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Original Article

Andrographis paniculata extract versus placebo in the treatment of COVID-19: a double-blinded randomized control trial

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Abstract

Background and purpose: *Andrographis paniculata* (Burm.f.) Nees has been recommended to relieve symptoms and decrease the severity of COVID-19. The clinical study aimed to investigate the efficacy and safety of *A. paniculata* ethanolic extract (APE).
Experimental approach: The efficacy and safety of APE in asymptomatic or mildly symptomatic COVID-19 patients compared with placebo were investigated through a prospective, double-blind randomized control trial. Patients received APE containing 60 mg of andrographolide, three times a day for five days. WHO progression scale, COVID-19 symptoms, and global assessment evaluated the efficacy and adverse events, liver and renal functions were monitored for safety.
Findings/Results: 165 patients completed the study (83 patients in the APE group and 82 patients in the placebo group). The highest WHO progression scale was 4 and COVID-19 symptoms were significantly relieved on the last day of intervention in both groups, with no significant difference between groups. APE significantly relieved headache symptoms on day 1 and olfactory loss symptoms on day 2 compared to placebo. The global assessment showed that 80.7% of patients had total recovery after 5-day treatment with APE. Mild diarrhea was the most common side effect with a high dose that resolved within a few days. No hepatic or renal toxicity was associated with treatment.
Conclusion and implications: APE at 180 mg/day for 5 days did not reduce COVID-19 progression in asymptomatic or mildly afflicted COVID-19 patients, however, it shortened the symptoms of olfactory loss with no adverse effects over 5 days of use.

Keywords: Capsule; Clinical trial; Coronavirus, HPLC.

INTRODUCTION

Coronavirus disease 19 (COVID-19) is a respiratory infection disease caused by severe acute respiratory syndrome Coronavirus-2 (SARS-CoV-2). COVID-19 became a pandemic on March 11, 2020, and threatened global health systems until May 4, 2023, when the International Health Regulations (IHR) and Emergency Committee of the World Health Organization (WHO) downgraded the COVID-19 pandemic. Clinical manifestations of COVID-19 are varied and range from asymptomatic infection to severe and life-threatening diseases.

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Most patients usually present with no to mild symptoms such as fever, upper respiratory tract symptoms, headache, fatigue, and loss of taste and smell without lower respiratory tract symptoms (1). The guidelines of COVID-19 treatment recommend conservative and symptomatic treatment for mild and uncomplicated diseases while antiviral therapy, corticosteroid, and other advanced treatments are recommended in moderate to severe infections or in patients with comorbidities at risk for disease progression (2).

In Thailand, the number of COVID-19 cases increased significantly during the delta-variant wave in 2021 and the omicron-variant wave in 2022. Most of the patients in these 2 pandemic surges were asymptomatic or had mild symptoms, except for those with underlying diseases and had no prior COVID-19 vaccination. According to the recommendations by the Ministry of Public Health of Thailand, patients with no or mild symptoms should self-medicate and isolate themselves at home. The basis of Thai traditional medicine rests upon the utilization of herbal medicine derived from *A. paniculata*, also known as "Fa-Ta-Lai-Chon," for its antipyretic properties, sore throat relief, and its potential to alleviate COVID-19 symptoms. This practice is supported by clinical rationale in viral disease (3), leading the Ministry to recommend the use of *A. paniculata* for symptomatic relief.

The principle of Thai traditional medicine uses bitter herbs as antipyretic drugs, with *A. paniculata* published in the Thailand National List of Essential Medicines as a first-line herbal medicine for the treatment of sore throat and to reduce the fever with no serious bacterial and fungal infection such as inflammation, myalgia, and the common cold. *A. paniculata* has various pharmacological activities which are associated with therapeutic effects such as anti-inflammatory (4), antioxidant, antipyretic (5), and immunomodulatory activity (6). A recent *in-vitro* study has shown that *A. paniculata* extract and andrographolide which is the main active compound of *A. paniculata* can prevent SARS-CoV-2 replication in human lung epithelial cells (7). A molecular docking study has demonstrated that andrographolide binds M protease and angiotensin-converting enzyme-2 receptors of SARS-CoV-2 which may be associated with COVID-19 prevention (8-10). Furthermore, a pilot study on *A. paniculata* in COVID-19 patients with mild symptoms was conducted and determined that it significantly relieved headaches and the severity and frequency of cough (11). Although *A. paniculata* is currently recommended for use in mild COVID-19 in Thailand based on *in-vitro* findings and pilot clinical studies, data from well-designed double-blinded clinical studies are currently limited, especially in a randomized placebo-control trial. The use of Thai traditional medicine has a supportive role in reducing fever and inflammation of sore throat, and some research is consistent with potential effectiveness against COVID-19 (8-10). However, thereis no published clinical data for high-dose andrographolide treatment in COVID-19 therapy. Consequently, this study was conducted to evaluate the potential efficacy and safety of a high dose of andrographolide, the main component in *A. paniculata* extract, for the treatment of mild COVID-19 in comparison with placebo.

MATERIALS AND METHODS

Research design and setting

This study was a prospective, double-blind randomized control trial that evaluated the safety and efficacy of *A. paniculata* methanolic extract (APE) in the treatment of COVID-19 patients compared with a placebo. This study was conducted in Thammasat University Field Hospital (TUFH), a 308 in patient bed hospital, all COVID-19 detected patients either asymptomatic or with moderate symptoms were quarantined in the hospital for 14 days according to the Ministry of Public Health guidelines during enrollment between July 2021 to September 2021. This study was conducted in accordance with the Declaration of Helsinki approved by the Human Research Ethics Committee of Thammasat University Faculty of Medicine and registered online at Thaiclinicaltrials.org under Ethical No. TCTR20210809004.

Participants

The COVID-19 patients aged between 18-65 years who were confirmed positive with SARS-CoV-2 detected *via* a real time-polymerase chain reaction (RT-PCR) method, were admitted to TUFH within 72 h of confirmed virus detection, with mild severity of symptoms based on the WHO progression scale (12) (Table 1). Patients with a score of not more than 3 (green color), were included in this study. Patients were quarantined in the hospital for 14 days. Patients who had an abnormal chest X-ray, oxygen saturation less than 95%, started treatment before the screening, and received aspirin more than 81 mg/day and APE or medicine derived from *A. paniculata*. Patients presenting with allergic history to APE, liver and renal function rising more than 2.5 times of upper normal limit, pregnancy, or breastfeeding were excluded from this study.

According to a previous report, the progression of moderate and severe COVID-19 symptoms in patients treated with *A. paniculata* was 0.97% compared to the control group which was 14.64% (11). A calculated sample size of 74 participants for each group was estimated based on a statistical power of 0.8 and a significance level of 0.05. A dropout rate of 10% was considered, 83 participants were required in each group, 166 participants in total.

Interventions

The APE capsule was manufactured by Good Manufacturing Practices (GMP) by Thai Herbal Products Co., Ltd, Thailand and contained 20 mg of andrographolide (Fig. 1) in each capsule. The andrographolide content was repeatedly measured using a validated high performance liquid chromatography (HPLC) method for content stability during the study by the Center of Excellence in Applied Thai Traditional Medicine Research (CEATMR), Faculty of Medicine Thammasat University. Corn starch was used in a matching placebo. Both interventions were encapsulated with the same size and color of the capsule, sealed in blister foil, and placed in an opaque container to ensure blinding to trial participants. The APE dosage regimen, including three capsules, was administered three times a day which totaled 180 mg/day of andrographolide. The recommended effective dose followed guidance from the Thailand Ministry of Public Health based on efficacy and tolerability in the previous pilot studies (11,13).

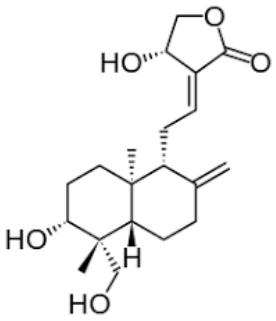

Fig. 1. Chemical structure of labdane diterpenoid andrographolide.

Table 1. The WHO COVID-19 progression scale (12).

Patient State	Descriptor	Score
Uninfected	Uninfected; no viral RNA detected	0
Ambulatory mild disease	Asymptomatic; viral RNA detected	1
	Symptomatic; independent	2
	Symptomatic; assistance needed	3
Hospitalized: moderated disease	Hospitalized; no oxygen therapy*	4
	Hospitalized; oxygen by a mask of nasal prongs	5
Hospitalized: severe diseases	Hospitalized; oxygen by NIV or high-flow	6
	Intubation and mechanical ventilation, $pO2/FiO_2 \geq 150$ or $SPO_2/FiO_2 \geq 200$	7
	Mechanical ventilation $PO_2/FiO_2 < 150$($SpO2/FiO_2<200$) or vasopressors	8
	Mechanical ventilation $PO_2/FiO_2 < 150$ and vasopressors, dialysis, or ECMO	9
Dead	Dead	10

*If hospitalized for isolation only, record status as for ambulatory patient.

The data collection was performed *via* telecommunication and an online platform until discharge on day 14 after quarantine. The demographic data, WHO progression scale, clinical COVID-19 symptoms according to WHO guidelines, and laboratory tests (complete blood count, renal function, and liver function tests) were collected at baseline and on day 14. WHO progression scale was evaluated every day until patient discharge. Clinical COVID-19 symptoms were assessed by a 0-10 numeric rating scale (no symptoms to severe symptoms) which were monitored daily for five days during treatment and the last day before being discharged from the TUFH. The global assessment was evaluated after the last dose of intervention or placebo. The adverse events and compliance were monitored by patient self-reporting through daily telecommunication and an online questionnaire *via* a Google form and Line official account. The clinical chemistry including complete blood count, renal, and liver function tests were performed within 48 h after completion of the last intervention dose.

Statistical analysis

Descriptive statistics are represented for this study with mean ± SD for normally distributed data and median (interquartile range; IQR) for non-normally distributed data. The independent Students t-test, repeated measure ANOVA or Chi-square, and Mann-Whitney U test were used to compare differences between groups. Data were analyzed using SPSS software (version 16.0, USA) and a *P*-value < 0.05 was set as the level of significance.

RESULTS

Baseline characteristics

A total of 229 patients with COVID-19 were screened, and 181 met the study criteria. However, 12 patients declined to participate in the study. 169 patients participated and were randomized to intervention groups which resulted in 85 and 84 patients in the APE group and placebo group, respectively. Four patients elected to drop out of the study (2 in APE and 2 in the placebo group) before the treatments commenced. In total, there were 83 and 82 patients who completed screening and were included in the APE and placebo groups, respectively (Fig. 2).

The baseline data including age, gender, body mass index (BMI), WHO progression scale at enrollment, COVID-19 symptoms and laboratory results (liver and renal functions) were not significantly different between the two groups. The number of participants provided standard supportive treatment was also not statistically different between the two groups. All patients demonstrated an excellent compliance average of greater than 90% (Table 2).

APE capsule 400 mg contained an amount of andrographolide 20.11 ± 0.80 mg. The standard andrographolide concentration range was linear between 20-200 µg/mL (R^2 = 0.9998) and the calibration curve equation was y = 35944x + 16082 (Fig.3).

Efficacy

According to the WHO progression scale, all patients in the trial experienced mild symptoms at enrollment. The scores ranged from baseline (2 and 3) to 4 during the study, for 4 and 8 participants in APE and placebo groups, respectively. The number of patients where elevations in score to greater than 3 in the APE group was less than twice that of the placebo group, and not statistically significant between the groups. However, these participants, who had a WHO progression scale score greater than 3, were provided with anti-viral drug (favipiravir) treatment according to standard clinical guideline treatment together with our research intervention. All patients recovered without any serious adverse events within 14 days of quarantine (Table 3).

The COVID-19 symptoms were not significantly different in most symptoms evaluated (Table 4). The results showed that mild symptomatic COVID-19 patients recovered in both groups within five days of consecutive treatment with no significant difference between the two groups. Nevertheless, APE significantly mitigated headache symptoms on day 1 in comparison with the placebo group. Interestingly, APE has a faster recovery in olfactory symptom loss than placebo.

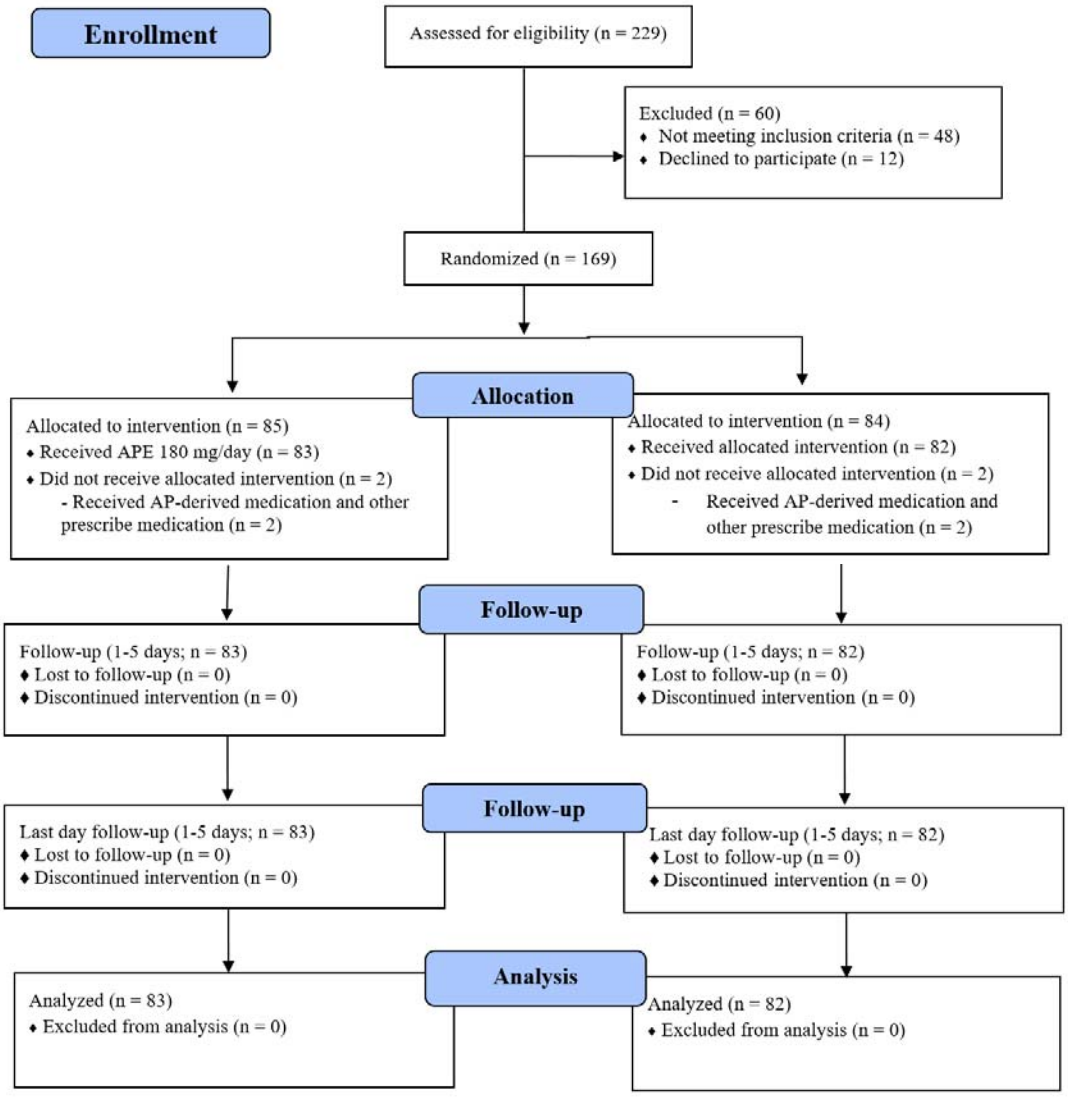

Fig. 2. Participants flow chart. APE, *Andrographis paniculata* ethanolic extract.

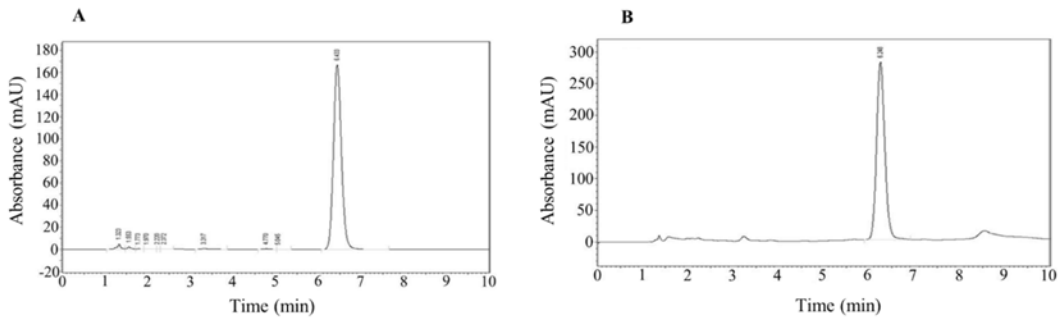

Fig. 3. HPLC Chromatograms and equivalent elution and retention of (A) andrographolide standard and (B) andrographolide in the capsule containing *Andrographis paniculata* extract.

Table 2. Baseline characteristics of the patients recruited in the study.

Data	APE (n = 83)	Placebo (n = 82)	*P*-value
Sex, n (%)			
Male	27 (32.53)	28 (34.15)	0.870 [c]
Female	56 (57.47)	54 (65.85)	
Age (years); median (IQR)	29 (23, 35)	33 (25, 42)	0.025 [m]
BMI (kg/m^2); median (IQR)	23.31 (20.50, 27.06)	22.29 (20.05, 26.07)	0.440 [m]
Body temperature (°C); median (IQR)	36.7 (36.4, 37.0)	36.6 (36.4, 36.9)	0.159 [m]
O_2 sat (%); median (IQR)	98 (98, 99)	98 (98, 99)	0.023 [m]
WHO progression scale n (%)			
2	30 (36.1)	19 (23.2)	0.069 [c]
3	53 (63.9)	63 (76.8)	
COVID-19 symptoms (NRS); median (IQR)			
Fever	1 (0, 5)	0 (0, 5)	0.371[m]
Severity of cough	3 (1, 4)	3 (1, 5)	0.774 [m]
Frequency of cough	2 (1, 4)	2.5 (1, 5)	0.681 [m]
Sputum production	3 (1, 4)	2 (1, 4)	0.782 [m]
Runny nose	2 (1, 3)	2 (0, 4)	0.835 [m]
Sore throat	0 (0, 3)	1 (0, 5)	0.456 [m]
Dyspnea	0 (0, 2)	1 (0, 3)	0.585 [m]
Shortness of breath	0 (0, 2)	0 (0, 2)	0.920 [m]
Olfactory loss	6 (0, 9)	7 (0, 10)	0.528 [m]
Loss of taste	0 (0, 7)	2 (0, 8)	0.130 [m]
Conjunctivitis	0 (0, 0)	0 (0, 0)	0.613 [m]
Skin rash	0 (0, 0)	0 (0, 0)	0.541 [m]
Diarrhea	0 (0, 1)	0 (0, 1)	0.550 [m]
Headache	2 (0, 5)	1 (0, 3)	0.138 [m]
Muscle aches (myalgia)	1 (0, 3)	0.5 (0, 3)	0.544 [m]
Nausea/Vomiting	0 (0, 0)	0 (0, 0)	0.252 [m]
Altered consciousness/confusion	0 (0, 0)	0 (0, 0)	0.323 [m]
Vaccinated, n (%)			
None	20 (24.10)	18 (21.95)	0.476[c]
1 dose	28 (33.73)	36 (43.90)	
2 doses	32 (38.55)	27 (32.93)	
3 doses	3 (3.61)	1 (1.22)	
Type of vaccine; n (%)			
None	20 (24.10)	18 (21.95)	0.524[c]
SV	30 (36.14)	26 (31.71)	
AZ	17 (20.48)	27 (32.93)	
PF	1 (1.20)	0 (0)	
SP	10 (12.05)	9 (10.98)	
SV + AZ	4 (4.82)	2 (2.44)	
SV +PF	1 (1.20)	0 (0)	
Supportive medicine (i.e., acetaminophen, anti-histamine) was used during the study; n (%)			
Yes	53 (63.86)	63 (76.83)	0.088[c]
No	30 (36.14)	19 (23.17)	
Lab results			
AST	26.45 (10.04)	27.44 (11.26)	0.551 [t]
ALT	25.13 (15.77)	27.35 (20.13)	0.431 [t]
ALP	59.22 (14.40)	61.38 (19.46)	0.418 [t]
BUN	10.83 (2.97)	10.42 (3.29)	0.401 [t]
Cr	0.80 (0.16)	0.78 (0.16)	0.303 [t]

Statistical analysis: c = chi-square test; t = independent t-test; m = Mann-Whitney U test; APE, *Andrographis paniculata* ethanolic extract; BMI, body mass index, IQR, interquartile range; SV, CoronaVac (Sinovac); AZ, ChAdOx1 nCoV-19 Astra Zeneca; PF, BNT162b2 Pfizer-BioNtech; SP, BBIBP-CorV Sinopharm; APE, *Andrographis paniculata* ethanolic extract; AST, aspartate aminotransferase; ALT, alanine transaminase; ALP, alkaline phosphatase; BUN, blood urea nitrogen; Cr, creatinine.

Table 3. WHO progression scale changes during clinical. The data were analyzed *via* a chi-square statistical test.

Progression score	APE (n = 83)	Placebo (n = 82)	*P*-value
Progression score changing ≤ 3	79 (95.2)	74 (90.2)	0.222
Progression score changing > 3	4 (4.8)	8 (9.8)	

APE, *Andrographis paniculata* ethanolic extract.

Table 4. Evaluation of COVID-19 symptoms. Data represent the median (IQR) analyzed via the Mann-Whitney U test. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 indicate significant differences from day 0 within group.

Symptoms	Day	Difference in score from day 0		*P*-value
		APE (n = 83)	Placebo (n = 82)	
Fever	1	0 (-2, 0) ***	0 (-2.25, 0) **	0.168
	2	-1 (-3,0) ***	0 (-2.25, 0) ***	0.21
	3	-1 (-4, 0) ***	0 (-3, 0) ***	0.153
	4	-1 (-4, 0) ***	0 (-3.25, 0) ***	0.336
	5	-1 (-4, 0) ***	0 (-4, 0) ***	0.546
Severity of cough	1	-1 (-2, 0) **	-1 (-2, 0) ***	0.226
	2	-1 (-2, 0) ***	-1 (-3, 0) ***	0.448
	3	-1 (-2, 0) ***	-1 (-3, 0) ***	0.448
	4	-2 (-3, 0) ***	-2 (-3, 0) ***	0.98
	5	-2 (-3, -1) ***	-2 (-3.25, 0) ***	0.836
Frequency of cough	1	-1 (-2, 0) ***	-1 (-2, 0) ***	0.579
	2	-1 (-2, 0) ***	-1 (-2, 0) ***	0.654
	3	-1 (-3, 0) ***	-1 (-3, 0) ***	0.793
	4	-1 (-3, -1) ***	-1 (-3, 0) ***	0.862
	5	-2 (-3, -1) ***	-1 (-3, 0) ***	0.919
Sputum production	1	-1 (-1, 0) ***	-1 (-2, 0) ***	0.884
	2	-1 (-2, 0) ***	-1 (-2, 0) ***	0.911
	3	-1 (-3, 0) ***	-1 (-2, 0) ***	0.253
	4	-1 (-3, 0) ***	-1 (-2, 0) ***	0.439
	5	-1 (-3, 0) ***	-1 (-3, 0) ***	0.786
Runny nose	1	-1 (-2, 0) ***	-0.5 (-2,0) ***	0.837
	2	-1 (-2, 0) ***	-1 (-2, 0) ***	0.927
	3	-1 (-2, 0) ***	-1 (-2, 0) ***	0.859
	4	-1 (-3, 0) ***	-1 (-2, 0) ***	0.776
	5	-1 (-3, 0) ***	-1 (-3, 0) ***	0.958
Sore throat	1	-1 (-2, 0) ***	0 (-2, 0) ***	0.167
	2	-1 (-3, 0) ***	-0.5 (-2, 0) ***	0.297
	3	-1 (-3, 0) ***	0 (-2, 0) ***	0.180
	4	-2 (-3, 0) ***	-0.5 (-2.25, 0) ***	0.061
	5	-2 (-3, 0) ***	-1 (-3, 0) ***	0.138
Dyspnea	1	0 (-1, 0) **	0 (-1, 0) ***	0.594
	2	0 (-2, 0) ***	0 (-2, 0) ***	0.425
	3	0 (-2, 0) ***	-0.5 (-2, 0) ***	0.386
	4	0 (-2, 0) ***	0 (-2, 0) ***	0.638
	5	0 (-2, 0) ***	0 (-2, 0) ***	0.522
Shortness of breath	1	0 (0, 1) **	0 (-1, 0) **	0.744
	2	0 (0, 1) ***	0 (-1, 0) ***	0.210
	3	0 (-1, 0) ***	0 (-1, 0) ***	0.682
	4	0 (-1, 0) ***	0 (-1, 0) ***	0.739
	5	0 (-1, 0) ***	0 (-1, 0) ***	0.564
Olfactory loss	1	0 (-2, 0) *	0 (-1, 0)	0.521
	2	0 (-3, 0) *	0 (-2, 0)	0.731
	3	0 (-3, 0) *	-1 (-3, 0)	0.678
	4	-1 (-4, 0) **	-1 (-5, 0) *	0.948
	5	-1 (-5, 0) ***	-1 (-6, 0) ***	0.928
Loss of taste	1	0 (-1, 0) *	0 (-1, 0)	0.68
	2	0 (-1, 0)	0 (-2, 0) *	0.583
	3	0 (-1, 0) *	0 (-3.25, 0) **	0.176
	4	0 (-2, 0) **	0 (-4, 0) **	0.650
	5	0 (-2, 0) **	-0.5 (-4.25, 0) ***	0.153

Table 4. continued

Conjunctivitis	1	0 (0, 0) ***	0 (0, 0) *	0.562
	2	0 (0, 0) *	0 (0, 0) **	0.709
	3	0 (0, 0) *	0 (0, 0) **	0.964
	4	0 (0, 0) *	0 (0, 0) ***	0.827
	5	0 (0, 0) ***	0 (0, 0) ***	0.887
Skin rash	1	0 (0, 0)	0 (0, 0)	0.443
	2	0 (0, 0) *	0 (0, 0)	0.769
	3	0 (0, 0) *	0 (0, 0) *	0.760
	4	0 (0, 0)	0 (0, 0)	0.997
	5	0 (0, 0) *	0 (0, 0)	0.811
Diarrhea	1	0 (0, 0)	0 (0, 0)	0.995
	2	0 (-1, 0) *	0 (-0.25, 0)	0.822
	3	0 (0, 0) ***	0 (-0.25, 0) ***	0.811
	4	0 (-1, 0) ***	0 (-1, 0) ***	0.598
	5	0 (-1, 0) ***	0 (-1, 0) ***	0.306
Headache	1	-1 (-2, 0) ***	0 (-1.25, 0) ***	0.027
	2	-1 (-3, 0) ***	0 (-2, 0) ***	0.157
	3	-1 (-3, 0) ***	0 (-2, 0) ***	0.086
	4	-1 (-4, 0) ***	-1 (-2, 0) ***	0.120
	5	-1 (-4, 0) ***	-1 (-2, 0) ***	0.177
Muscle aches (myalgia)	1	0 (-2, 0) ***	0 (-1, 0) ***	0.425
	2	0 (-2, 0) ***	0 (-2, 0) ***	0.175
	3	0 (-3, 0) ***	0 (-1.25, 0) ***	0.348
	4	0 (-3, 0) ***	0 (-3, 0) ***	0.348
	5	-1 (-3, 0) ***	0 (-2.25, 0) ***	0.458
Nausea/vomiting	1	0 (0, 0)	0 (0, 0)	0.838
	2	0 (0, 0) *	0 (0, 0)	0.808
	3	0 (0, 0)	0 (0, 0) *	0.327
	4	0 (0, 0) *	0 (0, 0) ***	0.177
	5	0 (0, 0) *	0 (0, 0)	0.670
Altered consciousness/confusion	1	0 (0, 0) ***	0 (0, 0) ***	0.521
	2	0 (0, 0) ***	0 (0, 0)	0.306
	3	0 (0, 0) ***	0 (0, 0) *	0.169
	4	0 (0, 0) ***	0 (0, 0) ***	0.500
	5	0 (0, 0) ***	0 (0, 0) ***	0.915

The olfactory symptom loss was significantly relieved after treatment for 2 days meanwhile the placebo group showed symptomatic and significant relief on day 4. In the same way, the global assessment was monitored at the last day of intervention and it was found that most of the patients improved or recovered with no statistical difference between both groups (Table 4). However, the APE-received group showed a higher number in terms of total recovery than that of the placebo group (Table 5).

Safety

The APE at 180 mg/day was continuously administered for five days and demonstrated no serious adverse events. Diarrhea was the most common side-effect found after APE capsule administration and all patients resolved within two days after the commencement of APE. Diarrhea was also found in the placebo group which was not significantly different from the APE group. Other side-effects were also found in the APE group including dizziness, constipation, flatulence, nausea, peripheral numbness, and abdominal pain which were not significantly different from the placebo group. In addition, some side-effects were determined before receiving treatment with either APE or placebo such as dizziness, constipation, peripheral numbness, and abdominal pain. Several symptoms were related to COVID-19 infection (Table 6).

The liver and renal function tests were monitored at baseline before the start of the interventions and within 48 h after the last dose of interventions. The results demonstrated an absence of liver and renal function tests higher than acceptable values (2.5 times of upper normal limit in aspartate transaminase (AST), alanine transaminase (ALT), blood urea nitrogen (BUN), and 1.5 times of upper normal

limit in creatinine (Cr)) at baseline without any significant difference between the two groups. Participants had a higher AST and ALT in the APE group compared to the placebo group after treatment, but all within acceptable values. In addition, liver and renal function were not statistically significant between the two groups after treatment (Table 7).

Table 5. Global assessment on day 5 after enrollment. The data were analyzed *via* the chi-square statistical test.

Global assessment score; n (%)	APE (n = 83)	Placebo (n = 82)	*P*-value
0 (Undefined)	16 (19.3)	14 (17.1)	0.457
1 (Worse)	0 (0)	1 (1.2)	
2 (Better)	48 (57.8)	54 (65.9)	
3 (Totally recovery)	19 (22.9)	13 (15.9)	

APE, *Andrographis paniculata* ethanolic extract.

Table 6. Adverse events. Data represent n (%) analyzed *via* Fisher's Exact statistical test.

Adverse effects	APE (n = 83)	Placebo (n = 82)	*P*-value
Diarrhea	11 (13.3)	6 (7.3)	0.306
Dizziness	8 (9.6)	3 (3.7)	0.211
Constipation	6 (7.2)	2 (2.4)	0.277
Flatulence	4 (4.8)	0 (0)	0.120
Nausea	3 (3.6)	1 (1.2)	0.620
Skin rash	0 (0)	1 (1.2)	0.497
Peripheral numbness	1 (1.2)	0 (0)	1.000
Abdominal pain	1 (1.2)	1 (1.2)	1.000

APE, *Andrographis paniculata* ethanolic extract.

Table 7. Laboratory results of baseline and after treatment. Data are presented as n (%). analyzed *via* the Mann-Whitney U statistical test.

Laboratory results	Baseline						*P-value
	APE (n = 83)			Placebo (n = 82)			
	Normal	Higher within acceptable value	Higher than acceptable value	Normal	Higher within acceptable value	Higher than acceptable value	
AST	76 (91.6)	7 (8.4)	0 (0)	72 (87.8)	10 (12.2)	0 (0)	0.455
ALT	72 (86.7)	11 (13.3)	0 (0)	68 (82.9)	14 (17.1)	0 (0)	0.522
ALP	83 (100)	0 (0)	0 (0)	81 (98.8)	1 (1.2)	0 (0)	0.497
BUN	83 (100)	0 (0)	0 (0)	81 (98.8)	1 (1.2)	0 (0)	0.497
Cr	80 (96.4)	3 (3.6)	0 (0)	82 (100)	0 (0)	0 (0)	0.245
	After treatment						
AST	55 (66.3)	14 (16.9)	2 (2.4)	73 (89.0)	8 (9.8)	1 (1.2)	0.329
ALT	82 (98.8)	25 (30.1)	3 (3.6)	64 (78.1)	16 (19.5)	2 (2.4)	0.240
ALP	83 (100)	1 (1.2)	0 (0)	80 (97.6)	2 (2.4)	0 (0)	0.620
BUN	80 (96.4)	0 (0)	0 (0)	81 (98.8)	1 (1.2)	0 (0)	0.497
Cr	80 (96.4)	3 (3.6)	0 (0)	81 (98.8)	1 (1.2)	0 (0)	0.620

APE, *Andrographis paniculata* ethanolic extract; AST, aspartate aminotransferase; ALT, alanine transaminase; ALP, alkaline phosphatase; BUN, blood urea nitrogen; Cr, creatinine; *, between APE and placebo.

DISCUSSION

The principle of Thai traditional medicine uses plants with a bitter taste as antipyretic drugs, so *Andrographis paniculata* is used to reduce fever following the Thailand National List of Essential Medicine (NLEM) guidelines. It is a first-line drug used for the treatment of fever in Thailand and therefore was chosen for its potential to treat COVID-19-induced fevers. A preliminary study in COVID-19 determined that *Andrographis paniculata* can reduce fever but there was no reported clinical data. Thus, this study aimed to provide further clinical and scientific evidence for APE efficacy and safety in COVID-19 treatment. From the results of this investigation of APE in mildly afflicted COVID-19 patients, it was demonstrated that the WHO progression score was not statistically significantly different between APE and a placebo-controlled group; however, the APE indicated a lower incidence of participants who had disease progression during treatment than the control group. The study results further demonstrated that short-term oral high doses of APE decreased the severity of disease according to the WHO progression score results. This clinical efficacy is consistent with a previous study that demonstrated pneumonia occurred in 0/29 (0%) and 3/28 (10.7%) of patients receiving APE and placebo respectively with p=0.112 (13). It was also consistent with reports from the Department of Thai Traditional and Alternative Medicine, Ministry of Public Health, Thailand that patients who received APE had significantly less severe disease progression such as pneumonia and intubation than patients who did not receive APE. The study reports that 77 out of 526 (14.64%) patients who did not receive APE and 3 out of 309 patients (0.97%) treated with APE had increased severity of disease progression (11). However, the current study did not show statistically considerable differences in this sample size compared to the previous report.

This study demonstrated that APE did not induce a statistically significant difference from the controlled placebo group in supportive medicine as needed in terms of COVID-19 symptoms. APE showed significant relief of headache symptoms compared to the control group on day 1. Intriguingly, APE also has provided significant relief of olfactory loss symptoms from day 1 after treatment while the olfactory loss symptoms significantly improved in the placebo group after day 4 compared to day 0. This effect was not statistically significant when compared between groups, however, it remains possible that the APE could potentially assist with the relief of olfactory loss compared to the placebo group. The various known pharmacological activities of APE might relieve complex symptoms (14). Particularly, anosmia in COVID-19 patients is associated with downregulation of the olfactory nerve (15) with direct inflammation of olfactory epithelium (16). The APE anti-inflammatory inhibition of various cytokines related to olfactory sensation, *i.e.* immunomodulator and neuroprotective (17), could play a role in relieving olfactory loss. No reports are indicating the efficacy of andrographolide doses lower than 180 mg/day in COVID-19 patients. However, a report has indicated that andrographolide at a dosage of 60 mg/day, when used for 5 days, can alleviate symptoms of acute and uncomplicated upper respiratory tract infections (18,19). Furthermore, a higher dose of andrographolide, ranging from 180-360 mg/day and administered for 7 days, has been recommended for diseases characterized by more severe inflammation, such as pharyngotonsillitis (20). This study is consistent with previous reports of APE that demonstrate an oral dose of APE for five days could relieve mild symptoms and decrease disease severity (11,13). The orally short-term high dose of APE has good safety as measured by liver and renal function clinical chemistry. Some study participants who received APE had slight liver enzyme elevations, although this was also found in the control group and is commonly determined in COVID-19 patients. This study supports the previous pilot study report on the safety of APE which appears safe in short-term use (21). Given the potential of APE to induce severe inflammation and systemic effects, considering the appropriate dosage of andrographolide for COVID-19 treatment is of importance. In addition, four participants received anti-viral drugs during the

study combined with APE and they did not show any significant difference in liver and renal function laboratory values. The robust study of a combination of APE and anti-viral should be further examined in a larger clinical trial. Interestingly, the most common side effect of APE is diarrhea which contrasts with its traditional as an anti-diarrheal medicine. The possible reason explaining this phenomenon could be using a high dose of the APE which contains diterpene lactone as andrographolide (Fig. 1), and lack of appreciable tannins and other potential active ingredients in other formulations.

This study was conducted in July-September 2021 during the delta COVID-19 variant outbreak. There was no first-line drug treatment for COVID-19 treatment in patients with mild WHO symptomology which was a limitation of this study because supportive drugs were required for trial inclusion and asymptomatic and mild COVID cases were all inpatient. However, the supportive treatment used in the study is commonly recommended drugs such as acetaminophen and antihistamines which were given similarly to both groups of this study.

CONCLUSION

The high dose of APE has been used in COVID-19 patients for short periods without appreciable side effects. The intervention with AP provided COVID-19 patients with early to mild symptoms prompt relief of headache and symptoms especially, olfactory loss symptoms. A decrease in the severity and progression of the disease was not determined. AP extract requires more clinical evidence and experience to confirm its therapeutic role and efficacy for COVID-19 patients and further research on the influence on inflammation and immunomodulatory effects of cytokines in the blood of COVID-19 patients is needed.

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Conflicts of interest statement

The authors declared no conflict of interest in this study.

Authors' contributions

Conceptualization, P. Kanokkangsadal, N. Mukkasombat, C. Mingmalairak and A. Itharat; methodology, P. Kanokkangsadal, C. Mingmalairak, P. Kuropakornpong, T. Khawcharoenporn, I. Sakpakdeejaroen and A. Itharat; validation, P. Kanokkangsadal, A. Itharat, N. Mukkasombat and P. Kuropakornpong; formal analysis, N.M. Davies and A. Itharat; investigation, P. Kanokkangsadal, C. Mingmalairak, N. Mukkasombat, P. Kuropakornpong, P. Worawattananutai and A. Itharat; resources, P. Kanokkangsadal and A. Itharat; data curation, P. Kanokkangsadal, N.M. Davies, A. Itharat and N.M. Davies; writing original draft preparation, P. Kanokkangsadal, N.M. Davies and A. Itharat; writing—review and editing, P. Kanokkangsadal, N.M. Davies and A. Itharat; visualization, P. Kanokkangsadal, N.M. Davies and A. Itharat; supervision, A. Itharat; project administration, P. Kanokkangsadal and A. Itharat; funding acquisition, A. Itharat All authors have read and agreed to the published version of the manuscript.

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