RESEARCH ARTICLE

The Combination of *Gardenia jasminoides*, *Boswellia serrata*, *Commiphora myrrha*, *Foeniculum vulgarae*, and *Daucus carota* Essential Oil Blend Improved the Inflammatory and Clinical Status in Respiratory Tract Infection of COVID-19 Patients: A Multicentre, Randomized, Open-label, Controlled Trial

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Abstract

BACKGROUND: Essential oils (EO) are complex volatile, naturally synthesized compounds from aromatic plants. It considers as healthy, effective, and safe since they were coming from nature. *Gardenia jasminoides*, *Boswellia serrata*, *Commiphora myrrha*, *Foeniculum vulgarae*, and *Daucus carota* are known to have antimicrobials, antioxidants, antiinflammation properties against respiratory tract infection. However, despite its natural content, a safety profile needs to be observed. Therefore, in this study, EO blend (EOB) made from the combination of these 5 plants was assessed for its efficacy and safety for respiratory tract infection in human.

METHODS: A multicentre, randomized, open-label, phase II controlled trial involving 80 hospitalized adults with COVID-19 was conducted. One group of subjects only received standard of care (SoC), while the other group receive SoC and EOB orally for 10 days.

RESULTS: There were significant decrease in interleukin (IL)-6 level (p=0.016) and interferon (IFN)-γ level (p=0.012), as well as better respiratory rate (p=0.024) for the group receiving SoC and EOB compared to the group receiving SoC only. However, there was no significant differences in aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, and the corrected QT interval (QTc) prolongation value in both groups. All subjects with adverse effects were improved and recovered, and there were no serious adverse events found.

CONCLUSION: The combination of *G. jasminoides*, *B. serrata*, *C. myrrha*, *F. vulgarae*, and *D. carota* EOB could improve the inflammatory and clinical status and safe to be used as adjuvant therapy for treating COVID-19 in adults.

KEYWORDS: essential oils, COVID-19, inflammation, safety

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Introduction

Respiratory tract infection (RTI) constitutes a substantial disease burden in adults and elderly individuals. As much as 151 million RTI incidences per year were recorded in Indonesia.(1) In the last decades, the interest of back to nature has been increased in healthcare industries, due to the efficacy and safety reasons of synthetic drugs, for example, the carcinogenic effects, acute toxicity, and environmental hazard potential.(2) Natural medicine also get easier to be accepted in developed countries due to its affordability, accessibility and cultural acceptability. The natural compounds extracted from plants and herbs are considered more healthy, effective, and safe since they were coming from nature. The extrinsic and intrinsic factors of the plant itself can cause variation in phytochemical constituents and may affect the efficacy and safety of the medicine.(3)

Essential oils (EO) are complex volatile, naturally synthesized compounds from aromatic plants. EO are usually characterized by 2-3 major components and some other components.(4) A lot of study found that EO have pharmacological actions not limited to antiinflammation, antioxidant or carminative effects but also can perform as antimicrobial or antifungal.(2) Due to their lipophilic features, the bioactive components in EO can easily pass the barriers in our body with good absorption either through oral, pulmonary, or cutaneous pathways. EO usually present in its full natural form and need to use in higher concentrations. Many studies have demonstrated that many traditional treatments show a synergistic effect by acting at different levels on multiple targets and pathways. The combination of EO commonly shows a higher antiviral activity than single compound although the mechanism of action for each EO are different.(5) Treatment using EO blends can significantly attenuates influenza virus PR8 infectivity by inhibit the viral NP and NS1 protein.(6) Various EO contain phenols, terpenes, to have the antiviral activity by interfering with virion envelopment and inhibit the virus entry into host cells, or by directly interacting with the virus particles.(7)

Since March 2020, COVID-19 has become a global pandemic. COVID-19 virus mainly affects the respiratory system and causing hyperinflammation with features of cytokine storm syndrome (CSS).(8) Infected people may or may not have symptoms such as fever, myalgia, headache and cough. In worse condition, especially elderly or individuals with chronic diseases, it can develop into pneumonia.(9)

Despite of the vaccine and antiviral therapies, millions of people globally are experiencing a prolonged symptom profile especially on respiratory system that is reported to last months and even years.(10) A lot of traditional medicines were then used to manage the symptoms but not all have been identified for the safety profiles.(11) Recent studies showed a better approach to fight RTI by combining the trinity of antimicrobials, and immunity, and anti-inflammatory properties.(12) Thus, EOs from formulation of some potential herbal medicine were made to enhance the protection against viral infections in human, especially through its antimicrobial, anti-inflammatory, antioxidant, and immunomodulatory activities. Even though most of the EO products were recommended to be safe and only used as additive agents, however the safety and toxicity issues cannot be ignored.(13)

Many studies have shown the efficacies and benefits of *Gardenia jasminoides, Boswellia serrata, Commiphora myrrha, Foeniculum vulgarae*, and *Daucus carota* both extracts and EO as antimicrobials, antioxidants, antiinflammation, etc (14), as well its safety profile in animal with acute hepatopancreatic necrosis disease and white spot syndrome virus (15,16). Somehow, only a limited number of studies had revealed the efficacy and safety of EO, especially in human.

Several *in vitro* and *in vivo* studies have been conducted earlier to confirm the potential of the combination of *G. jasminoides*, *B. serrata*, *C. myrrha*, *F. vulgarae*, and *D. carota* EO blend (EOB). Our previous study showed that at its minimum concentration up to 0.01 mL of 0.1%, the EOB was effective against the avian infectious bronchitis virus (AIBV) H120 strain. The EOB concentration lower than 0.1% was not sufficient to deactivate the AIBV H120 strain. The embryos up to 0.1% of EOB treated groups and negative control embryos were alive and tested negative to AIBV H120 strain, whereas the embryos in positive control died and tested positive.(17) The anti-inflammation of the EOB has been confirmed by the suppression of the production of interleukin (IL)-1β, tumor necrosis factor (TNF)-α, IL-6 and interferon (IFN)-γ in a dose-dependent manner by lipopolysaccharide (LPS)-stimulated single human nucleus cells. The production of anti-inflammatory IL-10 by cells equipped with LPS is also suppressed in a dose-dependent manner.(2) Whilst with quantitative flow cytometry, it appears that the EOB effectively reduces polymorphonuclear neutrophil chemotactic peptides in human N-formyl-Met-Leu-Phe (fMLP), human peripheral blood (fMLP), phorbol 12-myristate 13-acetate (PMA), and oxidative explosions induced by *Escherichia coli.*

Furthermore, the anti-oxidant activity of the EOB was confirmed *in vitro*, which examined the cleaning activity of DPPH free radical scavenging. The EOB interacts with DPPH through the transfer of electrons or hydrogen, by neutralizing its free radical character. At last, they has also shown its antiviral potential in *in vivo* and *in vitro* studies. In the in *in vivo* and *in vitro* studies using African swine fever virus (ASFV), treatment with the EO has effectively shown the inhibition of ASFV (16), inactivate infectious bronchitis virus (IBV) H120 virus, as showed by the live-ability of embryos in treatment groups inoculated with mixture of IBV virus and the EOB. The IBV is genetically similar to the SARS-CoV-2 virus and it uses the same mechanism for infection.(18) This was the first study to observe the mixture of *G. jasminoides, B. serrata, C. myrrha, F. vulgarae*, and *D. carota* EOB in human especially in the case of respiratory viral infection. In this clinical trial, the efficacy and safety profile of the EOB was evaluated to assessed its efficacy and safety for subject with RTI on hospitalized adults with mild to moderate levels of COVID-19.

Methods

Subject Recruitment

This was a multicenter, randomized, open-label, controlled trial to evaluate the effectiveness and safety of *G. jasminoides, B. serrata, C. myrrha, F. vulgarae*, and *D. carota* EOB in hospitalized adults with COVID-19. Eighty subjects, aged more than 18 to 50 years old, were recruited from in-patients at Rumah Sakit Darurat Penanganan COVID-19 Wisma Atlet Kemayoran, Jakarta, and Dr. Hasan Sadikin Central General Hospital, Bandung. Subjects were diagnosed with mild to moderate COVID19 (confirmed by positive polymerase chain reaction test (PCR) results) based on COVID-19 Treatment Protocol Ed. 1, 2020.(9)

All subjects have provided consent and have agreed to not participate in other study and not using any other immunomodulator or treatment that might bias the study. Subjects with any allergy to any composition of EOB; was pregnant or breastfeeding; and has retinopathy, cardiovascular diseases, diabetes, hypertension, renal dysfunction, liver impairment, chronic pulmonary disease, dementia and Alzheimer, autoimmunity, cancer, human immunodeficiency virus (HIV) and AIDS were excluded. The protocol of this study was approved by Universitas Padjadjaran Research Ethic Committee (No. 107/UN6. KEP/EC/2020) and registered with ClinicalTrials.gov (NCT04627519).

Subjects Randomization

Subjects were randomized 1:1 into Standard of Care (SoC) alone and SoC with EOB group (SoC+EOB). Randomization was be provided centrally and performed by an appointed independent statistician. Binomial randomization concept with following formula was used:

$$P(x) = \binom{n}{x} p^x q^{(n\ x)} = \frac{n!}{x!(n\ \ x)!} p^x q^{(n\ x)}$$

In which, n was number of sample and x was number of treatments, p was opportunity of treatment 1, and q (1- p) was opportunity of treatment 0. Computation from binomial randomization used R software (*https://www.r-project.org/*) by using equal allocation for treatment 1 and 0, which is 50%. Randomization list generated was inputted into RedCap application and randomization code for each subject would be issued after subjects were confirmed to be eligible to be randomized. RedCap was accessed by researcher in both study sites to obtain the randomization code and the recruitment was competitive among sites. In this study, the statistician only calculated the equal allocation based on treatment (SoC and SoC+EOB), while the subject criteria with mild or moderate would be only intended for inclusion and exclusion criteria during subject screening process. Therefore, the analysis process would be carried out based on the two treatments without comparing the effectiveness of EOB between different severity levels.

EOB Formulation

The EOB used in this study was Rhea Health Tone® (PT Rhea Natural Sciences, Tangerang, Indonesia), a natural herbal oil formulation consisting of EO derived from 5 various herbs. Each mL of the EOB contains 1.8 mg *G. jasminoides*, 1.8 mg *B. serrata*, 1.8 mg *C. myrrha*, 1.8 mg *F. vulgarae*, 1.8 mg *D. carota*, and 0.99 mg of *Olea europaea* oil as solvent (13). Each EO is obtained through a steam distillation process and should undergo thorough checking for the quality and chemical compositions.(17) The formation of EOB used in this study was already reported in previous study, and efficacy of the EOB was already tested against the AIBV H120 strain.(17) These five potential EO mostly have phenolic compounds to enhance the protection against viral infections in human, especially through the antimicrobial, anti-inflammatory, antioxidant, and immunomodulatory activities.

Subject Intervention

Subject's hospitalization duration was counted as the total days of subject stay in hospital, monitored up to 14 days.

Subjects were randomly divided into two groups, one group received SoC alone for 10 days, and the other group received SoC and 2 mL EOB every 12 hours after meal for 10 days. SoC was given based on COVID-19 Treatment Protocol.(3)

PCR Conversion

The PCR conversion defined as total days from confirmed positive by rRT-PCR to the first negative PCR result; PCR testing was performed on day-0, day-7, and day-10. PerkinElmer SARS-CoV-2 Realtime RT-CR Assay Kit (PerkinElmer, Waltham, MA, USA) was used to qualitatively detect SARS-CoV-2 nucleic acid from oropharyngeal and nasopharyngeal swab sample, targeting on nucleocapsid (N) and ORF1ab SARS-CoV-2 genes. PerkinElmer Nucleic Acid Extraction Kit (Cat. No. KN0212; PerkinElmer) through Pre-NAT II Automated Workstation system. The extract was processed in QuantStudioTM 5 Real-Time PCR System instrument using PerkinElmer® SARS-CoV-2 Nucleic Acid Detection Kit (Cat. No. 2019-nCoV-PCR-AUS; PerkinElmer). The detection limit was 9.3 to 30.5 copies/mL.

Clinical Status

Subjects clinical status were measured from total days of oxygenation, total days to receive ventilation, temperature, heart rate, respiratory rate, blood pressure, oxygen saturation, capillary filling time> 2 seconds, and percentage of subjects with reduced infiltrate based on chest X-ray result. Based on WHO, the clinical status was defined as improved after monitored on 3 consecutive days.(8)

Inflammatory Biomarkers Assessment

The inflammatory biomarkers, including serum IL-6, high-sensitivity C-reactive protein (hs-CRP) and IFN-γ level, were measured on day-0 and day-10. Serum IL-6 was measured by enzyme-linked immunosorbent assay (ELISA) Human IL-6 (Cat. No. D6050; R&D Systems, Minneapolis, USA) on Microplate Reader Bio-Rad Model 680 (Bio-Rad Laboratories, Hercules, CA, USA). The hs-CRP was measured on Abbott Architect CRP Vario (Cat. No. 6K26-30 and 6K26-41, Abbott Laboratories, Chicago, IL, USA). IFN-γ was measured by Human IFN-γ HS (Cat. No. BMS228HS; Bender MedSystems GmbH, Vienna, Austria) on Microplate Reader Bio-Rad model 680 (Bio-Rad Laboratories).

AST, ALT, Creatinine, and QTc Prolongation Assessment

AST and ALT were measured by International Federation of Clinical Chemistry (IFCC) with AST and ALT Reagent Kit (Abbott Laboratories), while the creatinine was measured by Jaffe Endpoint with Creatinine Kit Reagent (Abbott Laboratories). QTc prolongation was defined as the incidence of prolongation in the QT interval based on electrocardiogram (ECG). The QT interval was defined as the onset time of the QRS complex to the end of the T wave. Abnormal ECG included abnormal heart rate, irregular rhythm, abnormal waveforms or abnormal intervals.

Safety Endpoints

For adverse effects (AEs) and adverse drug reactions (ADRs) during the treatment period, the frequency was tabulated by the treatment group for the site of onset and its total. Similar tabulations were performed by severity and time of onset. In tabulation for the time of onset, time of the event occurred after the last administration were transposed to the duration of treatment, and proportion of subjects with AEs were calculated by using the number of subjects available at each time point as the denominator. Additionally, each frequency of AEs and ADRs were compared between the groups by $\chi 2$ test for the site of onset and its total. For laboratory test values and physical examination values, descriptive statistics (excluding Q1 and Q3) and changes from baseline (values after treatment – values at baseline) of continuous data were calculated for each treatment group and analysed by paired t-test group. Discrete data were tabulated by treatment group and analysed by McNemar test for normal/outliers.

Statistical Analysis

SPSS software v.22 (IBM Corporation, Armonk, NY, USA) was used for all statistical analysis. Two-sided hypothesis testing was performed with $p<0.05$ considered as significant. The general data description was presented in frequency, minimum, maximum, mean±SD, and median value. Comparisons between both groups' mean (for normally distributed variables) were conducted using two independent sample t-test, and paired test, while for the median comparisons (for not normally distributed variables) were conducted using non-parametric Mann-Whitney two independent sample test. A Chi-square test was later performed to compare variables with categorical data.

Results

The safety evaluation in this study was assessed by changes in AST, ALT, creatinine, QTc prolongation by ECG, and the number of reported AE or serious adverse events (SAE).

Subject Characteristics

There were total 80 subjects involved in this study, which were divided randomly into 2 groups: SoC (40 subjects) and the SoC+EOB (40 subjects) (Table 1). There were no clinical characteristics differences between both groups before the treatment. The SoC+EOB group had more subjects with moderate symptoms compared to SoC group (13 *vs.* 7), while SoC had more subjects with mild sympoms compared to SoC+EOB (33 *vs.* 27).

Subject Hospitalization Duration

There were no differences of hospitalization duration between both group in this study, despite the SoC+EOB group had more subjects with moderate symptoms. Subjects in SoC group were hospitalized for 11.55±5.46 days, and the SoC+EOB group 10.58±1.93 days (p=0.290). Cross tabulation data counting showed that the SoC+EOB groups had 38 subjects discharged from the hospital less than 14 days, compared to 34 subjects in SoC group.

Table 1. Subject's baseline demographic and clinical characteristics.

Variables	Total (n=80)		SoC (n=40)		SoC+EOB (n=40)		*p* -value
	n (%)	Mean±SD	n (%)	Mean±SD	n (%)	Mean±SD	
Gender							
Male	40 (50)		23 (57.5)		17 (42.5)		0.180
Female	40 (50)		17 (42.5)		23 (57.5)		
Age (years old)		30.79±8.46		31.50±8.50		30.10±8.50	0.455
Body weight (kg)		63.85±12.26		64.40±12.20		63.30±12.40	0.691
Body height (cm)		164.50±7.06		164.90±6.10		164.20±8.00	0.660
Covid severity							
Mild	60 (75)		33 (82.5)		27 (67.5)		0.121
Moderate	20 (25)		7 (17.5)		13 (32.5)		
PCR (CT)		34.06±3.80		34.06±3.80		34.06±3.87	1.000
ECG Qtc ND (ms)		400.6±36.38		398.53±36.82		402.85±36.28	0.598
External respiration							
Oxygenation	2 (2.5)		1 (2.5)		1 (2.5)		1.000
Ventilation	0 (0)		0 (0)		0 (0)		
Pulmonary infiltration	28 (35)		11 (27.5)		17 (42.5)		0.165
Laboratory parameters							
Haemoglobin (g/dL)		14.55±4.39		14.38±1.78		14.72±5.99	0.734
Haematocrit (%)		42.40±7.25		43.76±7.52		41.04±6.78	0.093
WBC Count (x10^3/µL)		6.19±1.82		6.15±1.84		6.22±1.83	0.851
RBC Count (x10^6/µL)		5.06±0.58		5.13±0.53		4.98±0.62	0.250
Plateletes (x10^3/µL)		266.00±80.82		263.78±90.16		268.20±71.37	0.808
Creatinine (mg/dL)		0.83±0.24		0.84±0.25		0.83±0.24	0.856
ALT/ALT (U/L)		31.15±19.16		32.10±21.25		30.20±17.04	0.660
AST/AST (U/L)		24.55±10.86		24.55±9.28		24.55±12.36	1.000
Neutrophil (%)		55.96±9.09		56.60±8.56		55.32±9.66	0.531
Lymphocyte (%)		33.45±8.34		32.14±8.35		34.77±8.21	0.159
Monocyte (%)		8.58±3.08		8.74±2.90		8.42±3.28	0.639
Eosinophil (%)		1.90±1.87		2.08±1.85		1.72±3.28	0.395
Basophil (%)		0.27±0.25		0.25±0.24		0.29±0.25	0.418
hs-CRP (mg/L)		4.72±8.34		4.79±9.37		4.64±7.29	0.935
Clinical status							
Temperature (°C)		36.50±0.27		36.49±0.23		36.54±0.31	0.415
Heart rate (bpm)		84.90±6.93		85.10±7.54		84.63±6.37	0.762
Respiratory rate (brpm)		19.90±1.09		19.90±1.10		19.80±1.09	0.685
Systolic (mmHg)		116.90±9.84		116.85±8.97		116.85±10.77	1.000
Diastolic (mmHg)		79.40±6.69		79.65±7.40		79.10±5.98	0.716
Oxygen saturation (%)		97.60±0.83		97.58±0.84		97.65±0.83	0.690

p<0.05 is considered as significant, analyzed with Chi-square analysis for categorical data, and independent t-test for normally distributed numerical data, and Kruskal Wallis analysis for not normally distributed numerical data. bpm: beats per minute; brpm: breaths per minute.

PCR Conversion Results

This variable was determined from PCR value conversion based on viral load (CT value) on day-7 and day-10. There were more subjects with negative PCR conversion in the SoC+EOB group compared to SoC alone both in day-7 (12 *vs.* 10) and day-10 (8 *vs.* 7) although not statistically significant (p=0.666 and p=0.951, consecutively). After day-10, we did not continue the observation on the subjects for PCR conversion.

Clinical Status Improvement

There was no significant differences for both group except for the respiratory rate, which showed that SoC+EOB group improved better respiratory rate compared to SoC alone (p=0.024), suggests that the EOB supplementation might improved the respiratory system via its anti-inflammatory effect (Table 2).

Another clinical status observed was the duration of finger capillary filling time to confirm the presence of circulatory shock when the filling time was more than 2 seconds. There was no subject with circulatory shock in this study (refill time >2 secs).

Inflammatory Biomarkers

The level of subjects' inflammatory biomarkers were measured on day-1 and on day-10 (or discharge), and then the results were compared. Paired difference analysis results of the subjects' inflammatory biomarkers analysis showed a significant decreased in IL-6 and IFN-γ level for the EOB group (p=0.016 and p=0.012, respectively), but not in the hs-CRP level.

Pulmonary Infiltration

Subjects' pulmonary infiltration improvement was assessed by thorax X-Ray. On the first day of subjects' admission, there were 11 subjects in SoC group with bilateral (6 subjects) and unilateral (5 subjects) pulmonary infiltration, and 17 subjects in the SoC+EOB group with bilateral (14 subjects) and unilateral (3 subjects) pulmonary infiltration. On day-10, there were 2 subjects with unilateral pulmonary infiltration in SoC group (2 subjects were not recorded) and 4 subjects with bilateral (3 subjects) and unilateral (1 subject) pulmonary infiltration in the SoC+EOB group. There were more subjects who recovered in the EOB group compare to SoC group (13 *vs.* 7), although the difference was not statistically significant (p=0.823).

AST, ALT, and Creatinine Level Evaluation

There were no significant differences of AST, ALT, and creatinine levels between both groups, which means that the supplementation showed no significant impact on AST, ALT, and creatinine levels of the subjects (Table 4).

QTc Prolongation Evaluation

QTc prolongation was determined by ECG examination and QTc (ms) prolongation value. On day-9, all subjects in both

Table 2. Subjects clinical status from day-1 to day-10/discharge.

Variables		Mean±SD		*p* -value
		SoC (n=40)	SoC+EOB (n=40)	
Temperature (°C)	Baseline (Day 1)	36.49±0.23	36.54±0.31	0.415
	Day10/Discharge	31.83±12.19	31.87±12.20	0.987
	Δ Day 1 to day 10	-0.21±0.48	-0.20±0.54	0.879
Heart rate (bpm)	Baseline (Day 1)	85.10±7.54	84.63±6.37	0.762
	Day10/Discharge	73.05±29.02	72.70±28.56	0.957
	Δ Day 1 to day 10	-3.43±14.37	-5.33±8.89	0.479
Respiratory rate (brpm)	Baseline (Day 1)	19.90±1.10	19.80±1.09	0.685
	Day10/Discharge	20.03±1.10	19.66±0.87	0.024*
	Δ Day 1 to day 10	0.17±0.98	-0.31±1.35	0.089
Systolic (mmHg)	Baseline (Day 1)	116.85±8.97	116.85±10.77	1.000
	Day10/Discharge	118.40±9.21	118.46±10.17	0.980
	Δ Day 1 to day 10	-1.51±11.18	-1.91±11.95	0.885
Diastolic (mmHg)	Baseline (Day 1)	79.65±7.40	79.10±5.98	0.716
	Day10/Discharge	80.09±7.45	80.80±6.69	0.674
	Δ Day 1 to day 10	1.37±11.08	1.43±10.79	0.983
Oxygen saturation (%)	Baseline (Day 1)	97.58±0.84	97.65±0.83	0.690
	Day10/Discharge	98.46±0.61	98.66±0.54	0.151
	Δ Day 1 to day 10	0.34±1.37	0.51±1.09	0.565

p<0.05 is considered as significant.

Table 3. Paired difference test of IL-6, IFN-γ, dan hs-CRP.

Groups	Variables			n	mean±SD	Δ Mean	*p*- value
SoC	Pair 1	IL-6 (pg/mL)	Day-1	39	3.86±2.73	-0.427	0.497
			Day-10/Discharge	39	3.43±2.92		
	Pair 2	IFN-γ (pg/mL)	Day-1	39	0.60±0.94	-0.217	0.204
			Day-10/Discharge	39	0.39±0.46		
	Pair 3	Hs-CRP (mg/L)	Day-1	40	4.79±9.37	-2.035	0.197
			Day-10/Discharge	40	2.76±4.63		
SOC+EOB	Pair 1	IL-6 (pg/mL)	Day-1	39	4.50±3.96	-1.594	0.016*
			Day-10/Discharge	39	2.90±1.24		
	Pair 2	IFN-γ (pg/mL)	Day-1	39	1.04±1.78	-0.661	0.012*
			Day-10/discharge	39	0.38±0.45		
	Pair 3	Hs-CRP (mg/L)	Day-1	40	4.64±7.29	-1.931	0.076
			Day-10/Discharge	40	2.71±3.87		

*p<0.05 is considered as significant.

groups showed normal ECG results, and no differences of QTc prolongation values between both groups (p=0.835) (Table 5).

AE or SAE Reported Number

AE and SAE were assessed for AE or SAE name, onset, severity, seriousness, and actions taken for the test products. AEs recorded were including nausea (5 subjects), vomiting (3 subjects), diarrhea (3 subjects), myalgia (2 subjects), insomnia (2 subjects), weakness (1 subject), chest pain (1 subject), rash (1 subject), and abnormality in safety profile biomarkers (9 subjects). All subjects with AEs improved and recovered in both groups without any additional treatments. There were no significant differences in AE between both groups, and no SAE was found in this study.

Discussion

The EOB recommended dose is 2 mL twice daily after the meal, 2 hours before the SoC, avoid eating and drinking 15 minutes before and after administration. Upon long-term delivery of oral EOB, the exposure of bioactive compounds can be cumulative, and the safety should be determined.(19) Among the bioactive ingredients in the EOB, most of them are classified as terpenes, flavonoids, phenolic compounds, and resins. Most of plant families rich in monoterpenes are safe as long as they were taken in an adequate dose. Despite of coming from natural ingredients, EO can also be harmful to humans, especially to induce hepatotoxicity and digestive issues.(19)

Subjects in our study show similar demographic and clinical characteristics, means we can assume the same starting points for all subjects, despite that the SoC+EOB group has more subjects with moderate symptoms rather than mild.

The hospitalization duration showed no differences between both groups. Many factors affect the parameter including regulation at the time this study was conducted, and administrative provision which did not let the subject to be discharged before 14 days despite of the PCR value or clinical status.

Our observation on day-7 and day-10 showed that there were more subjects having negative PCR results compared to SoC. This showed that the combination EO supplementation modulate the subjects' immune system to reduce the viral load. The 5 compositions of the EOB are loaded with high and diverse phenolic compound. Polyphenols were known to have a strong antimicrobial and immunomodulatory effect.(20)

Geniposides, the major iridoid glycoside ingredient of gardenia herbs, has demonstrated a multifunctional tissue-protective agent with anti-edematous and anti-inflammatory activities against respiratory tract viral infection by suppressing the phosphorylation of p38, JNK and nuclear factor kappaB (NF-κB), thus inhibit TNF-α, IFN-γ, and IL-6 to start an inflammatory cascade which can lead to a wide range of tissue damage. It also has been proven to down-regulate the expression of cleaved-caspase 3 and reduce its activation which is essential for efficient influenza virus propagation.

The flavonoids contained in *G. jasminoides* have been examined for the antioxidant activity by inhibiting nitric oxide (NO) production via inducible nitric oxide synthase (iNOS).(21) Volatile constituents of *B. serrata* showed an antiviral effect due to inhibition of influenza viral protein

Table 4. AST, ALT, and creatinine values for SoC and SoC+EOB groups.

Variables		Mean±SD		*p*-value
		SoC (n=40)	SoC+EOB (n=40)	
Creatinine (mg/dL)	Day-1	0.84±0.24	0.83±0.23	0.856
	Day 10/Discharge	0.78±0.19	0.76±0.21	0.636
	Δ Day 1 to day 10	-0.06±0.19	-0.07±0.21	0.796
ALT (U/L)	Day-1	32.10±21.25	30.20±17.04	0.660
	Day 10/Discharge	59.83±39.74	45.80±33.59	0.092
	Δ Day 1 to day 10	27.72±37.81	15.60±31.07	0.121
AST (U/L)	Day-1	24.55±9.28	24.55±12.36	1.000
	Day 10/Discharge	37.90±33.49	29.50±14.30	0.149
	Δ Day 1 to day 10	13.35±33.82	4.95±18.50	0.172

$p<0.05$ is considered as significant.

translation *in vitro*. The triterpenoids present in *B. serrata* have been reported to possess diverse biological activities via NF-κB pathway, that include immunostimulant, antimicrobial, anti-inflammatory, anti-cancer and antiviral properties.(23) The phenolic compounds of *C. myrrha* is believed to play an important role in quenching singlet and triplet oxygen or decomposing peroxides. *C. myrrha* also showed significant anti-inflammatory and analgesic activities.(22) The EO of fennel contains several bio reactive secondary metabolites, such as aldehydes, which effectively blocked the inflammatory processes, by regulating pro-inflammatory cytokine production, transcription factors, and NO.(23) *D. carota* oil showed a strong antioxidant and anti-inflammatory activity, also by NO scavenging mechanism partially attributed to the presence of phenolic compounds such as luteolin, kaempferol, apigenin, caffeic acid and quercetin. Its antimicrobial activity is most performed by the terpenes. Wild carrot also showed an immunomodulatory effect by inducing different patterns of cytokine release and enhancing production of IFN-γ in the spleens of mice.(24)

This study shows that the EOB has a strong effect in reducing inflammation especially IL-6 and IFN-γ level as shown in Table 3, as well as in subjects' clinical status assessment as shown in Table 2, including sore throat improvement. Stimulation of immune cells due to viral infection led to active inflammation through cytokine-mediated actions. EOB's sesquiterpenes suppress the excess secretion of IFN-γ by T-cell, and TNF-α, via NF-κB pathway, result in lower IL-6 level.(25) Strong inflammatory effect of the EOB furthermore improves the lung inflammation showed by improvements of pulmonary infiltration and respiratory rate in the subjects.

The hs-CRP level in this study showed no significant difference between both group (p=0.96). IL-6 acutely increased at the early phase of infection, and then induces synthesis of acute phase proteins such as CRP. Recovery of the infection soon reduces IL-6 activation by a negative regulatory system and continue by as degradation of IL-6 mRNA.(26) Thus IL-6 is the first to increase and decrease following the patient's status following by hs-CRP which was easily affected by many factors. In our study, the hs-CRP level on day-1 was 4.79 mg/L in SoC and 4.64 mg/L in the EOB group, then became 2.76 mg/L in SoC and 2.71 mg/L in the EOB group on day-10. This means that there was no more acute infection in both groups. Despite of the significant difference on respiratory rate, IL-6 and IFN-γ levels on day-10, it was not clinically significant since the level are still in the normal range. The EOB works works by suppressing the excess IL-6 and IFN-γ secretion by macrophages and other immune cells, thus improve the subjects' clinical status (Figure 1).

Table 5. QTc prolongation based on ECG examination for SoC and the EOB groups.

Day	ECG Examination (n)						QTc (ms)		*p*-value
	SoC (n=40)			SoC+EOB (n=40)			SoC (n=40)	SoC+EOB (n=40)	
	Normal	Abnormal	N/A	Normal	Abnormal	N/A	Mean±SD	Mean±SD	
Day-1	40	0	0	39	1	0	398.53±36.82	402.85±36.28	0.835
Day-3	37	3	0	34	5	1	388.68±54.69	402.82±35.41	
Day-6	35	4	1	39	1	0	404.74±37.28	407.90±32.15	
Day-9	35	0	5	40	0	0	401.17±36.16	407.78±44.10	

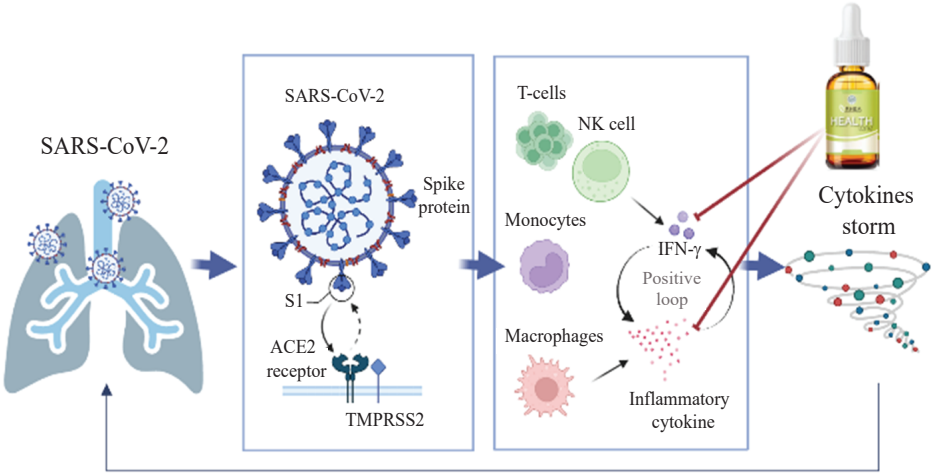

Figure 1. The EOB mostly works by suppressing the excess inflammatory cytokines (such as IL-6) and IFN-γ secretion by macrophages and other immune cells, thus improve the subjects' clinical status.

After 10 days of the EOB administration in this study, there was no significant difference between both groups in safety parameters as observed by the level of ALT, AST, and creatinine, the QTc prolongation value, and the number of AEs. This data showed the EOB administration did not induce any toxicity on the muscle, liver, and kidney function, as well as cardiovascular system. There was no SAE found in this study, and all AEs were recovered or improved.

G. jasminoides as an edible substance and has the low toxicity on the liver as observed by AST and ALT in mice.(21,27) The extract has shown to reduce liver injury caused by hepatic steatosis in rats fed with high fat diet and improve renal dysfunction.(22) There are no cytotoxic effects by taking *B. serrata* extract orally, and it was not lethal to animals up to 2000mg/kg/day when observed for 14 days and 600mg/kg/day when observed for 90 days.(28) A study using the combination of *B. serrata* and *C. myrrha* extract to rats per oral showed no acute or chronic toxicity for a period of two months on the metabolism, liver and kidney functions.(29) *In vitro* study of *F. vulgare* EO showed a safe profile for keratinocytes, hepatocytes and fibroblasts in concentrations up to 1.25 µL/mL (30), while study in mice exhibited no signs of toxicity or mortality up to the dose level of 3 g/kg body weight.(31) *D. carota* EO has long been used in food and pharmaceutical industry. An *in vitro* study on four mammalian cell lines (macrophages, keratinocytes, hepatocytes and microglia cells) showed no toxicity and should be safe for oral, topical or inhalation administration.(32)

Many natural metabolites include flavones, flavonols, fatty acids, tannins, terpenes, and alkaloids used to treat COVID-19 have low toxicity.(33) Some studies on terpenoid from plants and marine sponge also showed no mutagenicity, carcinogenicity, and toxicity in all aspects except immunotoxicity which was also observed in FDA approved drugs like paritaprevir, rilpivirine, glecaprevir, dolutegravir and grazoprevir.(34,35) Flavonoids have been used for a long time to treat respiratory tract infections. Flavonoids are also found to be beneficial to protect drug-induced toxicity in major organs (nephrotoxicity, hepatotoxicity, and cardiotoxicity) as evidenced in COVID-19 patients.(33,34,36) There are no signs of acute or chronic toxicity from several phytochemicals contain polyphenols.(37) There are also no toxicity toxic effects on blood parameters and vital organs function at therapeutic dose on herbal tea containing phenols and tannins.(38) Since, this study only evaluated the use of EOB in subjects with COVID-19, further research can be carried out to determine the efficacy and safety of EOB in RTI due to other bacteria or viruses.

Conclusion

The combination of *G. jasminoides*, *B. serrata*, *C. myrrha*, *F. vulgarae*, and *D. carota* EO can improve the inflammatory and clinical status and safe to be used as adjuvant therapy for treating respiratory tract infection due to COVID-19 infection in adults.

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Authors Contribution

KL and HB were involved in the research conception/design. IDK, FF, EI, YS, SS, PS, YH, and ARA were involved in the data acquisition. IPS and ES were involved in the data analysis. AM, IPS, and ES interpreted the results. KL, FF, YS, YH, ARA, and AM prepared the manuscript draft. KL and AM were involved in the figure and/or table design. KL, FF, YS, YH, ARA, AM, and IPS gave critical revision for the manuscript. All authors approved the final manuscript.

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