav, J.P., 2021. Identification and characterization of new potent inhibitors of dengue virus NS5 proteinase from *Andrographis paniculata* supercritical extracts on in animal cell culture and *in-silico* approaches. J. Ethnopharmacol. 267, 113541.10.1016/j.jep.2020.113541.

Kim, J.H., Marks, F., Clemens, J.D., 2021. Looking beyond COVID-19 vaccine phase 3 trials. Nat. Med. 27, 205–211. https://doi.org/10.1038/s41591-021-01230-y.

Liu, T., Zhang, J., Yang, Y., Ma, H., Li, Z., Zhang, J., Cheng, J., Zhang, X., Zhao, Y., Xia, Z., Zhang, L., Wu, G., Yi, J., 2020. The role of interleukin-6 in monitoring severe case of coronavirus disease 2019. EMBO Mol. Med. 12, e12421. https://doi.org/10.1101/2020.03.01.20029769.

Premnath, V., 2020. Task force on repurposing of drugs for COVID-19: charting the path for India. Coordinated by venture center and CSIR-NCL, Pune. https://nclinnovations.org/covid19/resources/Access on 25/02/2022.

Saxena, R., Singh, R., Kumar, P., Yadav, S., Negi, M., Saxena, V., Joshua, A., Vijayabalaji, V., Goudar, K., Venkateshwarlu, K., 2010. A randomized double blind placebo controlled clinical evaluation of extract of *Andrographis paniculata* (KalmCold™) in patients with uncomplicated upper respiratory tract infection. Phytomedicine 17, 178–185. https://doi.org/10.1016/j.phymed.2009.12.001.

Seifert, G., Jeitler, M., Stange, R., Michalsen, A., Cramer, H., Brinkhaus, B., Esch, T., Kerckhoff, A., Paul, A., Teut, M., Ghadjar, P., Langhorst, J., Häupl, T., Murthy, V., Kessler, C.S., 2020. The relevance of complementary and integrative medicine in the COVID-19 pandemic: a qualitative review of the literature. Front. Med. 7. https://doi.org/10.3389/fmed.2020.587749.

Sharifpour, M., Rangaraju, S., Liu, M., Alabyad, D., Nahab, F.B., Creel-Bulos, C.M., Jabaley, C.S., 2020. C-Reactive protein as a prognostic indicator in hospitalized patients with COVID-19. PloS One 15, e0242400. https://doi.org/10.1371/journal.pone.0242400.

Srikanth, L., Sarma, P.V.G.K., 2021. Andrographolide binds to spike glycoprotein and RNA-dependent RNA polymerase (NSP12) of SARS-CoV-2 by in silico approach: a probable molecule in the development of anti-coronaviral drug. J. Genet. Eng. Biotechnol. 19 https://doi.org/10.1186/s43141-021-00201-7.

United-Nations, 2022. Global perspective human stories. United Nations News- 22 May 2022 Health https://news.un.org/en/story/2022/05/1118752 accessed on 06 June 2022.

Valecha, N., Devi, C.U., Joshi, H., Shahi, V., Sharma, V., Lal, S., 2000. Comparative efficacy of AYUSH-64 vs chloroquine in vivax malaria. Curr. Sci. 1120–1122. https://www.jstor.org/stable/24103624.

Wanjarkhedkar, P., Sarade, G., Purandare, B., Kelkar, D., 2022. A prospective clinical study of an *Ayurveda* regimen in COVID 19 patients. J. Ayurveda Integr. Med. 13, 100365 https://doi.org/10.1016/j.jaim.2020.10.008.

WHO, 2021. R&D blueprint novel coronavirus COVID-19 therapeutic trial synopsis, Word Health Organization, Geneva, Switzerland. https://www.who.int/teams/blueprint/covid-19.

Yiu, H.H., Graham, A.L., Stengel, R.F., 2012. Dynamics of a cytokine storm. PLoS One 7, e45027. https://doi.org/10.1371/journal.pone.0045027.

Zhou, Y., Fu, B., Zheng, X., Wang, D., Zhao, C., Sun, R., Tian, Z., Xu, X., Wei, H., 2020. Aberrant pathogenic GM-CSF+ T cells and inflammatory CD14+ CD16+ monocytes in severe pulmonary syndrome patients of a new coronavirus. bioRxiv. https://doi.org/10.1101/2020.02.12.945576.