ว กรมวิทย พ 2565; 64 (2): 93-105

Anti-SARS-CoV-2 Activity Screening of the Selected Thai Medicinal Plants and Potential Host-target Molecules

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ABSTRACT Coronavirus disease 2019 (COVID-19), the pandemic, is caused by a virus called SARS-CoV-2. The objective of this study was to investigate the anti-SARS-CoV-2 properties of some selected medicinal plants used as antipyretic in Thai traditional medicines. They were screened for anti-SARS-CoV-2 activity using plaque reduction assay. The extracts were further investigated for activity in enzymatic inhibition and gene expression assays of several host-target molecules. The results revealed that the aqueous extracts of *Mesosphaerum suaveolens* (L.) Kuntze (HSF, HSD) and *Helicteres isora* L. (HID) at the highest concentrations of 5, 5 and 10 mg/mL showed the highest anti-SARS-CoV-2 activities of 100%, 100% and 99.49%, respectively. Rosmarinic acid (RA), one of the phytochemicals found in *M. suaveolens* (L.) Kuntze and *H. isora* L., at 0.625 mg/mL, gave an antiviral activity of 94.49%. RA also showed the highest relative ACE2 inhibition of 70.25% at 100 μg/mL with no toxicity to the cells, whereas the other extracts showed a lower level of relative ACE2 inhibition. RA also reduced the expression of ACE2 and TMPRSS2 but not PIKfyve or cathepsin L in Calu-3, lung epithelial cell lines. In conclusion, HSF, HSD, HID and RA provided anti-SARS-CoV-2 activity, which could limit the viral infection in the early phase. The extracts should be further investigated for anti-SARS-CoV-2 activity in animals and clinical trials for development as herbal drugs.

Keywords: Anti-SARS-CoV-2 activity, ACE2 inhibition, Target molecules, COVID-19

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Received: 31 January 2022 *Revised: 9 March 2022* *Accepted: 2 June 2022*

Introduction

Coronavirus disease 2019 (COVID-19) is caused by a virus called SARS-CoV-2. Patients will develop respiratory symptoms, including fever, cough, and shortness of breath or difficulty breathing.[1] It can cause complications such as pneumonia, kidney failure or even death. It was first detected in December 2019 in Wuhan, China. The World Health Organization then declared an international public health emergency and a global pandemic in March 2020. Currently, Thailand has cumulatively 2.8 million infected cases and 22,849 deaths, whereas globally there have been 285 million cases and 5.4 million deaths (February 2022).[1] The Economist magazine reported the value of global economic losses in 2020–2021 due to the coronavirus pandemic at $10.3 trillion based on a 6.6% drop in global GDP compared to forecasts in the absence of COVID-19. The total economic damage to Thailand is estimated at 18.4 trillion baht.[2] The COVID-19 outbreak now has a huge impact on the people and economies around the world. The Thai government has issued various policies and measures to help alleviate the suffering of the affected people by developing or adopting vaccines, medicines and related medical products. It is very important for the treatment and prevention of this virus. However, vaccine and drug development takes a long time and costs a lot of money. The use of herbal medicines or traditional medicines that could prevent, cure or reduce illnesses from COVID-19 is another alternative measure. Thus, such health products or modern drugs have to be developed.

From such events, some people have turned to use medicinal plants or alternative medicines, which are recorded in the Thai Herbal Pharmacopoeia or other traditional pharmacopoeia. Most of them showed antipyretic property and alleviating viral diseases including *Andrographis paniculata* (Burm.f.) Nees (Family Acanthaceae), *Cinnamomum verum* J.Presl. (Family Lauraceae), *Cinnamomum bejolghota* (Buch.-Ham.) Sweet (Family Lauraceae), *Houttuynia cordata* Thunb. (Family Saururaceae), *Tiliacora triandra* (Colebr.) Diels (Family Menispermaceae), *Mesosphaerum suaveolens* (L.) Kuntze (Family Lamiaceae), *Cinchona calisaya* Wedd. (Family Rubiaceae), *Helicteres isora* L. (Family Malvaceae) or even *Kaempferia parviflora* Wall. ex Baker (Family Zingiberaceae). Some of them were reported to alleviate symptoms or inhibit the coronavirus. *A. paniculata* (Burm.f.) Nees extract capsules containing 60 mg andrographolide were reported to reduce fever, cough, sore throat, sputum volume and headache in COVID-19 clinical trials.[3] *Boesenbergia rotunda* (L.) Mansf. (Family Zingiberaceae) and *Zingiber officinale* Roscoe (Family Zingiberaceae) extracts showed potent activity against COVID-19 *in vitro* in the pre-entry and post-infection stages.[4] Procyanidin A2, procyanidin B1 and cinnamtannin B1 in *C. verum* J.Presl. posed activity against SARS-CoV by inhibiting clathrin-mediated endocytosis.[5] Moreover, quercetin, the active compound in *H. cordata* Thunb., was found to inhibit chymotrypsin-like cysteine protease (3CLpro) of SARS-CoV-2.[6]

The infection process of the virus begins with the virus entering the cell by attaching a spike (S) glycoprotein to human receptor cells. The main receptor is the host angiotensin-converting enzyme 2 (ACE2) receptor, which is

cleavaged together with transmembrane serine protease 2 (TMPRSS2) before endocytosis. Once it enters the cell, the virus also needs the PIKfyve enzyme to form an endosome and transport virus particles in the cytoplasm. This process also needs cathepsin L protease, which is responsible for endosome maturity. The inhibition of PIKfyve and cathepsin L protease has been shown to be effective against SARS-CoV-2, Ebola virus (EBOV) and African swine fever in the early and middle stages of the infection. The virus then gets disassembled to release the nucleocapsid and the viral genome. The transcription process utilizes RNA-dependent RNA synthesis to generate mRNAs to translate into structural and non-structural proteins. Finally, the virion progeny is generated via rough ER, Golgi apparatus and exocytosis.[7]

This research, therefore, aimed to investigate the antiviral property of the medicinal plant extracts to the main host receptors and proteases focusing on ACE2 and others such as TMPRSS2, PIKfyve and cathepsin L, which were involved in the pre-entry and early phases of infection.

Materials and Methods

Cell culture

Vero E6 cells, African green monkey kidney epithelial cells (ATCC, NY, USA), were used in antiviral property investigation. Calu-3, lung epithelial cell line (ATCC, NY, USA), was used in the gene expression assay. The cells were grown in Eagle's minimum essential medium (EMEM) (Gibco, NY, USA) containing 2 mM L-glutamine, 0.1 mM non-essential amino acid, 1 mM sodium pyruvate and 10% fetal bovine serum at 37°C in a 5% CO_2 incubator.

Virus

SARS-CoV-2 (Delta variant/EPI_ISL_3797061) was obtained from a human nasopharyngeal swab. The virus was propagated in Vero E6 cells by three passages to establish a high-titer stock and stored at −80°C for use in all experiments. Virus titration as $TCID_{50}$ titer/mL was performed. All the experiments with live SARS-CoV-2 were performed at a certified biosafety level 3 facility of the National Institute of Health, Department of Medical Sciences, Thailand.

Standards

Rosmarinic acid (RA) (> 98% HPLC grade) and quinine sulfate (QS) (> 98% HPLC grade) from Merck KGaA (Darmstadt, Germany) were used.

Plant Materials

Fresh aerial parts of *M. suaveolens* (L.) Kuntze and green-brown fruits of *H. isora* L. were collected from Kanchanaburi province, Thailand, in February 2019 and September 2020, respectively, while fresh leaves of *T. triandra* (Colebr.) Diels, fresh bark of *C. calisaya* Wedd. and fresh rhizomes of *K. parviflora* Wall. ex Baker were from Phetchaburi, Chiang Mai and Tak provinces, Thailand, in October 2020, December 2020 and January 2021, respectively. The plants were identified by Mr. Sakwichai Ontong, a botanist, based on the plant identification handbook of Royal Botanic Gardens, Kew, London, United Kingdom. Voucher specimens were collected as DMSC5291, DMSC5270, DMSC5240, DMSC5295 and DMSC5285 for *M. suaveolens* (L.) Kuntze, *H. isora* L., *T. triandra* (Colebr.) Diels, *C. calisaya* Wedd. and *K. parviflora*

Wall. ex Baker, respectively. All of them were deposited at the DMSc International Herbarium, Department of Medical Sciences, Ministry of Public Health.

Plant Extraction

The plant parts were cleaned up with tap water, cut into small pieces about 1 cm in length. For the dried plant extraction of *M. suaveolens* (L.) Kuntze, *H. isora* L., and *K. parviflora* Wall. ex Baker, the materials were dried in a hot air oven at 45–50 °C for 24–48 hours and then ground into powder. The dried powder then was refluxed with distilled water (360 g of the powder in 3 L of water), the plant part was filtered and passed through the extraction process with distilled water two times consecutively. The aqueous extract of each plant, *M.suaveolens* (L.) Kuntze, *H. isora* L., and *K. parviflora* Wall. ex Baker, was then pooled, concentrated and dried by rotary evaporation yielding HSD, HID and KPD extracts, respectively. The dried extracts were collected and kept in a light protective bottle at −20 °C until use. For the fresh parts extraction of *M. suaveolens* (L.) Kuntze, *T. triandra* (Colebr.) Diels and *C. calisaya* Wedd., fresh small pieces were ground and then the extractions were conducted using the same procedure, but with lyophilization instead of rotary evaporation to dry the powder, and yielding HSF, TTF and CCF extracts, respectively.

The extracts were also analyzed for its phytochemical compounds with quality control using high performance liquid chromatography (HPLC) with diode array detector. Rosmarinic acid was used as a standard for HSF, HSD and HID, whereas quercetin, quinine sulfate and 5-hydroxy-7-methoxy flavanone were used for TTF, CCF and KPD, respectively. The plant extractions with the quality control were conducted at the Herbal Quality Assurance Center, Medicinal Plant Research Institute, Department of Medical Sciences. The data will be further published elsewhere.

MTT assay

The cells were seeded at 5×10^3 cells in 100 µL medium per well of 96-well plates and left in the incubator for 24 hours. The medium was removed and the cells were treated with eight different concentrations of each compound in triplicate and then incubated for 48 hours. The medium was replaced with 200 µL MTT reagent and then continued to incubate for 4 hours. The MTT solution was discarded and 200 µL DMSO was added to each well to dissolve the purple formazan product. The absorbance of the formazan product of viable cells was read using the microplate reader at 570 nm. The background absorbance was reduced by the blank and %viability was calculated compared to the control.

Anti-SARS-CoV-2 activity screening using plaque reduction assay

This assay was designed to investigate the antiviral property at the pre-entry phase modified from Kanjanasirirat, P. *et al.*, (2020).[4] The extracts were pre-incubated with SARS-CoV-2 at 37 °C for 1 hour before transferring 200 µL of the solution with virus particles onto the monolayer of vero E6 cells. Viral adsorption was allowed for 1 hour in the CO_2 incubator. The cells were washed with a fresh medium to remove both the unbound viral particles and the extract/compound. Then 3 mL overlaid medium was added into the wells. The semi-solid medium was allowed to set and all plates were placed in the incubator for 7 days. The overlaid

medium was discarded and cells were fixed with 5% formaldehyde and then stained with 0.5% (w/v) crystal violet. The excess colour was washed with tap water. The plaques were counted and % inhibition was calculated compared to the controls (without the compound). All extracts were used at previously determined non-toxic concentrations.

ACE2 inhibition assay

The enzymatic inhibition assay was conducted using angiotensin II converting enzyme (ACE2) activity assay kit (Abcam, MA, USA) at the Toxicology Laboratory, Department of Medical Sciences. The enzyme solution and reagents were prepared following the procedure described in the manufacturer's protocol. The plant extracts were diluted with water at the highest concentration, which was not toxic to Calu-3 cells. Totally, 48 μL of ACE2 assay buffer was mixed with 2 μL diluted ACE2 enzyme solution and then the solution was added to the diluted extracts or the standard inhibitor (the background and enzyme controls were also tested). The mixed solutions were placed at room temperature for 15 min. Totally, 40 μL ACE2 substrate mix was then added to the wells and immediately placed in spectrofluorometer and the fluorescence signal (Ex/Em = 320/420 nm) was measured after being in kinetic mode for 1 hour. The relative inhibition activity (%) of the sample was calculated using a relative fluorescence unit (RFU) compared to enzyme control.

Gene expression using real-time reverse-transcription polymerase chain reaction (RT-PCR)

Calu-3 cells were seeded at 5×10^5 cells/well in 6-well plates for 24 hours and then treated with the compounds or extracts at concentrations that were not toxic to the cells for 24 hours. Total RNA of each sample was extracted using the RNeasy Mini Kit (Qiagen, Hilden, Germany). According to the manufacturer's protocol, RNA was re-suspended in 30 μL of nuclease-free water and then the products were run on agarose gels to check the quality of the RNA. cDNA was synthesized using QuantiTect Rev. Transcription Kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocol. Briefly, 2 μg template RNA was added to the reverse-transcription master mix and then the samples tubes were incubated at 42 °C for 15 min. The cDNA samples were tested in triplicate with quantitative PCR using a QuantiTect SYBR Green PCR Reagents kit (Qiagen, Valencia, CA, USA). Totally, 2 μL of each sample was mixed with SYBR Green PCR Master Mix and 10x QuantiTect Primers (Qiagen, Valencia, CA, USA), and then the real-time PCR (RT-qPCR) was performed using the manufacturer's protocol in Thermal cycler (PCR) (Analytik Jena GmbH, Valencia, Jena, Germany). mRNA ratios relative to the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) housekeeping gene were calculated for the standardization of gene expression levels. A melting curve analysis was also performed to verify the specificity and identity of PCR products. For selected genes, the data were analyzed using the equation described by Livak and Schmittgen[8] as follows: the amount of target = $2^{-\Delta\Delta Ct}$. The average ΔCt from the untreated cells is a calibrator for each gene tested. This assay was conducted at the Toxicology Laboratory, Medicinal Plant Research Institute, Department of Medical Sciences.

Statistical analysis

The total percentage of plaque reduction assay was expressed in the mean of duplicated

experiments. The percentage of relative ACE2 inhibition and relative quantity of mRNA expression was expressed as mean±SD (n = 3). Statistical analysis of ACE2 enzymatic inhibition and gene expression of mRNA was carried out using the one-way analysis of variance followed by Dunnett's test.

Results

The six aqueous extract - HSF, HSD, HID, TTF, CCF and KPD - were obtained using reflux method and dried with rotary evaporation or using lyophilization technique. The highest percentage yield was for TTF at 20.74%, while those for HSD, HID, KPD, CCF, and HSF were 20.11%, 14.32%, 11.72%, 10.12% and 2.18%, respectively. RA quantities determined by using HPLC analysis in HSF, HSD and HID extracts were 0.35±0.02%, 0.18±0.01% and 0.29±0.01% (w/w), respectively. The weight of the plant materials and the percentage yields for the six extracts were shown in Table 1 and the chemical profiles of RA in HSF, HSD and HID were provided in Figure 1.

Table 1 Percentage yields of the herbal extracts

Extracts	Plant	Parts used	Weight		Yield (%)
			Plant materials (g)	Extracts (g)	
HSF	*M. suaveolens* (L.) Kuntze	Fresh aerial parts	1,200	26.20	2.18
HSD	*M. suaveolens* (L.) Kuntze	Air-dried aerial parts	360	72.42	20.11
HID	*H. isora* L.	Air-dried fruits	800	114.56	14.32
TTF	*T. triandra* (Colebr.) Diels	Fresh leaves	160	33.19	20.74
CCF	*C. calisaya* Wedd.	Fresh barks	500	55.60	10.12
KPD	*K. parviflora* Wall. ex Baker	Air-dried rhizomes	600	70.33	11.72

The activities of the plant extracts to reduce the pre-entry phase of SARS-CoV-2 were evaluated. Vero E6 cells were used as host cells for the virus. The percentage of the plaque reduction was calculated compared with the virus controls. HSD and HSF showed the highest plaque reduction of 100% at 5 mg/mL while HID, TTF, CCF, KPD and RA gave lower plaque reduction of 99.49%, 95.95%, 88.95%%, 85.24% and 94.49% at 10, 10, 0.625, 2.5 and 0.625 mg/mL, respectively. The anti-viral activities at different concentrations of the six extracts were shown in Table 2.

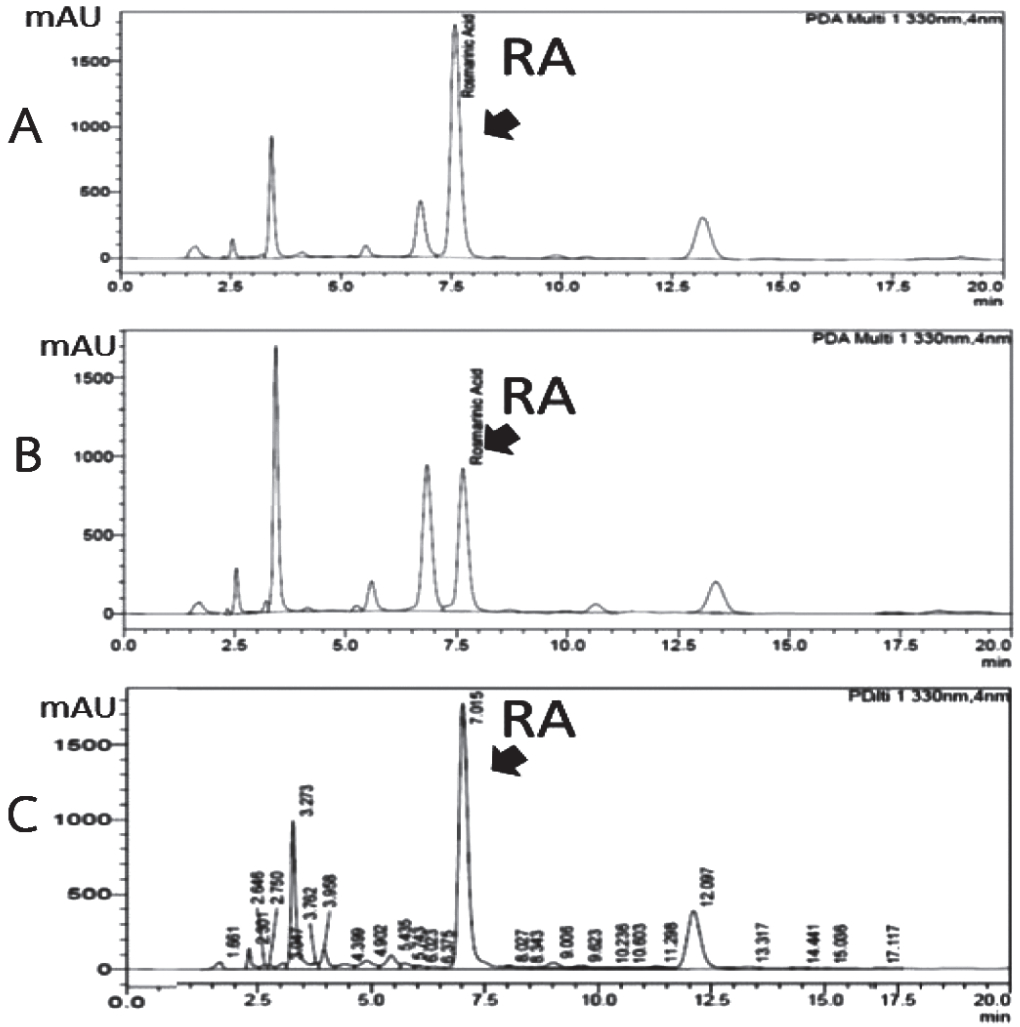

Figure 1 Chromatograms of rosmarinic acid in the three extracts, inj. vol. 10 μL, monitored at 330 nm A) HSF at 0.836 mg/mL; B) HSD at 0.479 mg/mL; C) HID at 0.747 mg/mL.

Table 2 Antiviral activities at the highest concentrations of the herbal aqueous extracts which were not toxic to the cells. The data was expressed as the mean, n = 2.

Herbal extracts/ compounds	The highest concentration tested (mg/mL)	Plaque reduction (%)
HSD	5.000	100.00
HSF	5.000	100.00
HID	10.000	99.49
TTF	10.000	95.95
CCF	0.625	88.95
KPD	2.500	85.24
RA	0.625	94.49
QS	0.250	81.65

ACE2 enzymatic inhibition of the extracts was evaluated at the highest concentration, which was not toxic to Calu-3 cells. HSD, HID and RA showed the inhibition activities in the dose-response relationship. RA showed the highest activity compared to others giving the relative inhibition values of 70.25%, 55.03% and 40.00% at the doses of 100, 50 and 25 µg/mL, respectively. HSD showed the inhibition values of 40.37%, 25.83% and 11.20% at the doses of 200, 100 and 50 µg/mL, respectively, while HID's inhibition values were 20.88%, 18.53% and 12.79% at the doses of 50, 25 and 12.5 µg/mL, respectively. ACE2 enzymatic inhibition values at different concentrations of HSD, HID and RA were shown in Figure 2.

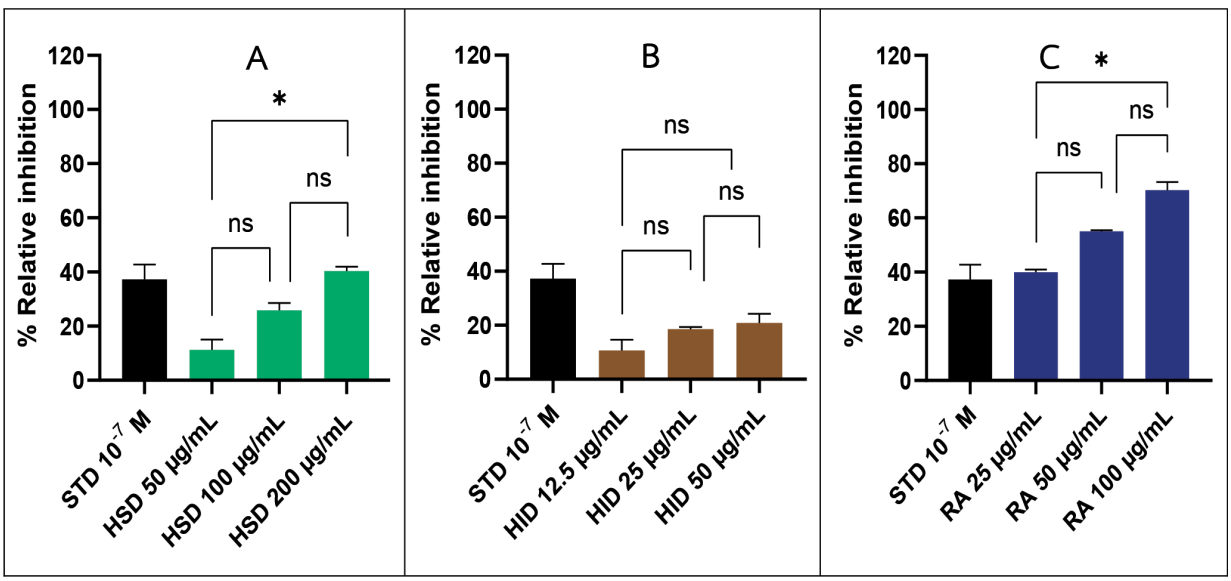

Figure 2 ACE2 enzymatic inhibitory activity at the concentrations which were not toxic to Calu-3 cells of the standard inhibitor (STD), (A) HSD, (B) HID and (C) RA. The data was expressed as mean ± SD, n = 3. Statistical analysis was carried out using One-Way analysis of variance followed by Dunnett's test (*$p < 0.5$).

Gene expression assay of four main genes for viral entry and progression processes was conducted. The lung cells were treated with RA at the concentrations of 25, 50 and 100 µg/mL. ACE2 and TMPRSS2 genes were down-regulated compared to the untreated control and demonstrated a dose-response relationship, whereas the expression of PIKfyve and cathepsin L remained the same at the doses of 25 and 50 µg/mL. At the highest concentration of 100 µg/mL, RA up-regulated PIKfyve and cathepsin L (around 1.4- to 2-fold increase at 100 µg/mL). The mRNA expression data of ACE2, TMPRSS2, PIKfyve and cathepsin L in Calu-3 cells treated with different RA concentrations were shown in Figure 3.

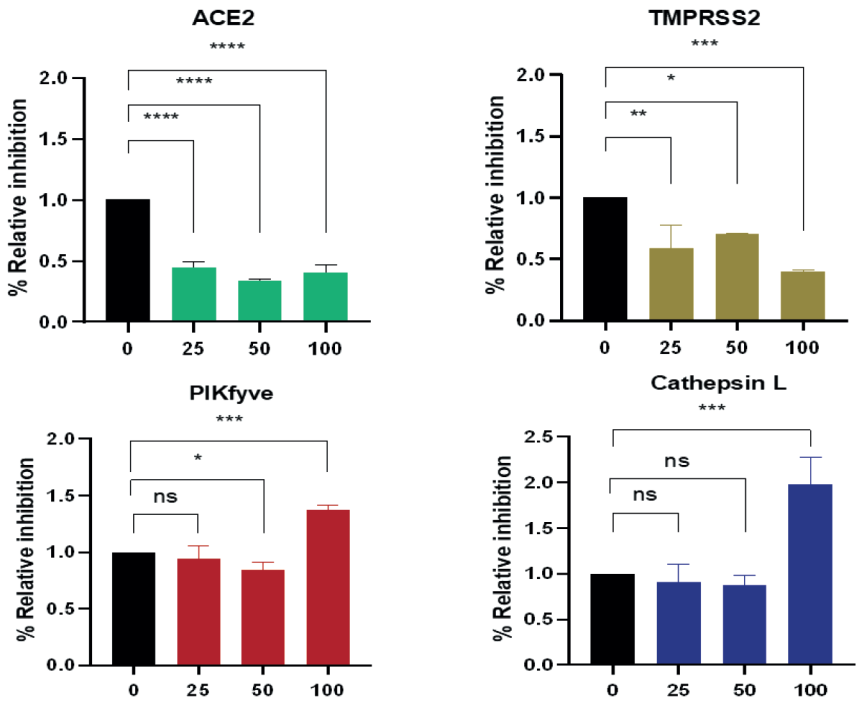

Figure 3 Relative quantity of mRNA expression of ACE2, TMPRSS2, PIKfyve and cathepsin L in Calu-3 cells treated with RA at 25, 50 and 100 μg/mL versus control data set for 24 h. The data was expressed as mean±SD, n = 3. Statistical analysis was carried out using One-Way analysis of variance followed by Dunnett's test (*$p < 0.5$, **$p < 0.005$, ***$p < 0.0005$ and ****$p < 0.0001$).

Discussion

Various Thai medicinal plants with traditional uses against viral diseases and antipyretic properties were screened for anti-SARS-CoV-2 activity. The aqueous extracts of *M. suaveolens* (L.) Kuntze and *H. isora* L. were reported here to pose anti-SARS-CoV-2 activity for the first time. The aqueous crude extracts were derived from fresh and air-dried plant parts. The fresh one has imitated the traditional extraction method[9] using fresh plants and then the filtrate was dried using lyophilization to reduce the effect of heat. On the other hand, the air-dried plant extraction was undertaken and then the filtrate was concentrated and dried using rotary evaporation.

RA is one of the constituents quantified in HSF and HSD. RA content determined in HSF was about twice that in HSD. RA in HSD might be degraded during the drying process. The extracts were screened for antivirus properties at high concentrations, which were not toxic to vero cells. HSF and HSD posed high anti-SARS-CoV-2 activity of 100% plaque reduction at 5 mg/mL. RA also showed anti-SARS-CoV-2 activity of 94.49% at 0.625 mg/mL. RA, therefore, may contribute to the antiviral property of the extracts. RA and its derivatives were reported to be the constituents of *M. suaveolens* (L.) Kuntze and *H. isora* L.,[10,11] which could cause the aqueous extract of *H. isora* L. exhibit the

activity. Besides, *M. suaveolens* (L.) Kuntze is an aromatic herb containing various compounds such as essential oils, phenolics, diterpenoids, triterpenoids, steroids, alkaloids and flavonoids.[10] Ursolic acid is one of the phytochemicals in the plants reported to show antispasmodic[11] and antiviral activity against rotaviruses, HIV, influenza and hepatitis B and C viruses.[12-15] Moreover, ursolic acid was identified as a strong protease inhibitor to the main protease (Mpro) of SARS-CoV-2 based on the molecular docking approach.[16]

RA inhibited ACE2 enzymatic reaction. However, the concentrations of HSD and HID depended on cytotoxic level of the extracts. ACE2 inhibition property of RA might be one of the important mechanisms contributing to the antiviral activity. Gene expression was investigated in Calu-3, lung epithelial cells, which expressed ACE2, TMPRSS2, PIKfyve and cathepsin L. The expression showed that RA down-regulated ACE2 and TMPRSS2 in the dose-response relationship but not PIKfyve and cathepsin L. The highest dose of RA was likely to up-regulate PIKfyve and cathepsin L expression but not higher than twofold. RA was also reported to show high efficacy in terms of molecular interaction and drug-likeness properties against ACE2 and TMPRSS2[17] or SARS-CoV 3CLpro[18] by molecular docking analysis. The mRNA expression analysis showed that the compound inhibited ACE2 along with TMPRSS2 which had been highlighted in recent studies to be one of the main targets of SARS-CoV-2 infection.[19,20] We also reported here for the first time that the aqueous extracts of fresh leaves of *T. triandra* (Colebr.) Diels (TTF), *C. calisaya* Wedd. (CCF) and *K. parviflora* Wall. ex Baker (KPD) were also found to possess *in vitro* anti-SARS-CoV-2 activity. *T. triandra* (Colebr.) Diels has been generally used in cuisine and traditional medicine in Southeast Asia. The main compounds of the aqueous extracts were analyzed as quercetin, cyanidin, gallic acid, chlorophyll, rutin, tannic acid and catechin.[21] Some sources reported that chlorophyll derivatives and quercetin could be potential therapeutic agents for treating COVID-19.[6,22] *C. calisaya* Wedd. has been identified to be the primary source of quinine, used as antimalarial. Quinine was reported that it targeted 6LU7 COVID-19 protease resulting in inhibition of the viral infection by in silico analysis.[23] *K. parviflora* Wallich. ex Baker belongs to the family Zingiberaceae, the same as *B. rotunda* (L.) Mansf. and *Z. officinale* Roscoe. This plant family has been reported to be the source of phytochemicals which were active against SARS-CoV-2 such as 6-gingerol from *Z. officinale* Roscoe and panduratin A from *B. rotunda* (L.) Mansf.[4]

Currently, the SARS-CoV-2 RNA-dependent RNA polymerase (RdRp) is being targeted by several inhibitors such as favipiravir, remdesivir and molnupiravir; and there are some pieces of evidence of drug resistance.[24] HSF, HSD, HID and RA provided anti-SARS-CoV-2 activity targeting the host molecules, which could limit the viral infection in the early phase and drug resistance. These extracts might be developed as anti-SARS-CoV-2 candidates in pre-clinical and clinical trials. Moreover, these phytochemical compounds should also be further isolated from the six extracts which were investigated for anti-SARS-CoV2 activity.

Conclusion

Based on the results, the two extracts of *M. suaveolens* (L.) Kuntze (HSF, HSD) showed the highest inhibitory activity (with no toxicity to the cells) against SARS-CoV-2 at 100% with the concentration of 5 mg/mL, while HID, TTF, CCF and KPD gave lower levels of plaque reduction of 99.49%, 95.95%, 88.95% and 85.24% at the doses of 10, 10, 0.625 and 2.5 mg/mL, respectively. HSD and HID showed ACE2 enzymatic inhibition. RA, one constituent of *M. suaveolens* (L.) Kuntze and *H. isora* L., also showed potent inhibition. RA down-regulated the expression of ACE2 and TMPRSS2 but not PIKfyve or cathepsin L in Calu-3 which were involved in the pre-entry and early phases of infection. The phytochemicals reported here in these selected medicinal plants should be developed as anti-SARS-CoV-2 candidates in animals and clinical trials or further isolated for other potential compounds against SAR-CoV-2. This is the first report on the *in vitro* anti-SARS-CoV-2 properties with detailed mechanisms of action of the selected Thai medicinal plant extracts.

Acknowledgements

This research was supported by the Department of Medical Sciences, Ministry of Public Health.

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การคัดกรองฤทธิ์ต้าน SARS-CoV-2 และโมเลกุลเป้าหมายการออกฤทธิ์ของพืชสมุนไพรไทย

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บทคัดย่อ โรคโคโรนาไวรัส 2019 หรือโควิด 19 เป็นโรคระบาดที่เกิดขึ้นทั่วโลกมีสาเหตุจากการติดเชื้อไวรัส SARS-CoV-2 การวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาว่าสมุนไพรไทยอาจมีฤทธิ์ยับยั้งเชื้อไวรัสดังกล่าว จึงได้คัดกรองฤทธิ์ต้าน SARS-CoV-2 ในหลอดทดลองด้วยวิธี plaque reduction assay ของพืชสมุนไพรบางชนิดที่มีสรรพคุณลดไข้ในตำรับยาแผนไทย โดยสารสกัดที่มีฤทธิ์ต้านไวรัสจะถูกนำมาศึกษาปฏิกิริยายับยั้งเอนไซม์ ACE2 และทดสอบการแสดงออกของยีนในเซลล์ปอดเพาะเลี้ยง ผลการวิจัยพบว่าสารสกัดด้วยน้ำของสมุนไพรแมงลักคา (HSF, HSD) และปอบิด (HID) ที่ความเข้มข้นสูงสุด 5, 5 และ 10 mg/mL มีฤทธิ์ต้าน SARS-CoV-2 ได้ 100, 100 และ 99.49% ตามลำดับ rosmarinic acid (RA) ซึ่งเป็นสารพฤกษเคมีชนิดหนึ่งในสมุนไพรแมงลักคาและปอบิดที่ความเข้มข้น 0.625 mg/mL มีฤทธิ์ต้านไวรัสที่ 94.49% และยับยั้งเอนไซม์ ACE2 สูงสุดที่ 70.25% ที่ความเข้มข้น 100 µg/mL ซึ่งไม่เป็นพิษต่อเซลล์ ในขณะที่สารสกัดสมุนไพรอื่นมีค่าการยับยั้งน้อยกว่า ซึ่ง RA ลดการแสดงออกของยีน ACE2 และ TMPRSS2 แต่ไม่ลดการแสดงออกของ PIKfyve หรือ cathepsin L ใน Calu-3 cells จึงสามารถสรุปได้ว่า HSF, HSD, HID และ RA มีฤทธิ์ต้าน SARS-CoV-2 ซึ่ง RA ในสมุนไพรอาจลดการแพร่กระจายไวรัสในระยะแรกของการติดเชื้อได้ ดังนั้นควรมีการศึกษาฤทธิ์ต้าน SARS-CoV-2 ของสารสกัดดังกล่าวในสัตว์ทดลองและทางคลินิกเพื่อพัฒนาเป็นยาสมุนไพรต้านไวรัสต่อไป

คำสำคัญ: ฤทธิ์ต้าน SARS-CoV-2, ฤทธิ์ยับยั้ง ACE2, โมเลกุลเป้าหมาย, โควิด 19