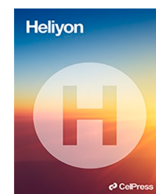
Heliyon 10 (2024) e40569

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Curcuma sp. “Khamin Oi” extracts inhibit human coronavirus OC43 replication in HCT-8 colorectal cell line

Piriyakorn Pichetpongtorn [a], Sasiporn Ruangdachsuwan [b], Theeraporn Churod [b], Jukrapun Komaikul [b], Promsin Masrinoul [b], Gorawit Yusakul [c], Tharita Kitisripanya [a,*]

[a] *Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand*
[b] *Center for Vaccine Development, Institute of Molecular Biosciences, Mahidol University, Nakhon Pathom, Thailand*
[c] *School of Pharmacy, Walailak University, Nakhon Si Thammarat, Thailand*

ARTICLE INFO

Keywords:
Curcuma
Khamin oi
Sesquiterpene
Curcuminoid
Curcumin
Virus
Bisdemethoxycurcumin
Demethoxycurcumin
HCT-8 colorectal cell
Human coronavirus OC43

ABSTRACT

Plants belonging to the genus *Curcuma* have shown promise in exerting antiviral activity. In this study, *Curcuma* sp. “Khamin Oi” (CKO), a traditional Thai medicinal herb listed in Thai herbal pharmacopoeia, was subjected to extraction using a variety of solvents. Consequently, LC-MS/MS and GC-MS/MS analyses were conducted to assess the phytochemical profiles of all the crude extracts and identify the compounds potentially responsible for their antiviral effects. The antiviral effects of these extracts against the human coronavirus OC43 (HCoV-OC43) were also investigated. An in-cell ELISA was used to investigate the anti-HCoV-OC43 replication activity of the crude rhizome extracts of CKO. Among the extracts, the crude hexane extract of CKO exhibited the lowest IC_{50} (3.62 μg/mL), with a high selectivity index (SI) of 31.90, despite having the lowest curcuminoid content. While the methanolic extract of *Curcuma longa* L., known for its high curcuminoid content among *Curcuma* species, showed an IC_{50} of 14.83 μg/mL with an SI of 2.80. Further *in vitro* and *in vivo* investigations of CKO crude extract are necessary to understand its potential anti-HCoV-OC43 properties.

1. Introduction

Respiratory viruses cause a significant global health burden, with millions of infections occurring annually. These viruses are extremely contagious and have a considerable impact on human health, causing a wide range of ailments, from the common cold to severe respiratory infections. While conventional medicine is the primary approach to manage respiratory illnesses, there is a growing interest in the potential of herbal medicine as an alternative or complementary treatment owing to the limitations of conventional medicine, for instance, potential side effects and limitations in effectiveness against certain viruses [1]. Certain phytochemicals and plant extracts inhibit respiratory viruses, such as andrographolide from *Andrographis paniculata* and quassinoids from *Eurycoma* species against the common cold virus [2,3].

The human coronavirus organ culture 43 (HCoV-OC43) belongs to the Betacoronavirus genus of the Coronaviridae family [4]. A notable member of this family is severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The predominant clinical symptoms

* Corresponding author. Mahidol University, Bangkok, Thailand.
E-mail address: tharita.kit@mahidol.edu (T. Kitisripanya).

https://doi.org/10.1016/j.heliyon.2024.e40569
Received 24 March 2024; Received in revised form 16 November 2024; Accepted 19 November 2024
Available online 20 November 2024
2405-8440/© 2024 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC license (http://creativecommons.org/licenses/by-nc/4.0/).

observed with HCoV-OC43 infection are fever and cough [5]. Similar to its relatives, HCoV-OC43 cause respiratory tract infections, which can result in severe lower respiratory tract infections such as bronchiolitis and asthma [6]. HCoV-OC43 is a respiratory pathogen that can also be detected in gastrointestinal tracts, causing acute gastroenteritis; the viral RNA was found in up to 45.5 % of children gastroenteritis cases in Finland [7].

A previous study demonstrated that plants belonging to the genus *Curcuma* possess diverse biological properties such as antiviral and anti-inflammatory activities [8]. *Curcuma longa* L. (CL) (turmeric), a the well-known plant belonging in this genus, demonstrated antiviral activities against several respiratory pathogens, including respiratory syncytial virus (RSV) and influenza A virus (IAV) [9]. Curcumin (CUR), a primary curcuminoid compound abundant in CL, is responsible for the biological activity [9]. Consequently, curcumin also showed antiviral activity against HCoV-OC43 and SARS-CoV-2 [10,11]. Since curcumin demonstrated antiviral capabilities, plants within this genus containing curcuminoids as major constituents warrant investigation as potential sources of similar antiviral activity. *Curcuma* sp. "Khamin Oi" (CKO), belonging to the *Curcuma* genus, has been reported to contain curcuminoids, including, bisdemethoxycurcumin (BIS), demethoxycurcumin (DEM), and curcumin (CUR) [12]. This plant has been included in Thai Herbal Pharmacopoeia (THP) and incorporated into many Thai traditional remedies for various ailments (e.g., anti-diarrhea, anti--flatulence, anti-cough, and anti-inflammation of muscle) [13]. Although the traditional uses and biological activities of CKO are significant, there is a lack of supporting evidence regarding its inhibition of HCoV-OC43. This highlights the need for further investigations in this area.

In this study, an in-cell ELISA was used to investigate the inhibitory effects of different crude extracts of CKO prepared from various solvents on HCoV-OC43 by comparing them with the crude methanolic extract of CL. The phytochemical profiles of the extracts were analyzed using HPLC, UPLC-HR-ESI-QTOF-MS/MS, and GC-MS/MS. These findings establish the efficacy of CKO extracts against pathogenic viruses and further validate the use of CKO as an herbal medicine endorsed by the Thai herbal pharmacopoeia.

2. Materials and method

2.1. Chemical and plant material

Dried CKO and CL rhizomes were obtained from Nakhon Pathom, Thailand, in June 2023. The plant material was identified and authenticated according to the Thai Herbal Pharmacopoeia 2021 (THP). Voucher specimens were deposited at the Project of Institute Establishment for Sireeruckhachati Nature Learning Park, Mahidol University. The curcuminoid standards of demethoxycurcumin (DEM) (purity ≥98.0 %), and curcumin (CUR) (purity ≥99.5 %) were purchased from Sigma-Aldrich (MO, USA). Bisdemethoxycurcumin (BIS) (purity ≥99.5 %) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan)

Ten grams of ground dried CKO rhizomes were mixed with 100 mL of hexane or toluene or ethanol or methanol, each in extraction ratio of 1:10, macerated for 2 days, and then ultrasonicated for 20 min before collecting the filtrates. The filtrates were air dried in a laminar flow cabinet at 30 °C to obtain CKO crude extracts. Meanwhile, CL rhizomes crude extract was prepared using methanol as a solvent. Briefly, 10 g of dried CL rhizome powder were mixed with 100 mL of methanol, macerated for 2 days, and ultrasonicated for 20 min. The filtrate was collected, and the solvent was evaporated at 30 °C under a laminar flow cabinet to obtain the CL crude extract.

2.2. Determination of curcuminoids content

High-performance liquid chromatography (HPLC) was performed as described previously [14], with modifications. Each of CKO and CL extracts was redissolved in methanol and diluted to obtain final concentrations ranging 0.11–3.11 mg/mL. For the crude CL methanol extract, a final concentration of 0.04 mg/mL was prepared. Quantification of the active compounds was performed using a Shimadzu machine equipped with an Acclaim™ 120 C18 column (5 μm, 250 × 4.6 I.D. mm) (Thermo Fisher Scientific, Waltham, MA, USA), with a photodiode array detector (PDA) at 210, 240, and 425 nm. The injection volume was 10 μL. The flow rate was 0.8. mL/min, with acetonitrile (solvent A) and 0.2 % phosphoric acid (solvent B) as mobile phase. The gradient system was as follows: 55 % B (0 min), 55–50 % B (0–5 min), 50–35 % B (5–20 min), 35–5 % B (20–25 min), 5 % B (25–30 min), 5–55 % B (30–35 min), 55 % B (35–40 min). The concentrations of the standard compounds used were as follows: BIS (0.029–1.875 μg/mL), DEM (0.391–25 μg/mL), and CUR (0.127–8.125 μg/mL). Standard calibration curves and chromatograms are shown in the Supporting Information (Figure S1 and Fig. 2S).

2.3. Ultra-high performance liquid chromatography-high-resolution electrospray ionization quadrupole time-of-flight mass spectrometer (UHPLC-HR-ESI-QTOF-MS/MS) analysis

Phytochemical profiles of the CKO hexane, toluene, ethanol, and methanol extracts were screened using an ultra-high performance liquid chromatography-high-resolution electrospray ionization quadrupole time-of-flight mass spectrometry (UHPLC-HR-ESI-QTOF-MS/MS). The system consisted of UltiMate 3000 UHPLC (Thermo Fisher Scientific, Waltham, USA) paired with Bruker Impact II UHR-QTOF mass spectrometer (Bruker, Billerica, MA, USA). The crude extracts were dissolved in methanol and separated using an Acclaim™ 120 C18 (2.2 μm, 2.1 mm I.D. × 100 mm) column (Thermo Fisher Scientific, Waltham, MA, USA). A gradient elution system was applied using 0.1 % formic acid in deionized water (A) and 0.1 % formic acid in acetonitrile (B): 5–20 % B (1–15 min), 20 % B (15–30 min), 20–50 % B (30–40 min), 50–100 % B (40–50 min). The flow rate was 0.3 mL/min, and the column temperature was 25 °C. The injection volume was 10 μL. QTOF-MS/MS detection was conducted in positive auto-MS^n mode with a detection mass range of 50–1300 *m/z*. The collision-induced dissociation (CID) energy was adjusted to 30–40 eV. The carrier gas was nitrogen at a flow rate

of 8.0 L/min at 220 °C. Other parameters were as follows: capillary voltage 3000 V, charging voltage 2000 V, end plate offset −500 V, nebulizer pressure 1.8 bar, a quadrupole ion energy 4.0 eV, collision energy 7.0 eV, and spectral rate of 2.00 Hz.

2.4. Gas chromatography-tandem mass spectrometry (GC-MS/MS) analysis

Gas chromatography-tandem mass spectrometry (GC-MS/MS) analysis was performed using a GC-MS-TQ8050 triple quadrupole system (Shimadzu) with a DB-5MS (Agilent) capillary column (30 m, 0.25 mm I.D.) coated with 0.25 μm film. Ultra-high-purity helium was used as the carrier gas. One microliter of each sample including CKO hexane, toluene, ethanol, and methanol extracts, as well as CL methanol extract, was injected into the instrument in splitless mode using an autosampler. The injection port temperature was set at 190 °C. The oven temperature programs for peak separation were as follows: initially maintained at 60 °C for 2 min, increased to 145 °C at a rate of 5 °C min^{-1} and held for 25 min, then increased to 200 °C at a rate of 5 °C min^{-1}, and finally increased to 250 °C at 20 °C min^{-1}, and then held for 3 min. Helium was used as the carrier gas at a flow rate of 1.23 mL/min. The temperature of interface and ion source were set at 250 °C. The mass analysis was operated in electron ionization scan mode, 70 eV with an acquisition scan rate of 0.34 s/scan from 40 to 550 *m/z*. Compounds were identified by comparing the mass spectra with the National Institute of Standards and Technology mass spectral library (National Institute of Standards and Technology (NIST).

2.5. Virus propagation

Human coronavirus OC 43 (HCoV-OC43, ATCC VR-1558) was cultured in the HCT-8 cell line (ATCC CCL-244) at the Center for Vaccine Development, Institute of Molecular Biosciences, Mahidol University, using a biosafety cabinet class II (BSC-2). The viral suspensions were collected from the cultivated supernatants and preserved at −80 °C until the experiment.

2.6. Detection of HCoV-OC43 infection using in-cell ELISA

Before conducting the experiment, the crude CKO hexane, toluene, ethanol, and methanol extracts, as well as CL methanol extracts were dissolved in methanol and subsequently diluted in DMEM with 1 % penicillin-streptomycin and 5 % FBS. The HCT-8 human colon cells were seeded in a 96-well plate at 2×10^5 cells/mL density under 37 °C and 5 % CO_2 for 48 h. The culture medium was removed, and then 40 μL of the virus dilution at 25 times the 50 % tissue culture infectious dose ($25TCID_{50}$) was added to each well and incubated in 5 % CO_2 at 33 °C for 1 h. Culture media with or without a diluted CKO extract or a standard compound were then added and gently mixed. The plates were then incubated at 33 °C for 4 days.

To determine the viral replication, the in-cell ELISA technique was validated and performed as previously described [2]. The cells were fixed with 80 % (v/v) acetone and blocked with 5 % skim milk and 0.5 % Triton-X100 in PBST. Following a 35-min incubation period at 37 °C, the cells were washed, stained with 1:10,000 diluted HCoV-OC43 nucleocapsid antibody (40,643-T62, Sino Biological, Beijing, China), and incubated for 1 h at 37 °C. After re-washing the cells with PBST, diluted HRP-goat anti-rabbit IgG (1:20,000) was added (Jackson ImmunoResearch, PA, USA) and incubated for 1 h at 37 °C. After washing, the substrate (3,3',5,5'-tetramethylbenzidine, TMB) (5120-0047, Seracare, MA, USA) was added. The changed in the color of the substrate, which expresses the content of HRP-goat anti-rabbit IgG residues, was measured at wavelengths of 450 nm and 630 nm (reference wavelength).

2.7. Cell viability assay

Prestoblue™ (Invitrogen, Life Technology, CA, USA), a resazurin-based reagent used to assess cell viability, was mixed in a culture media (1:9) and incubated for 2 h. The absorbance of the medium was measured at 570 and 600 nm using a Biochrom-EZ Read 800 microplate reader. The percentage of cell viability and 50 % cytotoxic concentration were calculated. The control group, which did not have a viral infection, was adjusted to achieve a cell viability of 100 %.

2.8. Data analysis

All experiments were conducted in triplicates (n = 3). The HPLC results for the quantitation of curcuminoids in CKO and CL rhizomes are presented as mean ± standard deviation (SD) using Microsoft Excel. One-way ANOVA and post-hoc Tukey's tests were conducted using SPSS software (version 29.0). The HCoV-OC43 experimental results, including virus inhibition activity and cell cytotoxicity, were analyzed using GraphPad Prism version 9.

3. Results and discussion

3.1. Plant materials

The morphology of both the CKO and CL plant materials was examined by referring to the plant descriptions provided in the THP. CKO and CL had exhibited differences in rhizome structure, plant size, leaf morphology, inflorescence characteristics, and flower details, which can distinguish the two plants from each other. An important difference is that the rhizome of CKO is well developed, mostly above ground, obconical to oblong-ovoid, branched, orange-yellow or pale yellow within, and aromatic (Fig. 1A). Meanwhile, that of CL is thick, the ellipsoid-ovate, orange, and rises to short, with blunt daughter rhizomes called fingers (Fig. 1B). Based on several

previous studies, curcuminoids, especially curcumin, exhibit antiviral activities [10,11,15]. In this study, CL was used for comparative purposes because it belongs to the *Curcuma* genus, which contains an abundant amount of curcuminoids.

3.2. Analysis of curcuminoids content in Curcuma sp. "khamin oi" and Curcuma longa using HPLC

Curcuminoids (bisdemethoxycurcumin (BIS), demethoxycurcumin (DEM), and curcumin (CUR)) were determined using HPLC as described previously with modification [14]. All method validation data were presented in Supporting Information Tables S1 and S2. The standard calibration curves and regression equations of each standard were established and demonstrated satisfactory linearity at the working concentration ranges (Supporting Information Figs. S1 and S2). The curcuminoid content of CKO and CL extracts obtained via HPLC analysis is shown in Fig. 2.

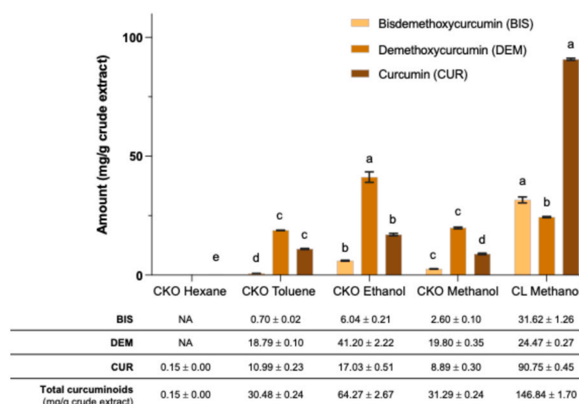
Among all extracts analyzed, the crude methanol extract of CL demonstrated the highest total curcuminoid content (146.84 ± 1.70 mg/g). Among CKO samples, the crude ethanol extract exhibited the highest amount of total curcuminoids (64.27 ± 2.67 mg/g), followed by methanol (31.29 ± 0.24 mg/g), toluene (30.48 ± 0.24 mg/g), and hexane (0.15 ± 0.00 mg/g). In a previous study, the crude ethanol extract of CL showed the highest curcuminoid content, while the crude hexane extract of CL provided the lowest curcuminoid content [15], which supported the results of the present study. Of the three compounds, DEM was predominantly present in all CKO crude extracts, except for the crude hexane extract. In CL crude extract, CUR was found to have the highest curcuminoid content.

Fig. 3A–C shows the chromatograms of the CKO and CL crude extracts at three different wavelengths. Several peaks are observed at various wavelengths. A wavelength of 425 nm was selected for quantification of curcuminoids. Different wavelengths were used to compare the characteristics of the compounds eluted in the HPLC system. Various phytochemical groups absorb UV light within their characteristic wavelength ranges. Analysis of the retention time and intensity of the peaks observed at 210 and 240 nm offered evidences about the potential presence of certain groups, such as terpenoids. All crude extracts showed curcuminoid peaks, except for the crude hexane extract, where only the curcumin peak was present. At 210 and 240 nm, the hexane extract demonstrated prominent peaks at approximately 15 and 30–37 min retention times, compared to the other extracts. The *Curcuma* genus has been reported to have sesquiterpenes as the main constituents [8]. These wavelengths could represent terpenoids (e.g. triterpenic acids and sesquiterpenes) and flavonoid-based compounds (e.g. flavones and flavonols) [16,17]. Ar-turmerone has been reported to be found in the *Curcuma* sample quantitating by HPLC at 240 nm [18]. Moreover, many sesquiterpenes such as curcumenol, curdione, furanodienone can be detected using HPLC at 210 nm [14]. The aforementioned data support that the multiple peaks found at 210 and 240 nm could be compounds in the sesquiterpene class. Because this information is crucial for the initial screening technique, LC-MS/MS and GC-MS/MS analyses were conducted to verify the phytochemical profiles of the extracts.

3.3. UHPLC-HR-ESI-QTOF-MS/MS analysis

To clarify the phytochemical profiles of the extracts from different solvents, crude extracts of CKO rhizomes were analyzed using UHPLC-HR-ESI-QTOF-MS/MS in the positive mode. The compounds identified in the crude extracts are listed in Table 1. The mass

Fig. 1. The rhizome and cross-section of *Curcuma* sp. "Khamin Oi" (A) and *C. longa* (B).



	CKO Hexane	CKO Toluene	CKO Ethanol	CKO Methanol	CL Methanol
BIS	NA	0.70 ± 0.02	6.04 ± 0.21	2.60 ± 0.10	31.62 ± 1.26
DEM	NA	18.79 ± 0.10	41.20 ± 2.22	19.80 ± 0.35	24.47 ± 0.27
CUR	0.15 ± 0.00	10.99 ± 0.23	17.03 ± 0.51	8.89 ± 0.30	90.75 ± 0.45
Total curcuminoids (mg/g crude extract)	0.15 ± 0.00	30.48 ± 0.24	64.27 ± 2.67	31.29 ± 0.24	146.84 ± 1.70

Fig. 2. Curcuminoids content in *Curcuma* sp. "Khamin Oi" (CKO) and *C. longa* (CL) crude extracts. The statistical significance of the difference between extracts was indicated by different alphabets (One-way ANOVA, post-hoc Tukey's test at a significance level of $p < 0.05$).

spectra of the compounds are shown in Supporting Information Figure S3-S20.

Consistent with the HPLC findings, curcuminoids (BIS, DEM, and CUR) were detected in all the extracts. Notably, the crude hexane extract lacked curcuminoids but contained abundant sesquiterpenes. Sesquiterpenes (α-curcumene, ar-turmerone, α-farnesene, and α-cubebene) were identified, aligning with literature on phytochemical variation within the *Curcuma* genus [19,20]. Since the different extraction solvents produce different phytochemical profiles, using hexane as the extraction solvent for CL extraction has demonstrated many types of sesquiterpenes in a previous study, including ar-curcumene, ar-turmerone, curcumenol, and *trans*-β-farnesene [21].

LC-MS/MS is a highly effective analytical tool for the identification of compounds. However, the sensitivity and detection limit of an instrument is influenced by various parameters, such as the concentration of the sample, sample preparation, column, and detector [22]. The same concentration of CKO crude extracts was evaluated to compare the phytochemical profiles. The absence of CUR in the hexane crude extract and BIS in the toluene crude extract, which was consistent with the HPLC results, may be attributed to the low concentration of curcuminoids in the samples. Optimizing the sample preparation and other parameters could help overcome these analytical limitations in further studies.

3.4. GC-MS/MS analysis

LC-MS/MS analysis identified sesquiterpenes in CKO crude extract. Therefore, GC-MS/MS analysis was performed to identify the volatile components, including sesquiterpenes, in the crude CKO and CL extracts (Table 2). Based on the percentage of the peak area, the results showed that all crude extracts of CKO contained high amounts of ar-tumerone, tumerone and beta-tumerone. The major sesquiterpenes in the crude methanol extract of CL were alpha-zingiberene, alpha-santalene, ar-turmerone, and beta-sesquisabinene. The difference between the majority of sesquiterpenes found in CKO and CL may lead to the differences in anti-HCoV-OC43 activities.

3.5. HCoV-OC43 inhibition using in-cell enzyme-Linked immunosorbent assay (in-cell ELISA) and cell cytotoxicity assays

In-cell ELISA is an immunochemical technique commonly employed for quantitatively analyzing intracellular proteins [23]. This method is widely recognized in research for detecting intracellular viruses, as demonstrated by previous studies such as those by Schöler et al. [24] and Conzelmann et al. [25] The results were expressed as half maximal inhibitory concentration (IC_{50}) and 50 % cytotoxic concentration (CC_{50}). Based on our preliminary study conducted in MRC-5 (human lung fibroblast) cells, the hexane extract of CKO demonstrates a higher IC_{50} (19.29 μg/ml), lower CC_{50} (77.87 μg/ml), and a lower selectivity index (4.04) compared to those of HCT-8 cells. Considering the concentrations of phytochemicals in CKO, they are likely to be lower after absorption and distribution to the lungs, and the lungs also require higher concentrations of these compounds. These findings suggest that the anti-HCoV-OC43 properties of CKO may not affect the lung tissue. Because HCoV-OC43 can remain in the gastrointestinal tract and replicate well in intestinal cells [26], intestinal epithelial cells may serve as effective antiviral targets for CKO. As CKO is traditionally administered orally, the gastrointestinal tract is the primary site of absorption. Utilizing HCT-8 cells, which represent the epithelial cells of the large intestine, offers a promising model for conducting an anti-HCoV-OC43 assay [23]. Our study found that CKO may be effective in inhibiting HCoV-OC43 replication in the intestine but not in the lungs.

The HCoV-OC43 inhibition activities of CKO crude extract prepared in different extraction solvents, CL crude methanol extract, and the standard compounds (remdesivir and curcuminoids) are shown in Fig. 4. Crude plant extracts obtained from different solvents inhibited HCoV-OC43 replication in HCT-8 cells. The crude hexane extract demonstrated the lowest half-maximal inhibitory concentration (IC_{50}) of 3.62 μg/mL (Fig. 4A), followed by the crude toluene extract, 3.69 μg/mL (Fig. 4B). The half-maximal cytotoxic concentrations (CC_{50}) of crude hexane and toluene extracts were promising, with values of 115.50 μg/mL and 61.15 μg/mL, respectively. The CKO crude ethanol and methanol extracts also exhibited anti-HCoV-OC43 activity with IC50 values of 5.31 μg/mL

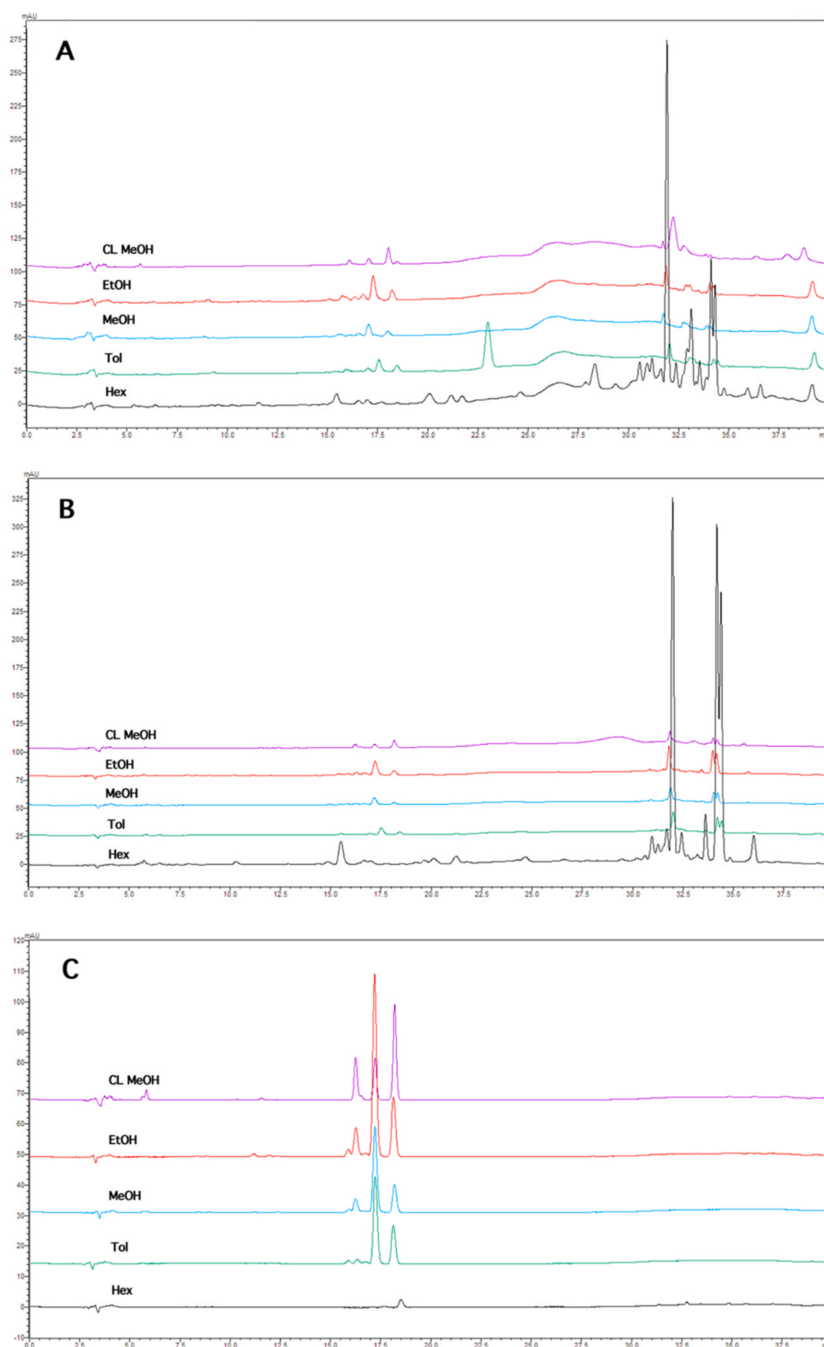


Fig. 3. HPLC chromatograms of *Curcuma* sp. "Khamin Oi" crude extracts (hexane, toluene, ethanol, and methanol) and *C. longa* (CL) crude methanol extract at different wavelengths: at 210 nm (A), at 240 nm (B), and at 425 nm (C).

and 6.01 μg/mL, respectively (Fig. 4C and D). While the CL crude methanol extract, produced the lowest anti-HCoV-OC43 activity, with IC50 of 14.83 μg/mL (Fig. 4E). The selectivity index (SI), which was used to evaluate the safety and efficacy of an antiviral compound, was calculated (CC_{50}/IC_{50}). The CKO crude hexane extract demonstrated the highest SI value (31.90), indicating decent potency and safety. The standard compounds remdesivir (an antiviral drug (Fig. 4F), and curcuminoids (BIS, DEM, and CUR, as shown in Fig. 4G–I) were evaluated for their anti-HCoV-OC43 activity. The IC_{50} of remdesivir was 0.24 μM ($CC_{50} > 20.00$ μM). Among the curcuminoid standards (BIS, DEM, and CUR), BIS had the lowest IC_{50} of 4.71 μM ($CC_{50} = 34.96$ μM).

Interestingly, the findings demonstrated an inverse proportion of total curcuminoid content in the CKO crude hexane extract, which provided the lowest IC_{50} with a high CC_{50} value, indicating strong antiviral activity against HCoV-OC43 with less toxicity to the host

Table 1
Chromatographic retention time and mass spectra data of constituents found in *Curcuma* sp. "Khamin Oi" crude extracts using UHPLC-HR-ESI-QTOF-MS/MS Analysis.

Cpd. No.	Retention time (min)	Compound name	*Curcuma* sp. "Khamin Oi" crude extracts $[M-H]^+$ (Mass fragmentation)			
			Hexane	Toluene	Ethanol	Methanol
1	14.2	Bisdemethoxycurcumin[a] ($C_{19}H_{16}O_4$) 308.3 g/mol	NA	NA	**309.1138 (147.0444, 225.0917)**	**309.1114 (147.0444, 225.0916)**
2	14.4	Demethoxycurcumin[a] ($C_{20}H_{18}O_5$) 338.35 g/mol	NA	**339.1224** (255.1008, 177.0539)	**339.1237** (255.1024, 177.0548)	**339.1232** (255.1017, 177.0546)
3	14.5	Curcumin[a] ($C_{21}H_{20}O_6$) 368.38 g/mol	NA	**369.1330** (177.0544, 285.1114)	**369.1346** (177.0548, 285.1130)	**369.1339** (177.0544, 285.1125)
4	16.7	α-Curcumene[b] ($C_{15}H_{22}$) 202.33 g/mol	**203.1794** (105.0701, 147.1172)	**203.1789** (105.0701, 147.1167)	NA	NA
5	17.0	Ar-Turmerone[b] ($C_{15}H_{20}O$) 216.32 g/mol	**217.1587** (119.0855, 91.0543)	**217.1584** (119.0854, 91.0541)	**217.1596** (119.0858, 91.0544)	**217.1590** (119.0857, 91.0544)
6	17.2	α-Farnesene[b] ($C_{15}H_{24}$) 204.36 g/mol	**205.1949** (121.1016, 149.1322)	NA	NA	NA
7	17.4	α-Cubebene[b] ($C_{15}H_{24}$) 204.35 g/mol	**205.0857** (121.1017, 93.0698)	**205.0857** (121.1012, 93.0696)	**205.0865** (121.1017, 93.0700)	NA

[a] Comparison of mass fraction to the previous studies [35].
[b] Comparision of mass fraction to the database (Bruker MetaboBASE Personal Library).

Table 2
GC-MS/MS analysis of the volatile components in *Curcuma* sp. "Khamin Oi" (CKO) and *C. longa* (CL) crude extracts.

Peak number	Retention time (min)	Compound name	Molecular formula	Area (%)				
				CKO hexane	CKO toluene	CKO ethanol	CKO methanol	CL methanol
1	8.13	Eucalyptol	C10H18O	–	–	–	–	0.31
2	9.95	Undecane	C11H24	–	–	–	–	0.91
3	18.79	alpha-Santalene	C15H24	–	–	–	–	14.64
4	19.14	cis-alpha-Bergamotene	C15H24	–	–	–	–	1.76
5	19.53	cis-beta-Santalene	C15H24	–	–	–	–	1.6
6	19.67	trans-beta-Bergamotene	C15H24	–	–	–	–	5.02
7	19.79	alpha-Caryophyllene	C15H24	1.07	1.34	–	0.84	–
8	20.53	alpha-Curcumene	C15H22	4.31	6.33	4.29	4.9	4.79
9	20.61	β-Sesquiphellandrene	C15H24	–	–	–	–	1.54
10	20.97	Zingiberene	C15H24	3.1	3.99	3.06	2.89	17.33
11	21.40	beta-Bisabolene	C15H24	–	0.8	–	–	8.4
12	21.79	Teresantalol	C10H16O	–	–	–	–	9.34
13	21.97	Sesquisabinene	C15H24	5.53	7.56	5.76	5.72	10.13
14	22.22	Humulene epoxide I	C15H24O	–	–	–	–	0.46
15	23.18	Sesquisabinene hydrate	C15H26O	0.58	–	0.56	–	–
16	23.47	Nerolidol	C15H26O	0.47	–	0.52	–	–
17	24.23	aR-Tumerol	C15H22O	2.42	0.93	1.15	–	–
18	24.70	(E)-gamma-Atlantone	C15H22O	–	–	–	–	2.48
19	24.80	Sesquisabinene hydrate	C15H26O	1.18	–	1.09	–	–
20	25.66	Humulene epoxide II	C15H24O	1.51	1.66	1.46	1.9	–
21	26.01	Zingiberenol	C15H26O	2.55	2.4	2.29	2.31	–
22	28.89	aR-Turmerone	C15H20O	28.08	28.82	28.1	35.91	14.05
23	29.23	Tumerone	C15H22O	19.33	18.59	23.1	27.36	–
24	30.59	Ageratriol	C15H24O3	4.18	3.7	3.94	–	–
25	31.13	Cedrenol	C15H24O	1.55	–	1.57	–	–
26	31.57	beta-Turmerone	C15H22O	12.17	12.19	12.92	15.57	5.74
27	35.07	(6R,7R)-Bisabolone	C15H24O	3.73	3.44	3.7	–	–
28	36.07	(−)-Xanthorrhizol	C15H22O	2.16	–	1.52	–	–
29	38.18	(E)-Atlantone	C15H22O	3.18	2.82	3.39	–	–
30	51.88	Hexadecanoic acid, methyl ester	C17H34O2	1.9	3.05	0.96	1.6	0.83
31	56.55	9,12-Octadecadienoic acid, methyl ester	C19H34O2	–	0.52	–	–	–
32	56.60	9-Octadecenoic acid, methyl ester	C19H36O2	–	–	–	–	0.16
33	57.04	Methyl stearate	C19H38O2	1	1.86	0.62	1	0.51
				100	100	100	100	100

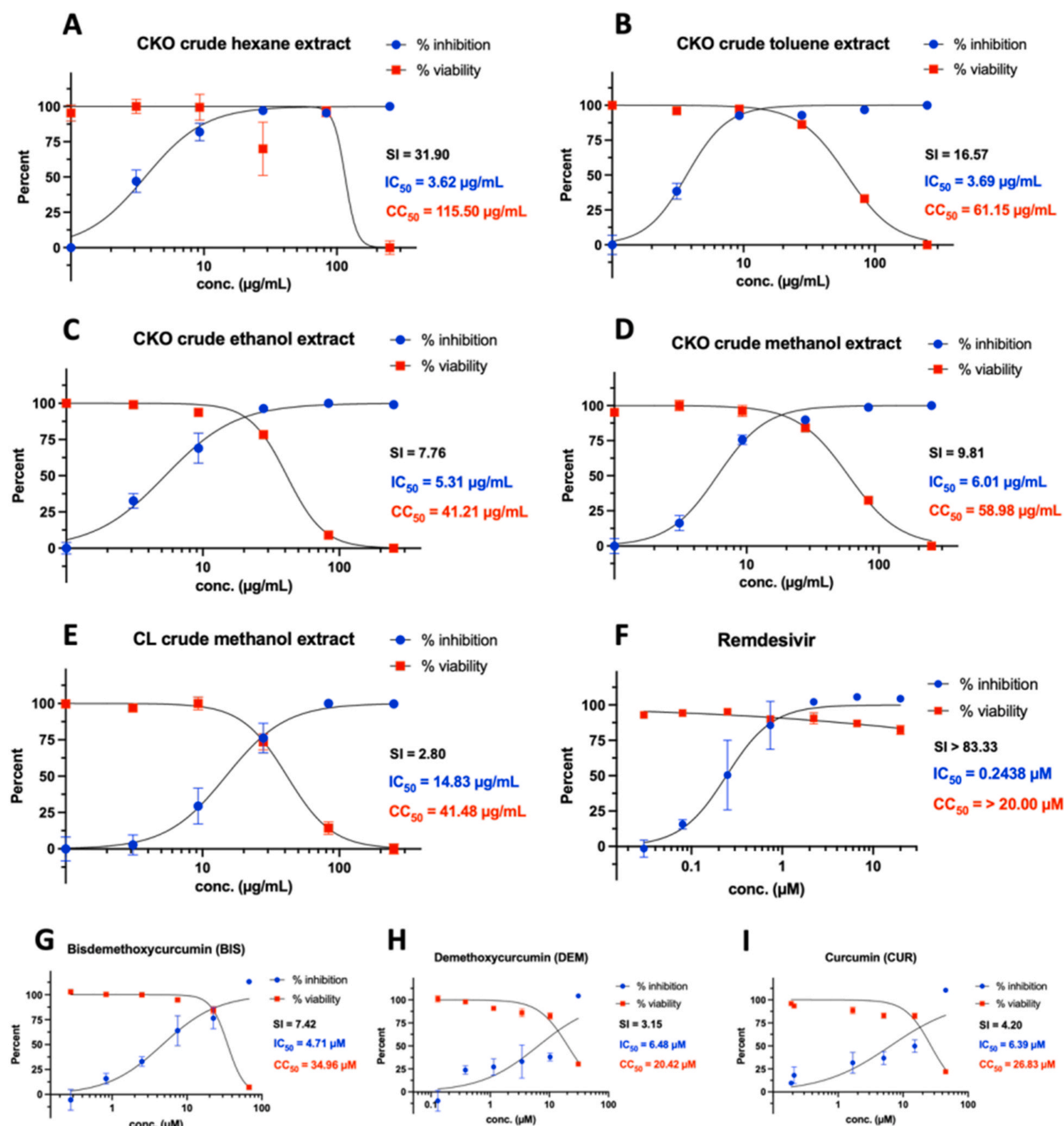


Fig. 4. HCoV-OC43 inhibitory effects of *Curcuma* sp. “Khamin Oi” (CKO), *C. longa* (CL) crude extracts, and standard compounds using in-cell ELISA. Dose-response curves of *Curcuma* sp. “Khamin Oi” crude extracts were demonstrated in (A) hexane, (B) toluene, (C) ethanol, and (D) methanol. Crude methanol extract of *C. longa* and the standard compounds including remdesivir, bisdemethoxycurcumin, demethoxycurcumin, and curcumin were demonstrated in (E)–(I), respectively.

cells. GC-MS/MS analysis revealed that tumerones may affect the anti-HCoV-OC43 properties of CKO. Additionally, bisabolone was identified in all crude extracts of CKO except for the extract obtained from the methanol extraction of CKO and CL. This may have contributed to higher IC_{50} against HCoV-OC43. A previous study suggested that bisabolane-type sesquiterpenoids also exhibit anti-influenza activity [27].

Other sesquiterpenes in plant essential oils demonstrated antiviral activities against several viruses [28]. Among the sesquiterpenes found in *Curcuma* species, germacrone, curcumol, and curdione were found to inhibit the influenza virus [29–31]. In addition, furanodienone and germacrone exhibited inhibitory effects on dengue virus type 2 (DENV-2) NS2B-NS3 protease activity comparable to

aprotinin, a potent NS2B-NS3 protease inhibitor [32]. In terms of sesquiterpenes found in other plants, brevilin A exhibits its anti-influenza A virus activity by impairing the viral RNA synthesis and protein translation [33]. Whereas beta-santalol can efficiently inhibit influenza A/HK (H3N2) virus replication [34]. Therefore, there is a compelling opportunity for further investigation of each sesquiterpene in CKO regarding its activity against HCoV-OC43 and other coronaviruses, like SAR-CoV-2. This included examining both the individual components and their combinations to explore their potential synergistic effects.

4. Conclusion

The curcuminoid content of CL was higher than that of all crude CKO extracts obtained using different extraction solvents. Interestingly, despite CKO having a lower curcuminoid content than CL, it exhibited the highest anti-HCoV-OC43 replication activity in the in-cell ELISA. Subsequently, GC-MS/MS analysis was conducted to identify the phytochemical profiles of CKO and CL crude extracts.

This study demonstrated that all the crude extracts of CKO and CL possessed antiviral activity against HCoV-OC43. The strong antiviral activity of CKO, with a lower curcuminoid content than that of CL, emphasizes that curcuminoids are not the only main component of CKO that inhibits HCoV-OC43. Further studies are necessary to identify the major active compounds, which may be sesquiterpenes, and to explore their potential synergistic or antagonistic effects. Additional quantitative determination of sesquiterpenes, *in vivo* experiments, and clinical studies are necessary to confirm the therapeutic efficacy of the CKO.

CRediT authorship contribution statement

Piriyakorn Pichetpongtorn: Writing – original draft, Visualization, Software, Investigation, Data curation. **Sasiporn Ruangdachsuwan:** Investigation. **Theeraporn Churod:** Investigation. **Jukrapun Komaikul:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Promsin Masrinoul:** Supervision, Resources. **Gorawit Yusakul:** Supervision, Resources. **Tharita Kitisripanya:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Data curation, Conceptualization.

Data availability statement

Data will be made available on request.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this study, the authors used ChatGPT for grammar and language checking. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Piriyakorn Pichetpongtorn reports a relationship with Mahidol University that includes: funding grants. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This research was supported by the scholarship “The 60th Year Supreme Reign of His Majesty King Bhumibol Adulyadej” from the Faculty of Graduate Studies, Mahidol University. The facilities for the HCoV-OC43 experiment and LC-MS/MS and GC-MS/MS analyses were supported by the Center for Vaccine Development, Institute of Molecular Biosciences, Mahidol University; the Center of Analysis for Product Quality (Division of Natural Products), Faculty of Pharmacy, Mahidol University; and the Central Research Unit, Faculty of Pharmacy, Mahidol University. The authors would like to thank Ms. Panyanuch Wongbunmak for her technical support with GC-MS/MS analysis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e40569.

References

[1] S. Ben-Shabat, L. Yarmolinsky, D. Porat, A. Dahan, Antiviral effect of phytochemicals from medicinal plants: applications and drug delivery strategies, Drug Delivery Transl. Res. 10 (2020) 354–367.

[2] J. Komaikul, S. Ruangdachsuwan, D. Wanlayaporn, S. Palabodeewat, S. Punyahathaikul, T. Churod, et al., Effect of andrographolide and deep eutectic solvent extracts of *Andrographis paniculata* on human coronavirus organ culture 43 (HCoV-OC43), Phytomedicine 112 (2023) 154708.

[3] R. Choonong, S. Ruangdachsuwan, T. Churod, S. Palabodeewat, S. Punyahathaikul, S. Juntarapornchai, et al., Evaluating the *in vitro* efficacy of quassinoids from *Eurycoma longifolia* and *Eurycoma harmandiana* against common cold human coronavirus OC43 and SARS-CoV-2 using in-cell enzyme-linked immunosorbent assay, J. Nat. Prod. 85 (12) (2022) 2779–2788.

[4] D.X. Liu, J.Q. Liang, T.S. Fung, Human coronavirus-229e,-oc43,-NL63, and-HKU1 (Coronaviridae). Encyclopedia of Virology, 2021, p. 428.

[5] C. Wen, L. Sun, M.-C. Zhao, S.-X. Duan, L. Wang, X.-W. Cui, Clinical study of human coronavirus NL63, OC43, 229E, HKU1 infentions in hospitalized children from 2015 to 2020, Infect. Drug Resist. (2022) 1093–1101.

[6] D. Ojha, F. Jessop, C.M. Bosio, K.E. Peterson, Effective inhibition of HCoV-OC43 and SARS-CoV-2 by phytochemicals *in vitro* and *in vivo*, Int. J. Antimicrob. Agents (2023) 106893.

[7] M. Risku, S. Lappalainen, S. Räsänen, T. Vesikari, Detection of human coronaviruses in children with acute gastroenteritis, J. Clin. Virol. 48 (1) (2010) 27–30.

[8] W. Sun, S. Wang, W. Zhao, C. Wu, S. Guo, H. Gao, et al., Chemical constituents and biological research on plants in the genus *Curcuma*, Crit. Rev. Food Sci. Nutr. 57 (7) (2017) 1451–1523.

[9] B.B.L. Srivastava, A.S. Ripanda, H.M. Mwanga, Ethnomedicinal, phytochemistry and antiviral potential of turmeric (*Curcuma longa*), Compounds 2 (3) (2022) 200–221.

[10] D. Marín-Palma, J.H. Tabares-Guevara, M.I. Zapata-Cardona, L. Flórez-Álvarez, L.M. Yepes, M.T. Rugeles, et al., Curcumin inhibits *in vitro* SARS-CoV-2 infection in vero E6 cells through multiple antiviral mechanisms, Molecules 26 (22) (2021) 6900.

[11] C.S. Enwemeka, V.V. Bumah, J.C. Castel, S.L. Suess, Pulsed blue light, saliva and curcumin significantly inactivate human coronavirus, J. Photochem. Photobiol., B 227 (2022) 112378.

[12] C. Ampasavate, U. Sotanaphun, P. Phattanawasin, N. Piyapolrungroj, Effects of *Curcuma* spp. on p-glycoprotein function, Phytomedicine 17 (7) (2010) 506–512.

[13] S.K.J. Urumarudappa, C. Tungphatthong, S. Sukrong, Mitigating the impact of admixtures in Thai herbal products, Front. Pharmacol. 10 (2019) 1205.

[14] F. Sun, X. Yang, F. Liu, Y. Zhang, S. Wang, H. Cao, J. Meng, Quality assessment of different species and differently prepared slices of zedoary rhizome by high-performance liquid chromatography and colorimeter with the aid of chemometrics, J. Anal. Methods Chem. 2020 (2020).

[15] R. Kisanthia, A.J. Hunt, J. Sherwood, L-o Somsakeesit, C. Phaosiri, Impact of conventional and sustainable solvents on the yield, selectivity, and recovery of curcuminoids from turmeric, ACS Sustainable Chem. Eng. 10 (1) (2021) 104–114.

[16] Z.A. Zakaria, F.H. Kamisan, T.L. Kek, M.Z. Salleh, Hepatoprotective and antioxidant activities of *Dicranopteris linearis* leaf extract against paracetamol-induced liver intoxication in rats, Pharm. Biol. 58 (1) (2020) 478–489.

[17] H. Lü, R.-P. Lee, J. Huang, J. Chen, V.-L.W. Go, Z. Li, Q.-Y. Lu, A new HPLC–UV method for the quantification of terpenoids and antioxidant activity of commercial loquat leaf tea and preparation, J. Food Meas. Charact. 14 (2020) 1085–1091.

[18] I.-C. Chao, C.-M. Wang, S.-P. Li, L.-G. Lin, W.-C. Ye, Q.-W. Zhang, Simultaneous quantification of three curcuminoids and three volatile components of *Curcuma longa* using pressurized liquid extraction and high-performance liquid chromatography, Molecules 23 (7) (2018) 1568.

[19] N.S. Dosoky, W.N. Setzer, Chemical composition and biological activities of essential oils of *Curcuma* species, Nutrients 10 (9) (2018) 1196.

[20] S. Mohanty, A. Ray, P.K. Naik, A. Sahoo, S. Jena, P.K. Das, et al., Variation in yield, chemical composition and biological activities of essential oil of three *Curcuma* species: a comparative evaluation of hydrodistillation and solvent-free microwave extraction methods, Molecules 28 (11) (2023) 4434.

[21] A.C.F. Amaral, L.A. Gomes, JRdA. Silva, J.L.P. Ferreira, AdS. Ramos, MdSS. Rosa, et al., Liposomal formulation of turmerone-rich hexane fractions from *Curcuma longa* enhances their antileishmanial activity, BioMed Res. Int. 2014 (2014).

[22] B.G. Keevil, LC–MS/MS analysis of steroids in the clinical laboratory, Clin. Biochem. 49 (13–14) (2016) 989–997.

[23] J. Zhou, C. Li, G. Zhao, H. Chu, D. Wang, H.H. Yan, V.K. Poon, L. Wen, B.H. Wong, X. Zhao, M.C. Chiu, D. Yang, Y. Wang, R.K.H. Au-Yeung, I.H. Chan, S. Sun, J. F. Chan, K.K. To, Z.A. Memish, V.M. Corman, C. Drosten, I.F. Hung, Y. Zhou, S.Y. Leung, K.Y. Yuen, Human intestinal tract serves as an alternative infection route for Middle East respiratory syndrome coronavirus, Sci. Adv. 3 (11) (2017) eaao4966.

[24] L. Schöler, V.T.K. Le-Trilling, M. Eilbrecht, D. Mennerich, O.E. Anastasiou, A. Krawczyk, A. Herrmann, U. Dittmer, M. Trilling, A novel in-cell ELISA assay allows rapid and automated quantification of SARS-CoV-2 to analyze neutralizing antibodies and antiviral compounds, Front. Immunol. 11 (2020 Oct 9) 573526.

[25] C. Conzelmann, A. Gilg, R. Groß, D. Schütz, N. Preising, L. Ständker, B. Jahrsdörfer, H. Schrezenmeier, K.M.J. Sparrer, T. Stamminger, S. Stenger, J. Münch, J. A. Müller, An enzyme-based immunodetection assay to quantify SARS-CoV-2 infection, Antiviral Res. 181 (2020) 104882.

[26] J. Harlow, M. Dallner, N. Nasheri, Protective effect of food against inactivation of human coronavirus OC43 by gastrointestinal fluids, Food Environ. Virol. 14 (2) (2022) 212–216.

[27] H. Ti, Z. Mai, Z. Wang, W. Zhang, M. Xiao, Z. Yang, P. Shaw, Bisabolane-type sesquiterpenoids from Curcuma longa L. exert anti-influenza and anti-inflammatory activities through NF-κB/MAPK and RIG-1/STAT1/2 signaling pathways, Food Funct. 12 (15) (2021) 6697–6711.

[28] L. Ma, L. Yao, Antiviral effects of plant-derived essential oils and their components: an updated review, Molecules 25 (11) (2020) 2627.

[29] Q. Liao, Z. Qian, R. Liu, L. An, X. Chen, Germacrone inhibits early stages of influenza virus infection, Antivir. Res. 100 (2013) 578–588.

[30] L. Li, Q. Xie, G. Bian, B. Zhang, M. Wang, Y. Wang, Z. Chen, Y. Li, Anti-H1N1 viral activity of three main active ingredients from zedoary oil, Fitoterapia 142 (2020) 104489.

[31] J.Y. Dong, X.Y. Ma, X.Q. Cai, P.C. Yan, L. Yue, C. Lin, W.W. Shao, Sesquiterpenoids from Curcuma wenyujin with anti-influenza viral activities, Phytochemistry 85 (2013) 122–128.

[32] S.S. Fazalul Rahiman, M. Al-Amin, N. Mohtar, I.I. Zakaria, U.M. Kahar, M. Khairuddean, S.M. Salhimi, Potential inhibitors isolated from Curcuma aeruginosa against dengue virus type 2 (DENV-2) NS2B-NS3 protease activity, J. Biol. Active Prod. Nature. 14 (1) (2024) 64–79.

[33] X. Zhang, Y. Xia, L. Yang, J. He, Y. Li, C. Xia, A. Brevilin, A sesquiterpene lactone, inhibits the replication of influenza A virus in vitro and in vivo, Viruses 11 (9) (2019) 835.

[34] M. Paulpandi, S. Kannan, R. Thangam, K. Kaveri, P. Gunasekaran, C. Rejeeth, In vitro antiviral effect of β-santalol against influenza viral replication, Phytomedicine 19 (3–4) (2012) 231–235.

[35] Z. Ayati, M. Ramezani, M.S. Amiri, A.T. Moghadam, H. Rahimi, A. Abdollahzade, et al., Ethnobotany, phytochemistry and traditional uses of *Curcuma* spp. and pharmacological profile of two important species (*C. longa* and *C. zedoaria*): a review, Curr. Pharm. Des. 25 (8) (2019) 871–935.