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Eucalyptus oil as adjuvant therapy for coronavirus disease 19 (covid-19): a randomized clinical trial

Arif Santoso[1*], Rasiha[2], Muthiah Nur Afifah[2], Munawir Muhammad[3], Andi Rofian Sultan[4], Muhammad Rezky Juni[2], Priady Wira Prasetia[2], Andreza[2], Idrianti Idrus[5], Andi Munawirah[6], Yuyun Widaningsih[6], Firdaus Hamid[4], Idrus A. Paturusi[7], M. Nasrum Massi[4], Asrul Abdul Azis[1], Niluh Putu Indi Dharmayanti[8], Harimurti Nuradji[8], Diana Nurjanah[9], Fadjry Djufry[9], Evie Safitri[9]

[1]Department of Pulmonology, Faculty of Medicine, Universitas Hasanuddin, Indonesia;
[2]Faculty of Medicine, Universitas Hasanuddin, Indonesia;
[3]Department of Pharmacology, Faculty of Medicine, Universitas Hasanuddin, Indonesia;
[4]Department of Microbiology, Faculty of Medicine, Universitas Hasanuddin, Indonesia;
[5]Department of Dermatovenereology, Faculty of Medicine, Universitas Hasanuddin, Indonesia;
[6]Department of Clinical Pathology, Faculty of Medicine, Universitas Hasanuddin, Indonesia;
[7]Department of Orthopaedic Surgery, Faculty of Medicine, Universitas Hasanuddin, Indonesia;
[8]Research Center for Veterinary Science, National Agency for Research and Innovation;
[9]Indonesian Agency for Agricultural Research and Development.

*Corresponding author:
Arif Santoso;
Department of Pulmonology, Faculty of Medicine, Universitas Hasanuddin, Indonesia;
arifs777@gmail.com

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ABSTRACT

Introduction: The coronavirus disease 19 (COVID-19) vaccination scheme is still being implemented worldwide, but problems with stock availability and other factors are still impeding this endeavor, especially in developing nations like Indonesia. Ethnopharmacology is known to provide a good source of a cheap candidate for anti-inflammation drugs. This study was conducted to determine the effectiveness of affordable adjuvant therapy, *Eucalyptus* oil, which has been used as a household remedy for respiratory tract infections in Indonesia.

Methods: 52 mild-moderate COVID-19 patients were randomly assigned for a prospective clinical trial. A group of 26 patients was given standard COVID-19 treatment as a control group, while another 26 patients as the treated group were given standard therapy and use *Eucalyptus* oil as an adjuvant. The clinical sign and symptoms, cytokines interleukin (IL) 6, IL-10, and transforming growth factor-beta (TGF-β) profiles were recorded during the study period.

Result: There was a trend of better improvement of clinical symptoms among patients who received adjuvant treatment than the control group. Altered smell ($p = 0.003$) and cough ($p = 0.641$) recovered faster in the treated group. While there was no marked difference in IL-10 and TGF-β levels among both groups, IL-6 level in the treated group was significantly lower and the neutrophil-to-lymphocyte ratio (NLR) was significantly decreased ($p < .05$) than the control.

Conclusion: Using *Eucalyptus* oil as an adjuvant of COVID-19 treatment promotes faster recovery of mild-moderate COVID-19 patients, probably via inhibition of the IL-6-mediated inflammation process.

Keywords: Adjuvant therapy, mild-moderate covid-19, *eucalyptus* oil, interleukin-6.
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INTRODUCTION

The coronavirus disease (COVID-19) vaccination program continues to be rolled out globally, but the stock limitation and other issues continue to hamper this global effort, particularly in developing countries, including Indonesia. Meanwhile, the need to provide effective and affordable treatments must be considered where vaccine rolling is slow. Up to today, around 400 drugs have been trialed for COVID-19 treatment, and only 4 of them were considered to have significant good results in COVID-19 treatment, namely baricitinib, remdesivir, dexamethasone, and tocilizumab.[1]

It has been known that the levels of the patient's interleukin 6 (IL-6) were correlated with COVID-19 severity.[2,3] The accumulation of inflammatory cells reflected this severity and sign of thrombosis and endothelial inflammation in the pulmonary vascular of COVID-19 patients. The potential role of IL-6 in these pulmonary inflammations provides a solid background for further evaluation of IL-6 signalling inhibitors.[4] Tocilizumab, an immunosuppressant, is a monoclonal antibody against IL-6 receptor-alpha used previously to treat other inflammatory diseases such as rheumatic.[5,6] Better outcomes in patients with severe COVID-19 pneumonia who received tocilizumab have been observed in earlier studies.[3] However, current work by Rosas *et al.* found that the use of tocilizumab did not result in significantly better clinical status or lower mortality than a placebo at 28 days of a randomized trial involving hospitalized patients with severe COVID-19 pneumonia.[7]

In addition to these contradictory findings, these repurposed immunosuppressive drugs were not affordable for most developing counties. Since ethnopharmacology may provide a good source for a cheap candidate of anti-inflammation drugs,[8] here we observed

the clinical application of *Eucalyptus* oil, which was previously proven could inhibit IL-6 expression in animal studies, as adjuvant therapy for COVID-19 patients.[9] This study was conducted to determine the effectiveness of affordable adjuvant therapy, *Eucalyptus* oil, which has been used as a household remedy for respiratory tract infections in Indonesia.

METHODS

Study Design

This prospective clinical trial was conducted at one of the academic hospitals in the eastern part of Indonesia. Confirmed COVID-19 patients, aged 20–60 years old, with mild clinical symptoms, fever, and other respiratory tract symptoms; minimal pneumonia manifestations were included. SARS-CoV-2 infections were confirmed by real-time reverse transcription-polymerase chain reaction (RT-PCR) from the nasopharynx swab.

Randomization

This study was designed as block randomization in parallel groups and performed with 1:1 allocation between the experimental and control arms by the research personnel. The randomization was performed using the platform randomization.com [http://www.jerrydallal.com/random/permute.htm]. The results were printed and inserted in sealed envelopes numbered 001 to 060. At the time of the randomization, each participant that meets the inclusion criteria will receive a consecutive participant number [001-060]. The sealed envelope with the assigned number will be opened in front of the participant and the arm allocation will be known. All physicians and outcome assessors were blinded to patient allocation. Research activities, including screening, enrolment, consent, baseline data collection, and randomization were conducted solely by research personnel.

Patients were excluded if they had or developed severe COVID-19 symptoms according to the COVID-19 diagnosis and treatment guidelines of the Indonesian Society of respirology; respiratory distress (RR ≥30 times/min, oxygen saturation ≤93% at a rest state, arterial partial pressure of oxygen (PaO2)/oxygen concentration (FiO2) ≤300 mmHg. Patients with critical symptoms of COVID-19 or a history of comorbidity such as coronary heart disease, congestive heart disease, renal insufficiency, chronic liver disease, diabetes mellitus, uncontrolled hypertension, immunocompromised, and CNS disorders were excluded. In addition, pregnant, lactating women and patients in the treatment group who did not use eucalyptus oil routinely according to predetermined standards were also excluded.

Patients were assigned into two groups. The number of participants who received treatment, lost and excluded, and analyzed for outcome measurement are shown in Figure 1. The first group was only given standard COVID-19 treatment; oseltamivir 75 mg 12 hourly a day, azithromycin 500 mg a day, vitamin C 300 mg 12 hourly a day, zinc 20 mg a day, and a tablet of complex vitamin B. In comparison, the treated group was given standard COVID-19 and adjuvant therapy. *Eucalyptus* oil as adjuvant therapy was given through inhalation by applying it on the surgery mask four times a day, 45 minutes duration in each session for 15 days.

Outcome

The primary outcome in this trial was the efficacy of *Eucalyptus* oil as adjuvant therapy in mild-moderate COVID-19 patients which was measured with clinical and laboratory changes from the baseline. After the report of PCR test positive was obtained, included patients were asked about their clinical symptoms and these were considered as the baseline. The questions were: any change in smell, presence of cough, and sore throat. The patients with those clinical symptoms were asked to rate the severity subjectively according to the 6-point Likert scale, scoring 0 for no problems, 1 for very mild, 2 for mild or slight, 3 for moderate, 4 for severe, and 5 for as bad as it can be. At follow-ups on days 5,10,15, they were asked about the disappearance of symptoms or their persistence. If persistent, patients were asked to rate it according to the same severity scale.

Conversion to negative of COVID-19 was evaluated using RT-PCR and viral load indirectly reflected by SARS-CoV-2 cycle threshold (Ct) on day 1,5,10,15. Blood samples of patients were also collected on days 1 and 15 to evaluate their serum cytokine level (interleukin 6, interleukin 10, and transforming growth factor-beta 2) and neutrophil-to-lymphocyte ratio (NLR). Safety outcomes included treatment-emergent, serious adverse events (SAEs), AEs, and toxicities (renal and liver function).

Eucalyptus Oil

The oil extraction process of *Eucalyptus corridor* and *Eucalyptus globulus* leaves was carried out by the distillation method using steam distillation tools with a temperature above 200°C and 1 atm pressure for approximately 4 hours. The leaves were harvested from *E. corridor* and *E. globulus* plants that are more than five years old and grown in Plant and Medicine Research Hall, West Java, Indonesia. The oil was then checked for quality standards at the Plant and Medicine Research Hall Quality Test Laboratory to determine its quality and quality. Based on the results of Gas Chromatography (GC) injection, the citronella oil content was 76.07% in *E. citriodora*, while in *E. globulus*, the main component was 1.8 cineole at 83%. The quality of the quality based on other parameters as follows (Table 1 and Table 2).

Data Analysis

A sample size of 46 subjects, 23 in each arm, is sufficient to detect a clinically important difference of 1.0 between groups in reducing COVID-19 clinical symptoms assuming a standard deviation of 1.195 using a two-tailed t-test of difference between means with 80% power and a 5% level of significance. Considering a dropout rate of 10%, the sample size required is 52 (26 per group).

Categorical variables were summarized using counts and percentages. Categorical variables were compared with the Chi-square test (or Fisher's exact test for low counts). Wilcoxon signed-rank test was used to test for differences in continuous variables between the two groups. Comparisons between means and medians of continuous variables were assessed with independent T-tests. Data were analyzed

using IBM SPSS 22 version software. Statistical significance was determined when the p-value <0.05.

RESULTS

Characteristics of the patients

Between November 2020 (first patient in) and January 2021 (last patient out), 52 patients from Hasanuddin University Hospital, Makassar, Indonesia, were treated with standard COVID-19 therapy plus *Eucalyptus* oil (treatment group, n = 26) or standard COVID-19 treatment alone (control group, n = 26). The baseline characteristics of patients are listed in Table 3. There was no significant difference between the gender and age of the two groups (p-value > 0.05). The baseline profiles of patients' blood tests and liver enzymes also show no significant differences (Table 4).

Effects of *Eucalyptus* on the Improvement of clinical symptoms

During 15 days, respiratory tract infection symptoms were monitored. There was a better trend and fewer symptoms (altered smell, cough, and sore throat) after 15 days of *Eucalyptus* oil administration in the treatment group than in the control group (Figure 1). In addition, the recovery duration (time between admission of the patients and disappearance during 2-week follow-up) of altered smell in the treatment group was significantly shorter (p = 0.003) (Table 5).

Effects of eucalyptus on SARS-CoV-2 RT-PCR and viral load

Adjuvant treatment with *Eucalyptus* showed a significant increase in the negative proportion of Covid-19 RT-PCR on day-10 and day-15 (on day-10: 53.85% vs 26.92%, p = 0.0239; on day-15: 80.77% vs 57.69%, p = 0.035) (Figure 2A) meaning that the time for patient's RT PCR results turning negative in the treated group was shorter than that in the control group.

Similarly, SARS-CoV-2 cycle threshold (Ct) values in the treatment group were significantly higher than the control group on Day-10 (p = 0.009) and 15 (p = 0.049). These results indirectly reflected the decrease in viral load (Figure 2B).

Effects of *Eucalyptus* on cytokines profiles

During 15 days of study, the median of IL-6 among treated patients was significantly lower (p < .05) in comparison to the control group on day-15 (Figure 3). There are no significant differences in IL-10 and serum TGF-β2 levels between the treated and control group.

Effects of *Eucalyptus* on the neutrophil-to-lymphocyte Ratio (NLR)

The neutrophil-to-lymphocyte ratio (NLR) was significantly decreased after 15 days of trial with *Eucalyptus* as an adjuvant (p = 0.0009). In contrast, patients in the control group had higher NLR on day-15 than on day-1 (Table 6).

Adverse Events

There were no adverse events and no serious adverse events.

Table 1. ***Eucalyptus citriodora oil*** **Quality Test Results**

Indicator	Result	Standard
Citronellal	76.07%	≥ 70 %
Color	Clear	Clear to yellowish
Spesific gravity	0.865	0.86-0.87
Optical loop	-2°	-0° -20°
Refractive index	1.4530	1.4510 -1.4640
Odor	Typical *E. citridora odor*	Typical *E. citridora* odor
Solubility in ethanol 70%	1:2	<1:3

Table 2. ***Eucalyptus globulus oil*** **Quality Test Results**

Parameter	Result	Standard
1,8 Cineole	83%	> 80 %
Colour	Clear	Clear to greenish yellow
Spesific gravity	0.915	0.905-0.927
Optical loop	+5	-0° -18°
Refractive index	1.4637	1.4580 – 1.4700
Odor	Typical *E. Globulus* odor	Typical *E. Globulus* odor
Solubility in ethanol 70%	1:4	1:5

Table 3. **Characteristics of the patients**

Variable	Total (*n* = 52)	Treatment (*n* = 26)	Control (*n* = 26)	p-value
Mean age (years), mean ± SD	32 ± 7.1	33 ± 7.0	31 ± 7.1	0.443[a]
Age (years), n (%)				
<30	23 (44.2)	12 (46.2)	11 (42.3)	0.358[b]
30-40	22 (42.3)	9 (34.6)	13 (50.0)	
>40	7 (13.5)	5 (19.2)	2 (7.7)	
Gender, n (%)				
Female	27 (51.9)	14 (53.8)	13 (50.0)	1.000[c]
Male	25 (48.1)	12 (46.2)	13 (50.0)	
Adverse event, n (%)	0 (0)	0 (0)	0 (0)	
Endpoint stage				
Cured	52 (100)	26 (100)	26 (100)	1.000[c]
Death	0 (0)	0 (0)	0 (0)	

**Analysis was carried out using; [a]Independent Sample T-test, [b] Fisher's exact test, and [c]Chi-square test. Results were considered significant if the p-value ≤0.05.*

Table 4. Patients' blood test, liver function, and renal function profile

Variables	Treatment (n=26),		Control (n=26),		p-value
	Mean ± SD	Median	Mean ± SD	Median	
Routine blood test					
Haemoglobin (g/dL)	14.38 ± 1.73	14.15	14.32 ± 1.35	14.0	0.783
Haematocrit (%)	42.42 ± 4.69	42.20	42.38 ± 3.19	42.55	0.783
Red cell count (x10^{12}/L)	5.04 ± 0.57	4.95	5.07 ± 0.56	5.06	0.583
White cell count (x10^{9}/L)	7.40 ± 2.08	7.63	6.91 ± 2.35	6.61	0.272
Platelet count (x10^{9}/L)	348.15 ± 76.18	362	302.54 ± 93.15	302	0.099
Neutrophil (%)	56.07 ± 18.27	62.40	287.77 ± 1206.03	53.20	0.272
Lymphocytes (%)	117.37 ± 465.71	24.95	86.03 ± 268.21	31.55	0.099
NL ratio	2.53 ± 1.20	2.39	1.97 ± 1.40	1.60	0.099
PT (sec)	12.95 ± 4.29	12.25	12.35 ± 2.16	12.50	0.272
aPTT (sec)	31.00 ± 5.73	31.60	29.77 ± 5.18	30.15	0.169
Liver function					
Ferritin (ng/mL)	185.49 ± 206.34	104.68	151.15 ± 153.93	111.55	1.000
Glucose (mg/dL)	109.15 ± 39.89	100.50	105.54 ± 22.42	106.50	0.583
AST (U/L)	25.92 ± 11.50	26.50	27.15 ± 15.28	21	0.583
ALT (U/L)	30.35 ± 17.51	29.80	34.15 ± 24.00	27	0.272
Renal function					
Urea (mg/dL)	18.37 ± 4.47	17.50	21.50 ± 6.41	20	0.401
Creatinine (mg/dL)	0.82 ± 0.26	0.80	0.81 ± 0.21	0.80	1.000

NL ratio: neutrophil lymphocytes ratio; PT: prothrombin time; aPTT: activated partial thromboplastin time; AST: aspartate transaminase; ALT: alanine transaminase; SD: standard deviation.

DISCUSSION

Eucalyptus oil is widely known as a traditional medicine to treat some respiratory diseases such as pharyngitis, bronchitis, and sinusitis. 1,8-cineole (eucalyptol), one of *Eucalyptus*'s active constituents, has shown muscle relaxant impacts by diminishing smooth muscle contractions of airways induced by distinctive microorganisms.[10,11] Additionally, clinical research has demonstrated that inhalation of cineole applied anti-inflammatory (by inhibiting cytokines release) and pain-relieving impacts; thus, it can be viably utilized in chronic obstructive pulmonary disease (COPD) and asthmatic patients. Nasal decongestant effects are also obtained from 1,8-cineole when applied to the nose or inhaled.[12] The results of our study demonstrated that *Eucalyptus* oil affects the symptoms of COVID-19, especially in altered smell, cough, and sore throat, which shows better outcomes than in the control group after 15 days *of Eucalyptus* oil administration.

Some studies have endeavored to investigate the antiviral viability of *Eucalyptus* oil and its active components against SARS-CoV-2 utilizing in vitro assays and molecular docking techniques. The data demonstrated that 1,8-cineole inhibits viral reproduction by binding with viral proteinase (Mpro). Hydrophobic interactions, solid ionic interactions, and hydrogen bond interactions were

Table 5. Recovery duration of clinical symptoms of patients

Clinical Symptom	Recovery Duration (days)		p-value
	Treatment (mean ± SD)	Control (mean ± SD)	
Altered smell	4 ± 1.840	8 ± 4.203	0.003*
Cough	9.23 ± 4.285	10 ± 4.188	0.641
Sore throat	5 ± 3.317	5 ± 2.000	1.000

**Analysis was carried out using Independent Sample T-test. Results were considered significant if the p-value ≤ 0.05*

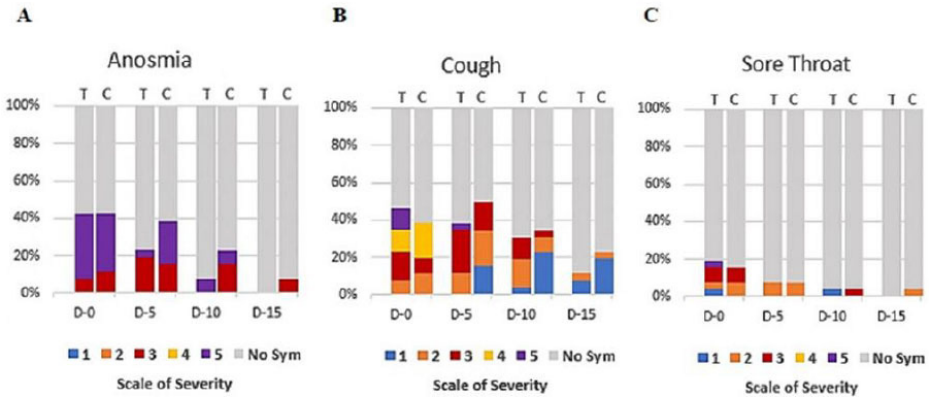

Figure 1. Improvement of clinical symptoms. Improvement of clinical symptoms (A) anosmia (altered smell) (B) cough and (C) sore throat in the Eucalyptus adjuvant treatment group (n = 26) and control (n = 26) were monitored on Day-1, 5, 10, and 15. Scale of severity; 5:as bad as it can be; 4:severe; 3:moderate; 2:mild or slight; 1:very mild; no problems.

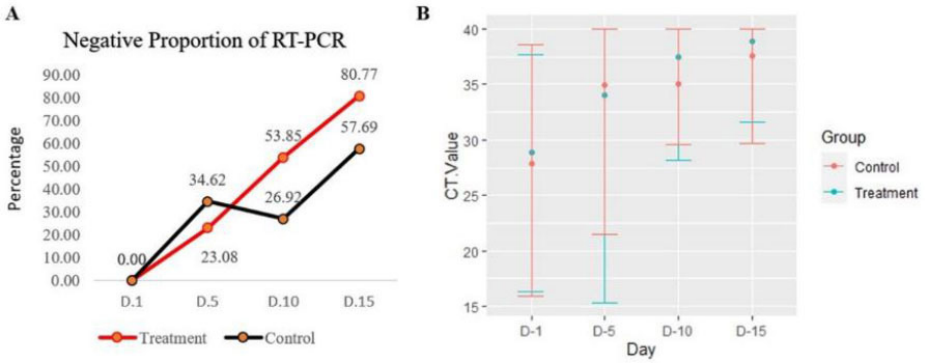

Figure 2. RT PCR result and viral load. (A) Negative proportion of RT-PCR test in the Eucalyptus adjuvant treatment group (n = 26) and control (n = 26) were monitored on Day-1, 5, 10, and 15. (B) Viral load is indirectly reflected by cycle threshold (Ct) values.

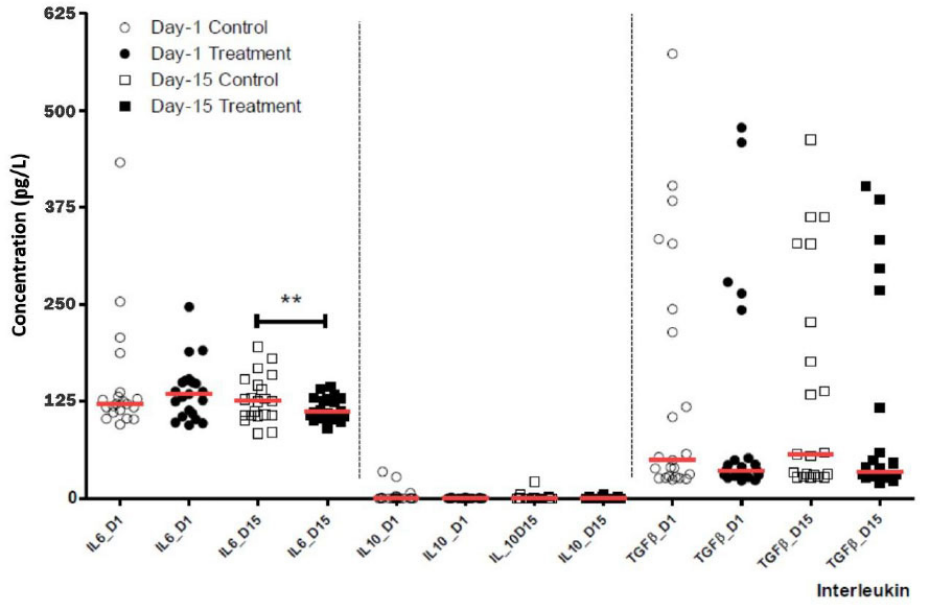

Figure 3. The kinetic of serum cytokines. Interleukin (IL) 6, 10, and serum TGF-β2 level of adjuvant treated (transparent circle or box) and control group (dark circle or box) were monitored on day-1 and day-15 using enzyme immunoassay (EIA)-based assay.

Table 6. The neutrophil-to-lymphocyte ratio between groups

Group	NLR (Mean)		*p-value*
	At admission	At follow-up	
Treatment	2.529 ± 1.200	1.998 ± 0.899	0.0009*
Control	1.965 ± 1.403	2.162 ± 1.006	0.740

**Analysis was carried out using Wilcoxon signed-rank test. Results were considered significant if the p-value ≤0.05.*

formed after Mpro/eucalyptol complexes emerged.[13] Another active constituent of *Eucalyptus* oil, jensenone, also formed a complex with Mpro via hydrophobic interactions with TRP207, PRO52, LEU29, TRY126, ALA7, and PRO184; hydrogen bond interactions with M4, D10, and T16, V18, L30; and ionic interactions with LYS3, ARG38, ASP34, and HIS163.[14] This study demonstrated a significant difference between the treated and control groups on the viral load reflected indirectly by the rRT-PCR cycle threshold (Ct). Cycle threshold values in the treatment group at day-10 and 15 are significantly higher (representing the viral load decrease) than in the control group. This finding is parallel with the significant increase of the negative proportion of Covid-19 RT-PCR on day-10 and day-15.

It has been known that the levels of patients' IL-6 were correlated with COVID-19 severity.[2] Abnormal and overactive inflammatory responses to the virus are proposed to be the significant causes of infection seriousness and death. This hyper-inflammatory state is related to the elevated level of IL-6 as a pro-inflammatory cytokine.[15] Subjects with SARS-CoV-2 had high levels of IL-6 that were associated with patient symptoms, including extensive lung damage and pulmonary inflammation.[16] A result of the meta-analysis indicated that increased levels of IL-6 are correlated with ICU (intensive care unit) admission, ARDS (acute respiratory distress syndrome), and death. Patients with complicated forms of COVID-19 had about triple higher serum IL-6 levels than those with non-complicated disease.[17]

Previous findings have identified the immunomodulatory effect of *Eucalyptus* oil by inhibiting monocytes and macrophages from releasing pro-inflammatory cytokines. It was observed that eucalyptol reduced IL-6 levels.[12] The mechanism of *Eucalyptus* oil on reducing IL-6 was shown in the study of applied lipopolysaccharides (LPS) to RAW 264.7 murine macrophages to trigger inflammatory reactions. Monocytes are attracted to chemokines when inflammation occurs. They enter the lesion and change into macrophages that undergo phagocytosis and release pro-inflammatory cytokines such as TNF-α and IL-6. It has been demonstrated that ERK1/2, JNK1/2, and p38 are the pathway for LPS to induce pro-inflammatory cytokines in macrophages.[18]

Furthermore, LPS-mediated protein kinase C (PKC) activation generates the downstream signaling pathways, such as NF-κB or MAPK, that modulate the expression of IL-6 and TNF-α in macrophages.[19] Here, *Eucalyptus* oil directly affects the expression level of IL-6 by inhibiting ERK1/2/p38 and PKC/NF-κB in the LPS-activated RAW264.7 macrophages.[9] This study showed that the treated group has a significantly lower level

of IL-6 in comparison with the control group, which was consistent with previous studies. The limitation of this study was single-centre studies and replication is required in multicentre studies.

CONCLUSION

Eucalyptus oil as an adjuvant of COVID-19 treatment promotes faster recovery of mild-moderate patients, probably via inhibition of the IL-6-mediated inflammation process. These results indicate that *Eucalyptus* oil could be the novel adjuvant treatment in COVID-19 patients at a more accessible and affordable price, especially for most developing countries.

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

AUTHOR CONTRIBUTION

AS, FH, IP, MNM, NPID, HN, DN, FD, and ES initiated and designed the study. AS, RR, MM, PWP, MRJ, AAA, MNA, and AA, performed, collected, and registered the data. AS, MM, AM, YW, II, ARS, and FH monitored the study and analyzed the data. AS, RR, ARS, FH, and MNA drafted the manuscript. All authors read and approved the final manuscript.

ETHICAL CONSIDERATION

All subjects gave written informed consent following the Declaration of Helsinki. The Ethical Committee of the Medical Faculty of Hasanuddin University approved the procedure applied in this study [718/UN4.6.4.5.31/PP36/2020]. The trial was registered at ClinicalTrials.gov (Identifier: NCT05398965). The trial was registered retrospectively (https://clinicaltrials.gov/ct2/show/NCT05398965) due to a lack of awareness of the requirement for registration and the need to be registered prospectively.

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