RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2025.049

ScienceAsia 51 (3): 2025: ID 2025049: **1–9**

Phytochemical fingerprint analysis, anti-SARS-CoV-2, and anti-inflammatory activities of Ya Mor Harak formulation

Yupaporn Wichian[a,*], **Navaporn Pimrat**[a], **Hatairat Lerdsamran**[b], **Suteema Sawadpongpan**[b]

[a] Abhaibhubejhr College of Thai Traditional Medicine Prachin Buri, Faculty of Public Health and Allied Health Science, Praboromarajchanok Institute, Ministry of Public Health, Prachin Buri 25230 Thailand

[b] Center for Research Innovation and Biomedical Informatics, Faculty of Medical Technology, Mahidol University, Nakhon Pathom 73170 Thailand

*Corresponding author, e-mail: yupaporn@acttm.ac.th

Received 25 Sep 2024, Accepted 7 May 2025
Available online 15 Jun 2025

ABSTRACT: In Thai traditional medicine, Ya Mor Harak formulation (YMH) is used for fever-relief in cases of high fever symptoms similar to COVID-19. This study aimed to screen anti-SARS-CoV-2, anti-inflammatory activities, and phytochemical fingerprint analysis of YMH. Anti-SARS-CoV-2 activity was investigated by plaque reduction assay. Anti-inflammatory IL-6 inhibition was determined by ELISA. This study showed that three YMH extracts from deionized water (AYMH), 70% EtOH (70EYMH), and 95% EtOH (95EYMH) exhibited anti-SARS-CoV-2 activity by plaque reduction assay with the IC_{50} values of 159.20, 79.80, and 71.02 µg/ml, respectively. Three YMH extracts demonstrated inhibitory effects against the IL-6 inflammatory cytokine secretion. The phytochemical constituents of YMH showed seven major compounds, including gallic acid, corilagin, chebulagic acid, chebulinic acid, ellagic acid, nortiliacorinine A, and one unidentified compound ($C_{27}H_{22}O_{18}$). The ellagic acid was found to be the most abundant in 95EYMH, which was consistent with the highest antiviral effect of 95EYMH against SARS-CoV-2. However, the results of this study cannot elucidate the specific antiviral mechanism of YMH extract or identify the active compound responsible for this effect. Further research is essential to investigate these extracts' mechanisms and chemical compounds for antiviral activity against SARS-CoV-2.

KEYWORDS: Thai traditional medicine, SARS-CoV-2, COVID-19, anti-inflammatory activity, Ya Mor Harak

INTRODUCTION

Currently, antiviral medicine for the treatment of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection has side effects such as headaches, nausea, vomiting, and abdominal pain [1, 2]. Antipyretic medicines, anti-inflammatory drugs, and antibiotics are common targets for treating COVID-19 patients. Although there are currently approved antiviral drugs to treat COVID-19, some patients with mild to moderate symptoms in Thailand prefer to use various herbal medicines as alternatives for relieving symptoms, such as *Boesenbergia rotunda*, *Artemisia annua*, *Harrisonia perforata*, *Capparis micracantha*, *Tacca leontopetaloides*, *Phyllanthus emblica*, *Ficus carica*, *Tiliacora triandra*, and *Terminalia bilaria* [3].

YMH is a medical recipe specified in the traditional Thai medicine textbook Phaet Tambon, Volume 3, on various fevers. The liquid obtained from decoction has potent fever-relieving properties (Kaiphit) for lethargy, feeling hot inside the body, thirst, fidgeting, delirium, and unconsciousness. It eliminates internal heat, prevents fever with associated skin symptoms (Phitkan), and improves appetite [4]. These symptoms are consistent with the general symptoms of a viral infection, including COVID-19 symptoms. It has 21 herbal components which are shown in Table 1. According to these properties, YMH was used to treat COVID-19 patients at Thai Traditional Medicine Clinics of Abhaibhubejhr Thai Traditional Medicine College, Prachin Buri province, during the COVID-19 outbreak. A major cause of severe illness or death in COVID-19 patients is a cytokine storm [5, 6]. The infection of SARS-CoV-2 into the epithelial layer triggers an innate immune response, causes a decrease in lymphocytes and CD8 T cells [7]. Additionally, adaptive immunity induces the production of numerous cytokines, including interleukin-6 (IL-6), interleukin-1 (IL-1), and TNF, and stimulates T cells that have never been exposed to infection, causing tissues to be affected [8]. The cytokine storm is induced by increased levels of inflammatory cytokines, such as IL-6. IL-6 has been proposed as the most crucial component responsible for inflammation in COVID-19 patients [9]. The review of the herbal components of YMH has found properties such as anti-SARS-CoV-2, anti-inflammatory, nitric oxide inhibition, antipyretic effects of *T. triandra* [10, 11], antipyretic properties of aqueous extracts from *P. santalinus* [12], and immune-boosting effects (IgM, IgA, and IgG) of *P. emblica* [13]. The various activities of these herbal components of YMH could reduce the severity of COVID-19 and help patients recover faster. A previous review of YMH has studied its antioxidant activity, Thin Layer Chromatography (TLC) analysis, and phytochemical screening, which found that YMH contained compounds such as alkaloids, terpenoids,

Table 1 List of scientific names, family, parts used, voucher specimen, and proportion of YMH.

Scientific name	Family	Plant part	Voucher specimen	Proportion (%)
Tiliacora triandra	MENISPERMACEAE	root	BKF no.194782	3.77
Clerodendrum indicum	LAMIACEAE	root	BKF no.194783	3.77
Ficus racemosa	MORACEAE	root	BKF no.194784	3.77
Capparis micrantha	CAPPARACEAE	root	BKF no.194839	3.77
Harrisonia perforata	SIMAROUBACEAE	root	BKF no.195575	3.77
Pterocarpus santalinus	FABACEAE	heartwood	TTM-c No.1000723	3.77
Myristica fragrans	MYRISTICACEAE	heartwood	TTM-c No.1000724	3.77
Chrysopogon zizanioides	POACEAE	root	TTM-c No.1000725	3.77
Mesua ferrea	CLUSIACEAE	flower	TTM-c No.1000726	3.77
Terminalia chebula	COMBRETACEAE	fruit	TTM-c No.1000727	7.55
Terminalia arjuna	COMBRETACEAE	fruit	TTM-c No.1000728	7.55
Terminalia bellirica	COMBRETACEAE	fruit	TTM-c No.1000729	7.55
Phyllanthus emblica	EUPHORBIACEAE	fruit	TTM-c No.1000730	7.55
Gymnopetalum chinense	CUCURBITACEAE	fruit	TTM-c No.1000731	1.89
Pinus kesiya	PINACEAE	heartwood	TTM-c No.1000732	0.94
Ligusticum sinense	APIACEAE	rhizome	TTM-c No.1000733	0.94
Nelumbo nucifera	NELUMBONACEAE	pollen	TTM-c No.1000734	1.89
Azadirachta indica	MELIACEAE	petiole	TTM-c No.1000735	3.14
Tinospora crispa	MENISPERMACEAE	vine	TTM-c No.1000736	1.60
Cassia fistula	LEGUMINOSAE	meat in the pod	TTM-c No.1000737	22.64
Bridelia ovata	PHYLLANTHACEAE	leaf	TTM-c No.1000738	2.83

coumarins, anthraquinones, and tannins [14].

However, YMH formulation has never been investigated for antiviral activity against SARS-CoV-2, anti-inflammatory, and phytochemical fingerprint identification. Therefore, this study aims to screen anti-SARS-CoV-2 and anti-IL-6 activities as well as identify the phytochemical fingerprint of YMH.

MATERIALS AND METHODS

Plant materials

The 21 plant components of YMH were purchased from traditional drug stores in Bangkok and Prachin Buri, Thailand, in March 2022 (Table 1). All plants were identified macroscopically by comparison with voucher specimens kept at the Forest Herbarium, National Park Wildlife and Plant Conservation Department, Ministry of Natural Resources and Environment, Thailand, and the Thai Traditional Medicine Herbarium, Department of Thai Traditional and Alternative Medicine, Ministry of Public Health, Thailand.

Extract preparation

Plant materials of YMH were cleaned and dried in a hot air oven at 60 °C. YMH components were mixed according to the traditional ratio shown in Table 1. YMH was extracted by 3 methods: decoction with deionized water (AYMH), maceration with 70% EtOH (70EYMH), and maceration with 95% EtOH (95EYMH). For decoction, 500 g of YMH powder was boiled in 3 l of deionized water until 1/3 remained (repeated 3 times) and filtered with Whatman No. 1 filter paper; the combined filtrate was freeze-dried. For maceration, 1000 g of YMH powder was mixed with 5 l of 70% EtOH or 95% EtOH for 3 days and filtered with Whatman No. 1 filter paper (repeated 3 times); the combined filtrate was dried with an evaporator [14]. The weight of each extract was calculated as a percentage yield. All extracts were stored at −20 °C until use.

Sample preparation

For the three crude extracts, AYMH was dissolved in deionized water at a concentration of 1 mg/ml, while 70EYMH and 95EYMH were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 1 mg/ml to prepare stock solutions. These solutions were stored at −20 °C until used in the assays.

Determination of IL-6 inflammatory cytokine inhibition by enzyme-linked immunosorbent assay (ELISA)

RAW 264.7 cells (ATCC, TIB-71™) were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS) and 1% (v/v) antibiotic (Gibco®, NY, USA). Cells were seeded in a 96-well plate at a density of 2×10^5 cells/well and incubated at 37 °C with 5% CO_2 for 24 h. YMH extracts at concentrations of 0.156, 0.313, and 0.625 mg/ml (non-cytotoxic) were added and incubated for 24 h. Subsequently, lipopolysaccharide (LPS) at a concentration of 10 mg/ml was added and incubated for an additional 24 h. The supernatant was then transferred to new 96-well plates, mixed with 50 µl of human IL-6 biotinylated antibody reagent (Abcam®, USA) and 50 µl of the sample, and incubated for 2 h at room temperature.

One hundred µl of streptavidin-HRP solution

(Abcam®) was added and incubated at room temperature for 30 min, followed by three washes with wash buffer. One hundred µl of TMB substrate solution (Abcam®) was added to each well and incubated at room temperature for 30 min. Finally, 100 µl of stop solution was added, and the optical density (OD) was measured at 450 nm using a microplate reader.

Investigation of anti-SARS-CoV-2 activity

Test virus: This study investigated the anti-SARS-CoV-2 activity of YMH using SARS-CoV-2 Variant 20I (Alpha, V1) or B.1.1.7 which was isolated from a clinical sample of a COVID-19 patient in Thailand. This virus was isolated and propagated in Vero cells (ATCC, CCL-81) maintained in Eagle's Minimum Essential Medium (EMEM) (Gibco®, Life TechnologiesTM, NY, USA) supplement with L-glutamine, antibiotic, and 2% FBS under humidified at 37 °C with 5% CO_2 condition in BSL-3 laboratory (the researcher received a license to produce pathogens and animal toxins from the Department of Medical Science, Ministry of Public Health, Thailand). After isolation and propagation, the isolated virus was identified by nucleotide sequencing and determined for virus titer by 50% tissue culture infection dose ($TCID_{50}$) assay in Vero cells. The aliquots of virus stock were stored at below −70 °C until used. However, before investigating the antiviral activity through plaque reduction assay, the virus stock was titrated to determine the viral titer in PFU/ml using a plaque assay in Vero cells.

Cytotoxicity assay: Three samples of YMH were screened for cytotoxicity on Vero cells using an MTS-based cytotoxicity assay with the CellTiter 96® AQueous One Solution Cell Proliferation Assay kit (Promega, USA). Briefly, monolayer cells in 96-well plates were treated with serial 2-fold dilutions of YMH extracts starting from 500 to 0.976 µg/ml in triplicate wells, and untreated cells served as mock controls. Cells were incubated at 37 °Cwith 5% CO_2 for 3 days, then MTS solution was added into each well and incubated under the same conditions for 3 h. Cell viability was determined by measuring the OD of the formazan product in each reaction well at 490 nm. Tests were performed in three independent experiments. The 50% cytotoxicity concentration (CC_{50}) was calculated by comparing the percentage of viable cells treated with YMH extracts to untreated control cells.

Antiviral testing by plaque reduction assay: Plaque reduction assay is the gold standard phenotypic method for *in vitro* antiviral susceptibility testing. Each YMH extract was included throughout the virus replication process for antiviral activity screening, including pre-treatment, co-treatment, and post-treatment. Briefly, confluent Vero cell monolayers were prepared in 12-well plates and pre-treated with various concentrations (at % cell viability ⩾50%) of the YMH extracts at 37 °C with 5% CO_2 for 1 h. Then, the test virus SARS-CoV-2 at a concentration of 50 PFU/well was added to each well and incubated for co-treatment at 37 °C with 5% CO_2 for 1 h [15, 16]. After that, the mixture was removed, and infected cells were overlaid with 1.2% Avicel containing the test compound for post-treatment and further incubated for 72 h. After fixing with 10% formaldehyde and staining with 1% crystal violet, viral plaques in each well were visually counted by the naked eye, and the CTL Analyzers, LLC (Model: S6 Universal V) was used to confirm the plaque counts when the morphology of plaque separation was unclear (Fig. S1). The percentage of inhibition at each concentration was calculated compared to untreated virus controls. Virus control (without drug) and cell control (without drug and virus) were run in parallel. The 50% inhibition concentration (IC_{50}) is the concentration of YMH extract that reduces the number of viral plaques by 50% compared to the untreated virus control.

Each YMH extract was analyzed in triplicate experiments and expressed as an average with standard deviation (SD) values. CC_{50} and IC_{50} were calculated using GraphPad Prism 10.0 Software. The selection index (SI) is the CC_{50}/IC_{50} ratio that measures the window between cytotoxicity and antiviral activity of YMH extracts.

Phytochemical characterization by ultra performance liquid chromatography coupled with diode array detector and tandem mass spectrometry (LC-DAD-MS/MS) method

All YMH extracts were analyzed using an UPLC-UV-Q-Orbitrap performed on a Vanquish UPLC system (Thermo Fisher Scientific Inc., USA) equipped with the Binary Pump F, the Split Sampler FT, Column Compartment H, and Diode Array Detector FG, coupled with an Orbitrap Exploris™ 120 mass spectrometer. The separation was done on a BDS Hypersil C18 column (50 × 2.1 mm i.d., 2.4 µm). The mobile phases were (A) 0.1% formic acid (Fish Scientific Inc.) in water and (B) 0.1% formic acid in methanol (Fish Scientific Inc.). A mobile phase program was set up with gradient elution with 100% A for 2 min, linear increasing from 0% to 100% B in A for 8 min, then 100% B for 2 min. The column temperature was controlled at 25 °C with a constant 1.0 ml/min flow rate. A Diode Array Detector (DAD) was set at the wavelength of 254 nm. The injection volume setting was 2 µl for all extracts.

Liquid chromatography-mass spectrometry (LC-MS) method

Mass spectrometry analysis was carried out by positive and negative mode internal mass calibration EASY-IC™. The ion source type was Heated-ESI. The spray voltage was set in static mode with positive ion 3500 V, and negative ion 2500 V. Nitrogen gas mode was used in static mode with flow settings of sheath gas 60 Arb,

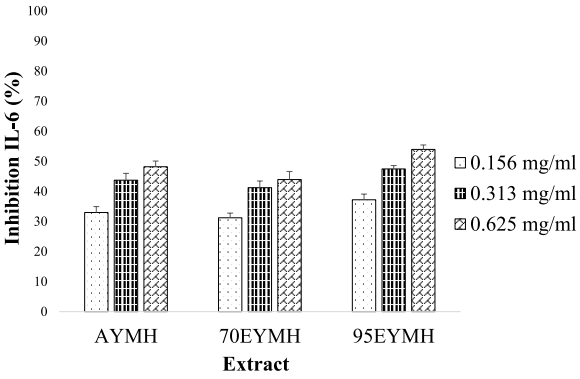


Fig. 1 Inhibition of IL-6 inflammatory cytokines by three YMH extracts as determined by ELISA.

Aux gas 15 Arb, and sweep gas 2 Arb. The ion transfer tube temperature was 350 °C. The vaporizer temperature was 350 °C. The full scan mode range was 200–1000 m/z with a resolution of 60000 and RF Len 70%. The ddMS^2 mode was triggered with an intensity threshold of 5.0×10^5. The MS^2 parameters were an isolation window of 1.5 m/z, a collision energy type that was normalized, an orbitrap resolution of 15000, and a scan range mode that was automatic.

The instrument was controlled, and the data were analyzed by Chromeleon™ Chromatography Data System software. The recorded chromatogram was visualized and analyzed using FreeStyle software. Compounds were identified or tentatively identified by their mass spectral data. The mass spectrum compounds were analyzed for molecular formula, molecular weight, and named compounds that were delivered up-to-date by the mZcloud database.

RESULTS AND DISCUSSION

The 21 herbarium specimens and the ratio of the YMH formulation according to traditional knowledge are shown in Table 1. The percentage of yield of AYMH, 70EYMH, and 95EYMH was 18.05, 31.67, and 16.87%, respectively.

Inhibition of IL-6 inflammatory cytokine by YMH

The macrophage cell line (RAW 264.7) was treated with AYMH, 70EYMH, and 95EYMH samples at different concentrations of 0.156, 0.313, and 0.625 mg/ml for 24 h, and analyzed for IL-6 secretion by ELISA. All three extracts had inhibitory effects of low to high concentrations. Cells stimulated with LPS exhibited an IL-6 cytokine level of 16.28 ± 0.61 pg/ml. In the control group, where cells were not stimulated with LPS, the expression level was 4.09 ± 1.53 pg/ml. Meanwhile, cells stimulated with LPS and treated with all three extracts at a concentration of 0.625 mg/ml showed IL-6 cytokine levels of 8.43 ± 0.31, 9.13 ± 0.19, and 7.50 ± 0.24 pg/ml, respectively, which were lower than those observed in cells stimulated with LPS alone. Additionally, these treatments exhibited an inhibitory effect on IL-6 cytokine secretion, with inhibition values of $48.19 \pm 1.89\%$, $43.94 \pm 1.14\%$, and $53.93 \pm 1.50\%$, respectively (Fig. 1).

Cytotoxicity effects of YMH

The percentage cytotoxicity of YMH extracts on Vero cells was determined by MTS assay (Fig. 2). The percentage cytotoxicity <30% is considered non-cytotoxic. At the highest concentration of 500 µg/ml, AYMH showed a cytotoxicity value <30%, which was considered non-toxic to Vero cells. The CC_{50} value of each extract was calculated. The CC_{50} values of AYMH, 70EYMH, and 95EYMH were >500, 186.10, and 125.10 µg/ml, respectively. AYMH extract showed the highest CC_{50} value >500 µg/ml, which indicated the lowest cytotoxicity compared with the ethanol extracts.

Antiviral activities of YMH against SARS-CoV-2

The antiviral activities of three YMH extracts against SARS-CoV-2 were assessed *in vitro* by plaque reduction assay (Fig. 3, Table S1, and Table S2). Dose-response curves for the antiviral activities of each compound against SARS-CoV-2 are shown in Fig. 4. The IC_{50} of AYMH, 70EYMH, and 95EYMH were 159.20, 79.80, and 71.02 µg/ml, respectively. The SI value was also determined for each extract. Among the three extracts, AYMH showed the highest SI value >3.14, followed by 70EYMH (SI = 2.33) and 95EYMH (SI = 1.76), respectively.

The results of this study revealed that all three extracts from the Thai traditional medicine "Ya Mor Harak", using three different solvents exhibited antiviral activity against SARS-CoV-2. YMH extracted by 95% EtOH exhibited the highest anti-SARS-CoV-2 activity (IC_{50} = 71.02 µg/ml). Specifically, the decoction of YMH that was prepared by boiling in water (AYMH) according to the traditional method described in "Tamra Phrae Tambon Volume 3" [4] showed anti-SARS-CoV-2 activity with an IC_{50} value of 159.20 µg/ml. Additionally, at the highest concentration tested of 500 µg/ml, AYMH was considered non-toxic to Vero cells, showing the highest SI value of >3.14. While, using EtOH extraction demonstrated higher cytotoxicity to the cells, this toxicity was also shown in plaque assay at concentrations of 250 and 500 µg/ml (Fig. 3). These research findings preliminarily confirm the traditional use of "Ya Mor Harak" in Thai medicine, indicating that it possesses antiviral properties and is non-toxic to cells. However, it is important to note that this antiviral effect was observed in screening assays only, and the specific processes or mechanisms responsible for viral inhibition have not yet been elucidated.

Therefore, future research should focus on elu-

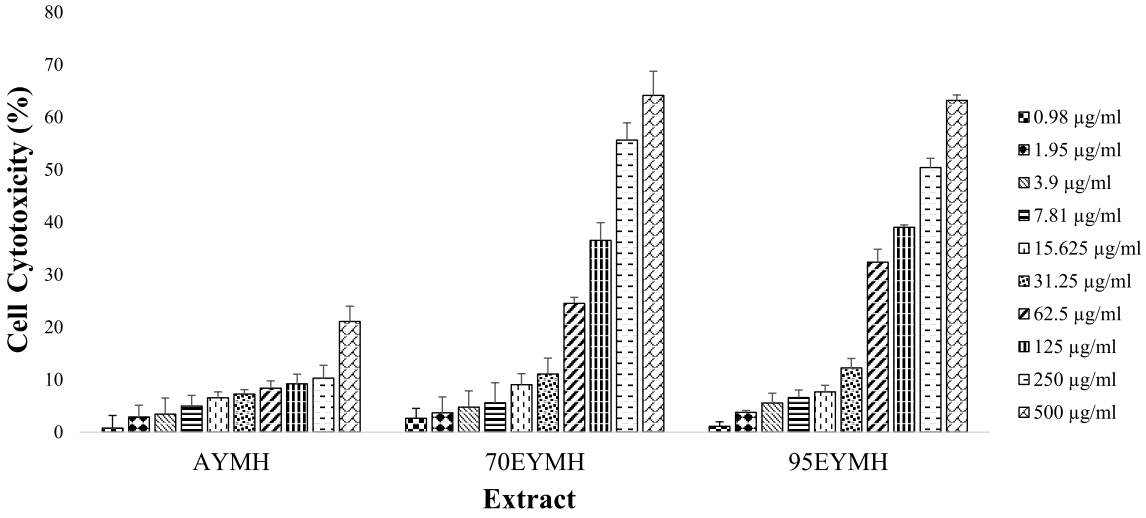


Fig. 2 The cytotoxicity of YMH extracts on Vero cells as determined by MTS assay.

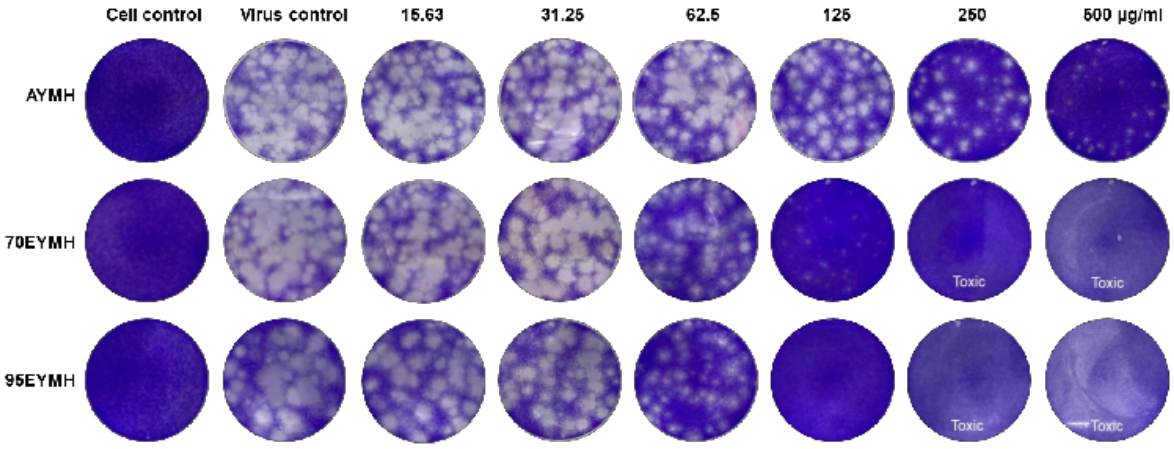


Fig. 3 Anti-SARS-CoV-2 activities of YMH extracts by plaque reduction assay.

cidating the mechanism of antiviral activity of these extracts. Additionally, *in vivo* studies are necessary to evaluate their efficacy and safety profiles. This includes the protease inhibitors that are important in anti-SARS-CoV-2 activity. It has been report that herbal plants or natural products have the ability to resist proteases and thus predicted to have potential antiviral activity [17]. These steps are crucial before proceeding

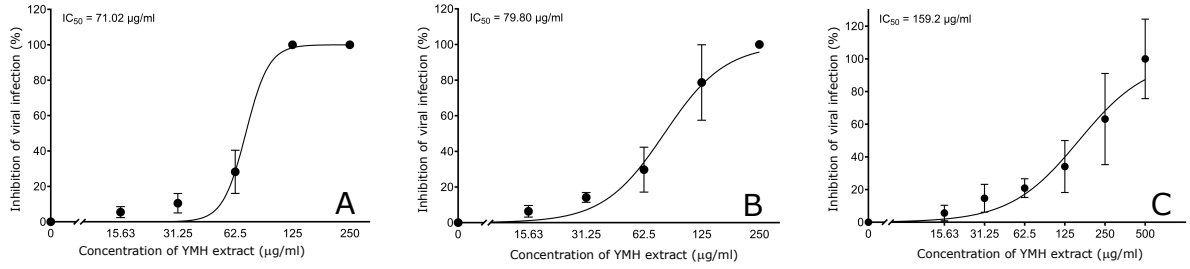


Fig. 4 Dose-response curve of YMH extracts for reduction of SARS-CoV-2 infection. The IC_{50} values of 95EYMH (A), 70EYMH (B), and AYMH (C) were indicated.

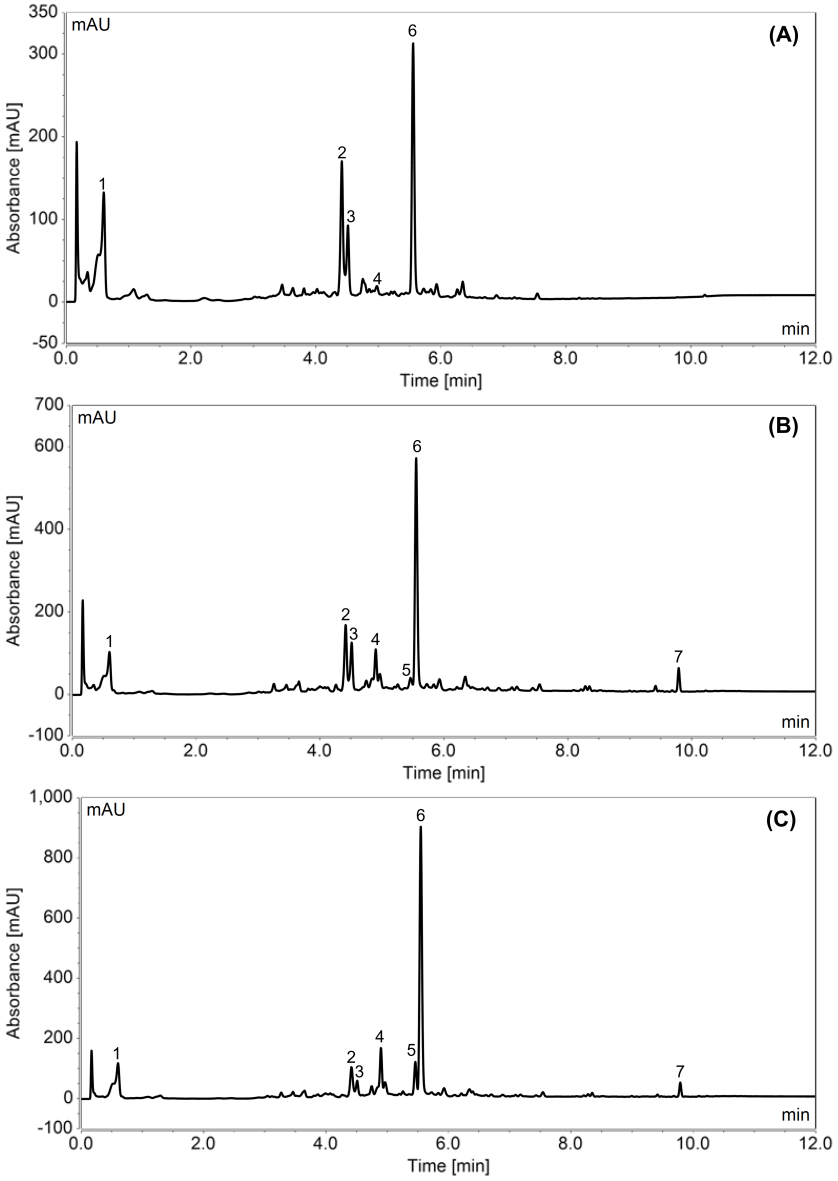


Fig. 5 UPLC-UV chromatogram of AYMH (A), 70EYMH (B), and 95EYMH (C) at a concentration of 1 mg/ml. Absorbance detection was at 254 nm.

to clinical trial research.

Fingerprint analysis by UPLC-UV-MS/MS

To get a comprehensive phytochemical profile, UPLC-UV-MS/MS analysis was conducted. The UV detector could measure a chromophore and cursorily compare the amounts, while the mass detector relied on ionizable compounds. Using these hyphenated methods, extensive phytochemical data could be achieved. In this study, seven major compounds were detected in YMH by the using UPLC-UV method, and their structures were tentatively identified using their mass spectra and co-chromatography with an authentic standard. They revealed gallic acid (1), corilagin (2), chebulagic acid (4), chebulinic acid (5), ellagic acid (6), and nortiliacorinine A (7), as shown in the UPLC-UV chromatogram in Fig. 5. One unidentified compound, $C_{27}H_{22}O_{18}$ (3), was found in all three extracts of YMH. Among these compounds, ellagic acid showed the highest peak area of 37.79, 20.20, and 16.97 in 95EYMH, 70EYMH, and AYMH, respectively, as shown in Table 2. The highest peak area of ellagic acid correlated with the highest anti-SARS-CoV-2 activity of 95EYMH. This might indicate that ellagic acid plays an important role in the anti-SARS-CoV-2 antiviral effect of YMH.

Table 2 Peak area of compounds of YMH at a concentration of 1 mg/ml.

No.	R/T	Compound	Peak area		
			A	70E	95E
1	0.60	Gallic acid	8.8826	6.0304	7.4803
2	4.42	Corilagin	8.5512	8.3822	5.3729
3	4.52	Unidentified	4.5813	6.2121	2.6297
4	4.90	Chebulagic acid	1.1351	4.8591	7.1593
5	5.46	Chebulinic acid	ND	2.3837	3.7556
6	5.56	Ellagic acid	14.8125	27.3356	43.6169
7	9.80	Nortiliacorinine A	ND	2.0190	1.6673

ND = Not detected, R/T = Retention Time, A = AYMH, 70E = 70EYMH, 95E = 95EYMH.

Table 3 Identification of phytochemical constituents in YMH extract by LC-MS/MS.

No.	R/T	Compound	Extraction			Mass data		MW
		(Molecular formula)	A	70E	95E	Mode	Base peak (m/z)	
2	4.43	Corilagin ($C_{27}H_{22}O_{18}$)	✓	✓	✓	Negative	[MS1] 633.0721 [MS2] 633.0734 → 300.9991	MW = 634 Calc for $[M+Na]^+$ = 657.06983
						Positive	[MS1] 657.0696	Calc for $[M-H]^-$ = 633.07334
3	4.52	Unidentified ($C_{27}H_{22}O_{18}$)	✓	✓	✓	Negative	[MS1] 635.0880 [MS2] 635.0883 → 483.0779, 465.0681, 169.0143	MW = 636 Calc for $[M+Na]^+$ = 659.08548 Calc for $[M-H]^-$ = 635.08899
						Positive	[MS2] 659.0853	
4	4.90	Chebulagic acid ($C_{41}H_{30}O_{27}$)		✓	✓	Negative	[MS1] 476.0412 (double charged) [MS2] 476.0412 → 300.9988, 169.0143	MW = 954 Calc for $[M-2H]^{2-}$ = 476.04145 Calc for $[M+Na]^+$ = 977.08667
						Positive	[MS1] 977.0853 (low signal)	
5	5.46	Chebulinic acid ($C_{41}H_{32}O_{27}$)		✓	✓	Negative	[MS1] 477.0489, 955.1045 [MS2] 477.0488 → 169.0142, 202.0789, 125.0245 [MS2] 955.1046 → 169.0142, 205.0506, 275.0197, 465.0675, 617.0786	MW = 956 Calc for $[M-H]^-$ = 955.10582 Calc for $[M-2H]^{2-}$ = 477.04927 Calc for $[M+Na]^+$ = 979.10232
						Positive	[MS1] 787.0986, 819.1251, 979.1021	
6	5.56	Ellagic acid ($C_{14}H_6O_8$)	✓	✓	✓	Negative	[MS1] 300.9987 [MS2] 300.9991 → 202.0789	MW = 302 Calc for $[M-H]^-$ = 300.99899
7	9.80	Nortiliacorinine A ($C_{35}H_{34}N_2O_5$)	✓	✓	✓	Negative	[MS1] 561.3221 [MS2] 561.3223 → 433.2392, 125.0610	MW = 562 Calc for $[M-H]^-$ = 561.23950 Calc for $[M+H]^+$ = 563.25405
						Positive	[MS1] 563.3366, 585.3185 [MS2] 563.3371 → 223.0965, 281.0807, 353.1385 [MS2] 585.3185 → 393.1310, 457.2344, 516.2490	Calc for $[M+Na]^+$ = 585.23599

R/T = Retention time, MW = Molecular weight, A = AYMH, 70E = 70EYMH, 95E = 95EYMH.

The fingerprint of YMH by the LC-MS/MS method could tentatively identify 6 compounds, including corilagin (2), chebulagic acid (4), chebulinic acid (5), ellagic acid (6), nortiliacorinine A (7), and unidentified (3), as shown in Table 3. Their mass spectral data were presented in Fig. S2-1 to Fig. S2-6. Gallic acid was detected by only the UPLC-UV method, because gallic acid with a molecular weight of 170 Da could not be detected in LC-MS due to the mass setting of 200–1000 m/z. It was identified using co-chromatography with an authentic standard and confirmed with its UV-spectrum. An unidentified compound ($C_{27}H_{22}O_{18}$) with a molecular weight of 636 Da (Calc for $[M+Na]^+$ = 659.08548, Calc for $[M-H]^-$ = 635.08899) could not be identified in this study. It is noted that identification using their mass spectrum could not

distinguish between isomers. However, due to the biosynthesis capabilities of plants, the structures could be indubitable.

Seven compounds of the YMH extracts were identified by chemical fingerprint analysis. Ellagic acid was found to be most abundant in *M. fragrans* (Nutmeg) [18], *T. chebula*, and *P. emblica* [19]. The content of ellagic acid in AYMH, 70EYMH, and 95EYMH extracts showed a consistent trend with their antiviral activity; 95EYMH exhibited the highest ellagic acid content and displayed the highest antiviral activity. A previous study demonstrated that ellagic acid inhibits the binding of spike glycoprotein and human angiotensin-converting enzyme 2 (ACE2) with an IC_{50} value of 2.5 µg/ml but showed no potential for antiviral activity in a plaque reduction assay [20]. Therefore, only the high ellagic acid content cannot explain the antiviral activity of YMH extracts against SARS-CoV-2 [21]. Although ellagic acid may not directly possess antiviral activity, it has been found to have other beneficial effects, such as anti-nociceptive [22] and antipyretic [23] properties, which may assist in the recovery of COVID-19 patients. In addition to the high ellagic acid content, this study also identified chebulagic acid in the YMH extracts. Previous research has shown that chebulagic acid exhibits antiviral activity against SARS-CoV-2 with an IC_{50} value of 9.76 ± 0.42 µM [24]. However, this study cannot definitively determine which specific chemical compound is responsible for the antiviral activity of YMH extract against SARS-CoV-2. Further research is necessary to identify the active compound(s) in YMH extract responsible for its antiviral activity against SARS-CoV-2.

The chemical compound analysis of the YMH extract found six identifiable chemicals were gallic acid, corilagin, chebulagic acid, chebulinic acid, ellagic acid, and nortiliacorinine A. Additionally, an unidentified compound with the molecular formula $C_{27}H_{22}O_{18}$ was detected in all three solvent extracts, with the highest concentration found in the AYMH. This compound ($C_{27}H_{22}O_{18}$) could not be identified since its data were not found in existing databases or literature. Therefore, future research should focus on the isolation and characterization of this compound to elucidate its structure and potential biological activities.

The YMH extracts from two different extraction methods exhibited different activities and chemical fingerprints. Macerate extraction with 70% EtOH and 95% EtOH showed stronger anti-SARS-CoV-2 and anti-IL-6 activities compared to decoction. However, the decoction of the traditional method for Thai medicine showed anti-SARS-CoV-2 activity and this study's findings are interesting as AYMH has a high level of safety. Among the 6 compounds identified in YMH, ellagic acid was found in the highest concentration. Literature has reported that ellagic acid increases innate immunity [25]. Decoction is an appropriate method for extracting YMH. This traditional Thai Medicine method, which is still used today for dispensing to patients in the form of a decoction, is suitable for achieving antiviral effects while ensuring safety.

CONCLUSION

The "Ya Mor Harak" demonstrated anti-SARS-CoV-2 activity. The AYMH extract showed the highest safety profile. Phytochemical fingerprint analysis of the YMH extract revealed seven compounds: gallic acid, corilagin, chebulagic acid, chebulinic acid, ellagic acid, nortiliacorinine A, and an unidentified compound ($C_{27}H_{22}O_{18}$). The results from this study cannot elucidate the specific mechanism by which the YMH extract exhibits antiviral activity and also cannot identify the exact active compound responsible for this effect. Further studies should be designed to investigate these extracts' mechanisms and chemical compounds for antiviral activity against SARS-CoV-2.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at https://dx.doi.org/10.2306/scienceasia1513-1874.2025.049.

Acknowledgements: This work was financially supported by the Fundamental Fund (FF) grant under Thailand Science Research and Innovation (TSRI) via Praboromarajchanok Institute, Ministry of Public Health, Thailand.

REFERENCES

1. Singh AK, Singh A, Singh R, Misra A (2021) Molnupiravir in COVID-19: A systematic review of literature. *Diabetes Metab Syndr* **15**, 102329.
2. Ergür FÖ, Yıldız M, Şener MU, Kavurgac S, Ozturk A (2022) Adverse effects associated with favipiravir in patients with COVID-19 pneumonia: a retrospective study. *Sao Paulo Med J* **140**, 372–377.
3. Nithikathkul C, Kijphati R, Krates J, Junto E, Thessingha C, Aneksak W, Amornmahaphun S, Pholputta L, et al (2023) Geographic information database of herbs against COVID-19 in Thailand: The medicinal plants those folk healers commonly used for treatment and boosting people's immunity. *Int J Geoinformatics* **19**, 67–82.
4. Wisutthatipphadee PP (Suntorawech S) (1927) *Phaet Tambon Volume 3*, Bangkok, Thailand.
5. Hu B, Huang S, Yin L (2021) The cytokine storm and COVID-19. *J Med Virol* **93**, 250–256.
6. Zeng F, Huang Y, Guo Y, Yin M, Chen X, Xiao L, Deng G (2020) Association of inflammatory markers with the severity of COVID-19: A meta-analysis. *Int J Infect Dis* **96**, 467–474.
7. Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S, Huang H, Zhang L, et al (2020) Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. *JAMA Intern Med* **180**, 934–943.
8. Monteleone G, Sarzi-Puttini P, Ardizzone S (2020) Preventing COVID-19-induced pneumonia with anticytokine therapy. *Lancet Rheumato* **2**, e255–e256.

9. Aziz M, Fatima R, Assaly R (2020) Elevated interleukin-6 and severe COVID-19: A meta-analysis. *J Med Virol* **92**, 2283.
10. Nutmakul T (2021) Phytochemical and pharmacological activity of *Tiliacora triandra* (Colebr.) Diels. *Songklanakarin J Sci Technol* **43**, 1264–1274.
11. Radapong S, Sahad T, Harnkit N, Suppajariyawat P, Okada PA, Meechalad W, Sincharoenpokai P, Niumsakul S, et al (2022) Anti-SARS-CoV-2 activity screening of the selected Thai medicinal plants and potential host-target molecules. *Bull Dept Med Sci* **64**, 93–105.
12. Shanti VCN, Bibu JK, I'ma N (2019) Antipyretic activity of aqueous extract of heart wood of *Pterocarpus santalinus* L. in yeast induced pyrexia. *J Pharmacogn Phytochem* **8**, 244–246.
13. Nguse M, Yang Y, Fu Z, Xu J, Ma L, Bu D (2022) *Phyllanthus emblica* (Amla) fruit powder as a supplement to improve preweaning dairy calves' health: effect on antioxidant capacity, immune response, and gut bacterial diversity. *Biology (Basel, Switz)* **11**, 1753.
14. Wichian Y, Pimrat N, Lairungruang K (2024) Quality control and antioxidant activities of Ya Mor Harak formulation. *J Thai Trad Alt Med* **22**, 164–177.
15. Nowakowska A, Choi H, Park K, Kim J, Jang Y, Chun J, Kim YB, Lee HJ (2022) *In vitro* antiviral activity of remdesivir against SARS-CoV-2 and its variants. *J Bacteriol Virol* **52**, 149–159.
16. Hu Y, Lewandowski EM, Tan H, Zhang X, Morgan RT, Zhang X, Jacobs LMC, Butler SG, et al (2023) Naturally occurring mutations of SARS-CoV-2 main protease confer drug resistance to nirmatrelvir. *ACS Cent Sci* **9**, 1658–1669.
17. Sanachai K, Wilasluck P, Deetanya P, Wangkanont K, Rungrotmongkol T, Hannongbua S (2023) Targeting the main protease for COVID-19 treatment: A comprehensive review of computational screening and experimental studies. *ScienceAsia* **49**, 477–484.
18. Odubanjo VO, Olasehinde TA, Oyeleye SI, Oboh G, Boligon AA (2018) Seed extracts from *Myristica fragrans* (Nutmeg) and *Moringa oleifera* (Drumstick tree) inhibits enzymes relevant to erectile dysfunction and metal-induced oxidative damage in rats' penile tissues. *J Food Biochem* **42**, e12452.
19. Ríos JL, Giner RM, Marín M, Recio MC (2018) A pharmacological update of ellagic acid. *Planta Med* **84**, 1068–1093.
20. David AB, Diamant E, Dor E, Barnea A, Natan N, Levin L, Chapman S, Mimran LC, et al (2021) Identification of SARS-CoV-2 receptor binding inhibitors by *in vitro* screening of drug libraries. *Molecules* **26**, 3213.
21. Sharopov FS, Salimov A, Numonov S, Bakri M, Sangov Z, Habasi M, Aisa HA, Setzer WN (2020) Chemical compositions and biological activities of essential oils: phytochemical study on the essential oils of tarragon (*Artemisia dracunculus* L.) growing in Tajikistan and its comparison with the essential oil of the species in the rest. *Nat Prod Commun* **15**, 1–7.
22. Abbasi M, Mansouri MT, Naghizadeh B, Ghorbanzadeh B (2018) Ellagic acid enhances the anti-nociceptive effects of cyclooxygenase inhibitors in a mouse visceral pain model. *Jundishapur J Physiol* **1**, e148265.
23. Xie F, Xu L, Zhu H, Li Y, Nong L, Chen Y, Zeng Y, Cen S (2022) Serum metabolomics based on GC-MS reveals the antipyretic mechanism of ellagic acid in a rat model. *Metabolites* **12**, 479.
24. Du R, Cooper L, Chen Z, Lee H, Rong L, Cui Q (2021) Discovery of chebulagic acid and punicalagin as novel allosteric inhibitors of SARS-CoV-2 3CLpro. *Antiviral Res* **190**, 105075.
25. Promsong A, Chuerduangphui J, Levy CN, Hladik F, Satthakarn S, Nittayananta W (2024) Effects of ellagic acid on vaginal innate immune mediators and HPV16 infection *in vitro*. *Molecules* **29**, 3630.

Appendix A. Supplementary data

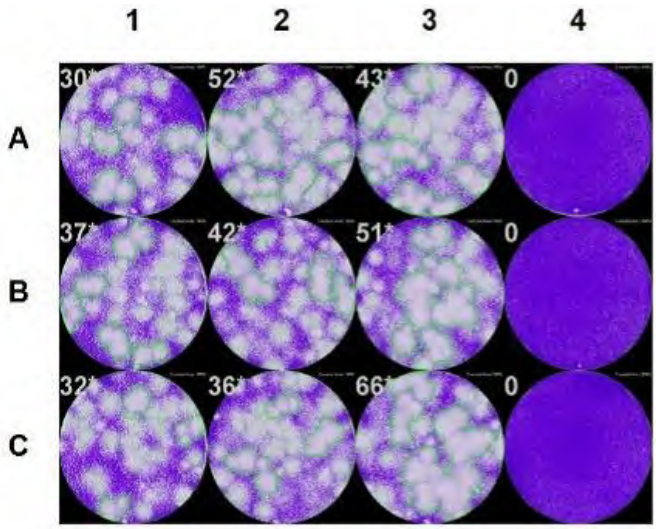
Table S1 Number of plaques on plaque reduction assay.

		Number of plaques at various YMH concentration (µg/ml)						0
Exp.	**Compound**	**500**	**250**	**125**	**62.5**	**31.25**	**15.625**	**(Control)**
Exp. 1	**AYMH**	0	12	48	43	46	51	57
			13	40	47	47	46	50
			24	36	46	46	51	56
	Average	0.00	16.33	41.33	45.33	46.33	49.33	54.33
	% inhibition	100.00	69.94	23.93	16.56	14.72	9.20	0.00
	70EYMH		0	0	30	52	43	56
			0	0	37	42	51	52
			0	0	32	36	66	54
	Average		0.00	0.00	33.00	43.33	53.33	54.00
	% inhibition		100.00	100.00	38.89	19.75	1.23	0.00
	95EYMH		0	0	45	52	65	63
			0	0	45	51	56	64
			0	0	49	60	62	68
	Average		0.00	0.00	46.33	54.33	61.00	65.00
	% inhibition		100.00	100.00	28.72	16.41	6.15	0.00
Exp. 2	**AYMH**	7	25	29	46	43	57	54
		3	20	36	44	48	46	57
		8	18	28	37	44	56	53
	Average	6.00	21.00	31.00	42.33	45.00	53.00	54.67
	% inhibition	89.02	61.59	43.29	22.56	17.68	3.05	0.00
	70EYMH		0	14	35	52	56	54
			0	12	41	53	59	65
			0	12	36	50	56	57
	Average		0.00	12.67	37.33	51.67	57.00	58.67
	% inhibition		100.00	78.41	36.36	11.93	2.84	0.00
	95EYMH		0	0	18	42	46	51
			0	0	39	44	40	49
			0	0	31	47	49	47
	Average		0.00	0.00	29.33	44.33	45.00	49.00
	% inhibition		100.00	100.00	40.14	9.52	8.16	0.00
Exp. 3	**AYMH**	20	46	45	44	46	53	54
		21	38	45	51	60	55	58
		25	40	48	51	55	57	56
	Average	22.00	41.33	46.00	48.67	53.67	55.00	56.00
	% inhibition	60.71	26.19	17.86	13.10	4.17	1.79	0.00
	70EYMH		0	24	45	46	48	50
			0	23	48	42	50	53
			0	20	41	49	49	55
	Average		0.00	22.33	44.67	45.67	49.00	52.67
	% inhibition		100.00	57.59	15.19	13.29	6.96	0.00
	95EYMH		0	0	37	47	49	47
			0	0	42	43	47	49
			0	0	44	48	47	50
	Average		0.00	0.00	41.00	46.00	47.67	48.67
	% inhibition		100.00	100.00	15.75	5.48	2.05	0.00

Exp. 1, 2, and 3 are the three repetitions of the experiment.

Table S2 Calculation of the percentage of inhibition and standard deviation (S.D.).

	% Inhibition at various YMH concentration (µg/ml)						0
Compound	**500**	**250**	**125**	**62.5**	**31.25**	**15.625**	**(Control)**
AYMH	100.00	69.94	23.93	16.56	14.72	9.20	0.00
	89.02	61.59	43.29	22.56	17.68	3.05	0.00
	60.71	26.19	17.86	13.10	4.17	1.79	0.00
Average	83.25	52.57	28.36	17.41	12.19	4.68	0.00
S.D.	20.27	23.23	13.28	4.79	7.11	3.97	0.00
70EYMH		100.00	100.00	38.89	19.75	1.23	0.00
		100.00	78.41	36.36	11.93	2.84	0.00
		100.00	57.59	15.19	13.29	6.96	0.00
Average		100.00	78.67	30.15	14.99	3.68	0.00
S.D.		0.00	21.20	13.02	4.18	2.95	0.00
95EYMH		100.00	100.00	28.72	16.41	6.15	0.00
		100.00	100.00	40.14	9.52	8.16	0.00
		100.00	100.00	15.75	5.48	2.05	0.00
Average		100.00	100.00	28.20	10.47	5.46	0.00
S.D.		0.00	0.00	12.20	5.53	3.11	0.00



Software version: BioSpot 7.0.38.8
Analyzer Serial number and Computer name: S6UNIV-02-7392
Counting window size: 600x600
Scanning: 8 bit
Counting: 8 bit

	Spot Counts					Mean spot sizes (1E-3 Sq.mm)			
	1	2	3	4		1	2	3	4
A	30	52	43	0	A	2983.32570	3383.01329	4165.47120	0.00000
B	37	42	51	0	B	3057.92900	2354.92800	2937.37295	0.00000
C	32	36	66	0	C	2793.23276	3063.26733	2876.40169	0.00000

QC changes are highlighted
-1 = Well is not counted -2 = Off scale well -3 = Rejected in QC -4 = Not Done -5 = N/A
Blue numbers mean spotcounts are not 100% certain

Fig. S1 Number of plaque count on plaque reduction assay of anti-SARS-CoV-2.

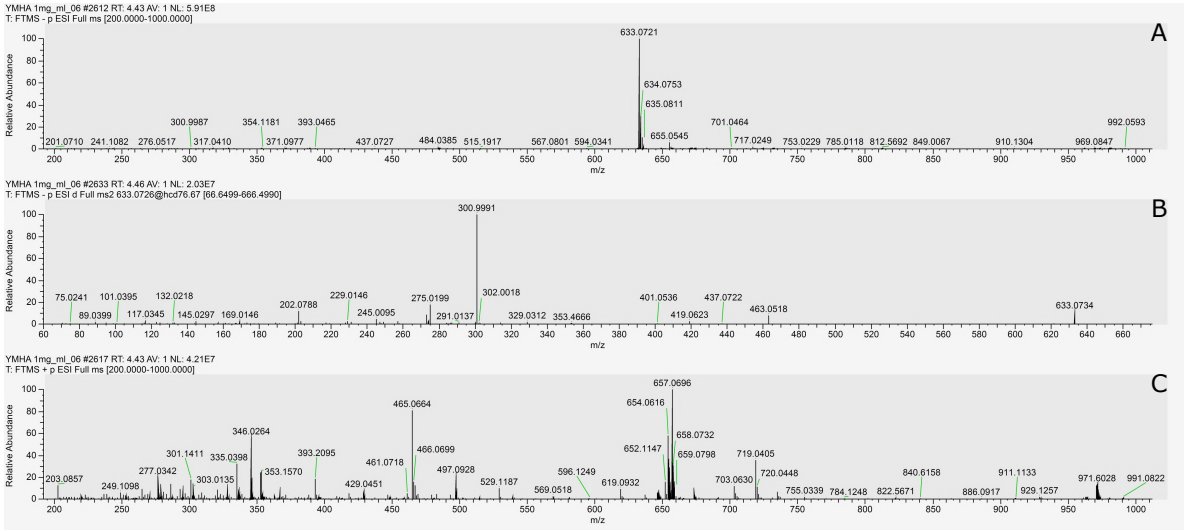


Fig. S2-1 Mass spectra of corilagin (2) at retention time of 4.43 min. (A) Full scan of 200–1000 m/z with negative mode, (B) MS^2 scan in negative mode of precursor of 633.0726 m/z, and (C) full scan of 200–1000 m/z with positive mode.

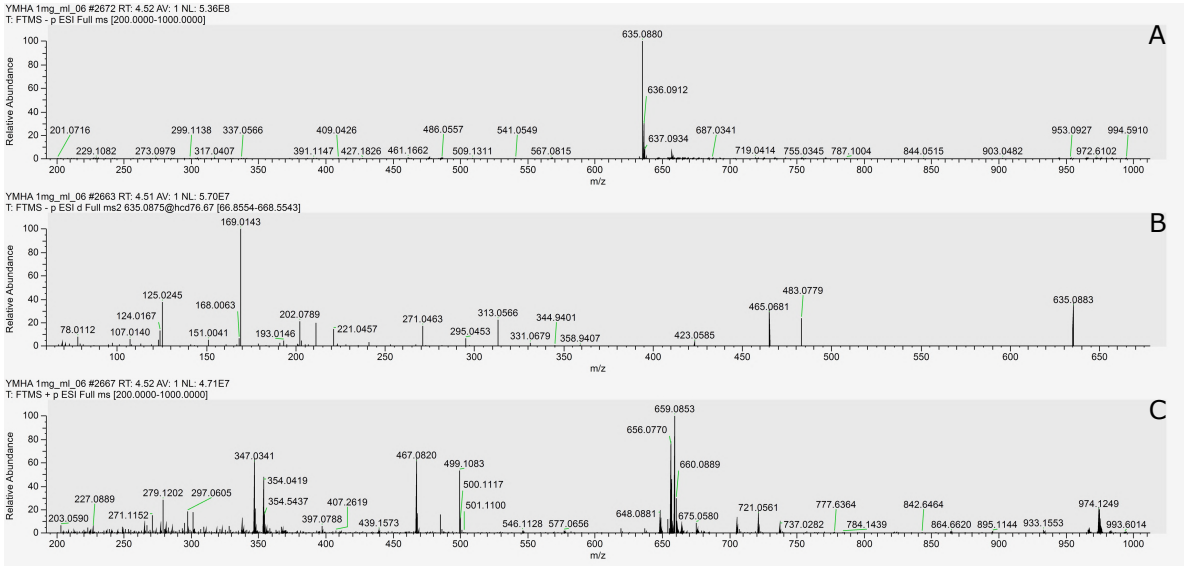


Fig. S2-2 Mass spectra of unidentified compound (3) at retention time of 4.52 min. (A) Full scan of 200–1000 m/z with negative mode, (B) MS^2 scan in negative mode of precursor of 635.0875 m/z, and (C) full scan of 200–1000 m/z with positive mode.

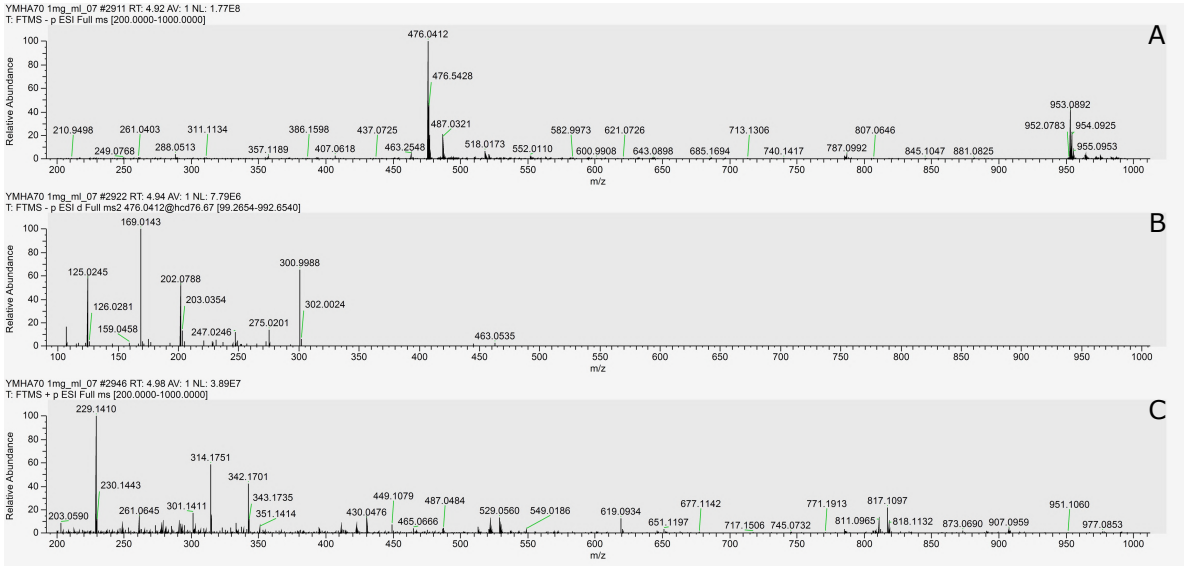


Fig. S2-3 Mass spectra of chebulagic acid (4) at retention time of 4.90 min. (A) Full scan of 200–1000 m/z with negative mode, (B) MS^2 scan in negative mode of precursor of 476.0412 m/z, and (C) full scan of 200–1000 m/z with positive mode.

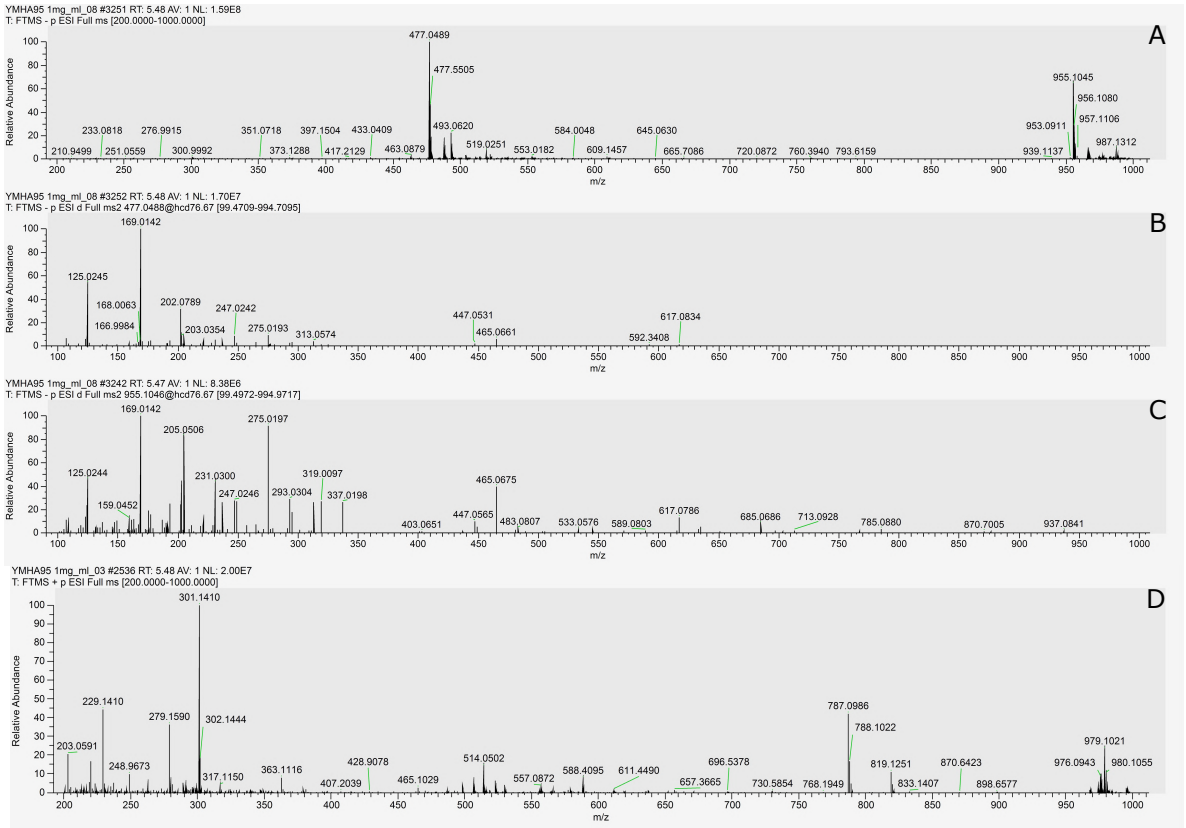


Fig. S2-4 Mass spectra of chebulinic acid (5) at retention time of 5.46 min. (A) Full scan of 200–1000 m/z with negative mode, (B) MS^2 scan in negative mode of precursor of 477.0488 m/z, (C) MS2 scan in negative mode of precursor of 955.1046 m/z, and (D) full scan of 200–1000 m/z with positive mode.

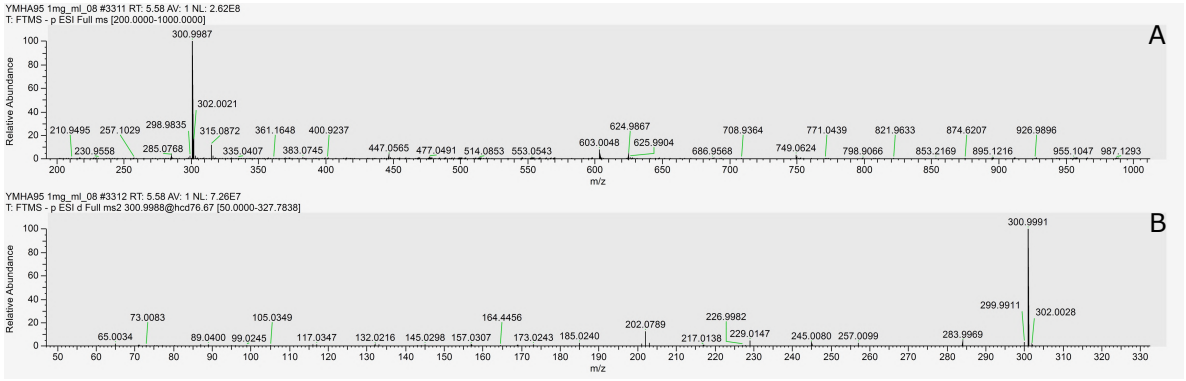


Fig. S2-5 Mass spectra of ellagic acid (6) at retention time of 5.56 min. (A) Full scan of 200–1000 m/z with negative mode, (B) MS^2 scan in negative mode of precursor of 300.9988 m/z.

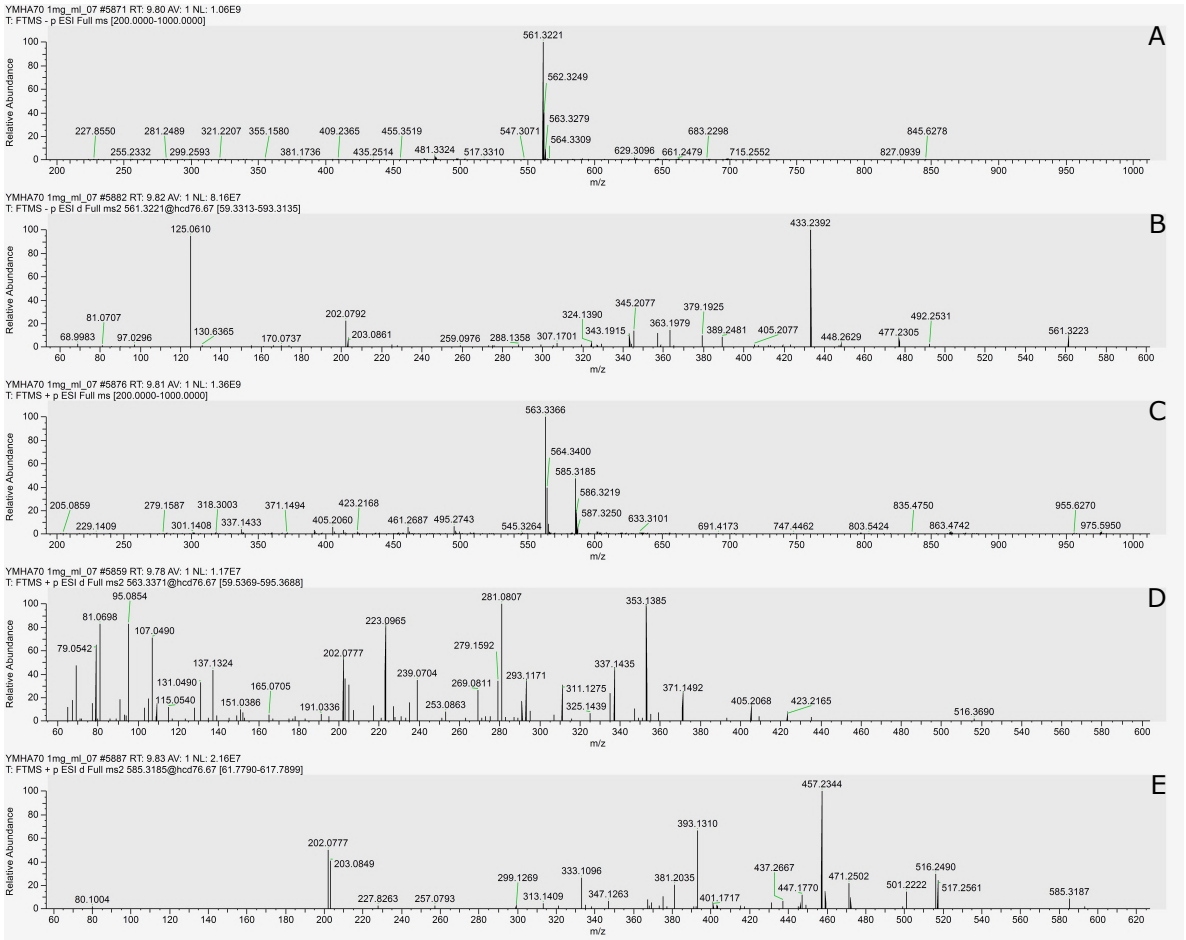


Fig. S2-6 Mass spectra of nortiliacorinine A (7) at retention time of 9.80 min. (A) Full scan of 200–1000 m/z with negative mode, (B) MS^2 scan in negative mode of precursor of 561.3221 m/z, (C) full scan of 200–1000 m/z with positive mode, (D) MS^2 scan in positive mode of precursor of 563.3371 m/z, and (E) MS^2 scan in positive mode of precursor of 585.3185 m/z.