**Curcumin and turmeric extract inhibited SARS-CoV-2 pseudovirus cell entry and Spike mediated cell fusion**

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**CONFLICT OF INTEREST**

The authors declared no potential conflict of interests.

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Not applicable.

**DATA AVAILABILITY**

All data and materials are provided within the research article.

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# Curcumin and turmeric extract inhibited SARS-CoV-2 pseudovirus cell entry and Spike mediated cell fusion

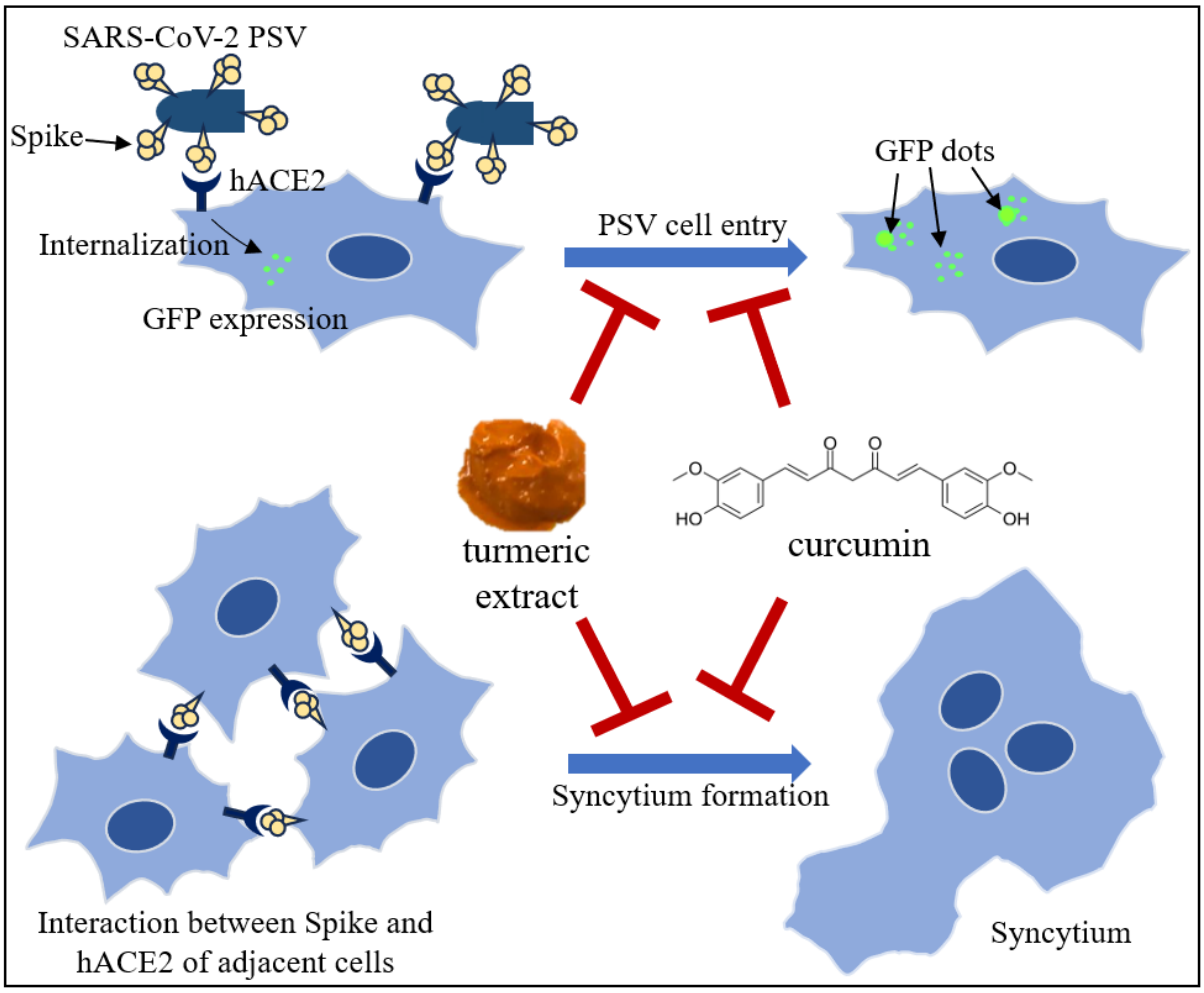

## ABSTRACT

Turmeric extract (TE) with curcumin as its main active ingredient has been studied as a potential COVID-19 therapeutic. Curcumin has been studied in silico and in vitro against a naive SARS-CoV-2 virus, yet little is known about TE's impact on SARS-CoV-2 infection**.** Moreover, no study reveals the potentials of both curcumin and TE on the inhibition of SARS-CoV-2 cell-to-cell transmission. Here, we investigated the effects of both curcumin and TE on the inhibition of SARS-CoV-2 entry and cell-to-cell transmission using pseudovirus (PSV) and syncytia models. We performed PSV entry assay in 293T or 293 cells expressing hACE2. The cells were pretreated with curcumin or TE, then treated with PSV with or without the test samples. Next, we carried out syncytia assay by co-transfecting 293T cells with plasmids encoding Spike, hACE2, and TMPRSS2 to be treated with the test samples. The results showed that in PSV entry assay on 293T/hACE/TMPRSS2 cells, both curcumin and TE inhibited PSV entry at concentrations of 1 µM and 10 µM for curcumin and 1 µg/ml and 10 µg/ml for TE. Moreover, both curcumin and TE also reduced syncytia formation compared to control cells. Based on our study, both TE and curcumin are potential inhibitors of SARS-CoV-2 infection at entry points, either by direct or indirect infection models.



**Keywords:** COVID-19, curcumin, pseudovirus, SARS-CoV-2, syncytia, turmeric extract.

**GRAPHICAL ABSTRACT:**



## 1. INTRODUCTION

SARS-CoV-2 can infect the target cells by direct viral infection and cell-to-cell transmission (Jackson et al., 2021; Rajah et al., 2021). The first mode of viral infection has been widely explored by studying cell and organ tropisms. SARS-CoV-2 infection toward various cell types has been performed, which includes studies using pulmonary cancer cells Calu-3, kidney cells 293FT, liver cells Huh7, colon cancer cells CaCO2, and Vero (Hoffmann et al., 2020; Katopodis et al., 2022; Saccon et al., 2021). In addition, organ tropisms have been studied using post-mortem tissue samples from COVID-19 patients and organoid models (Peng et al., 2022). Based on these studies, SARS-CoV-2 tropisms are strongly related to hACE2 expression as its target receptor and TMPRSS2 protease, which enhances the viral entry process. SARS-CoV-2 infection involves the interaction of Spike glycoprotein with hACE2, with or without Spike priming by TMPRSS2, to facilitate cell entry through endocytosis or membrane fusion mechanisms (Hirano et al., 2020; Jackson et al., 2021).

In addition, SARS-CoV-2 can transmit through a cell-to-cell mechanism, which induces cell-to-cell fusion and syncytia formation (Zheng et al., 2021). Syncytia has been found in the lungs of patients infected with SARS-CoV-2 (Lin et al., 2021). SARS-CoV-2 infection induces spike glycoprotein expression. Eventually, as a transmembrane protein, Spike will be transported to the plasma membrane with the receptor binding domain (RBD) at the extracellular position, ready for receptor binding. Similar to the virally integrated Spike as the outer protein, cellular Spike protein can also bind to the adjacent cells' hACE2 receptor, which further induces cell-to-cell fusion and finally forms a multinucleated giant cell or syncytium (Zheng et al., 2021; Lin et al., 2021). Syncytia formation has been implicated with the severity of COVID-19 prognosis by several mechanisms, including immune cell phagocytosis, antibody evasion, and induction of syncytial cell death (Rajah et al., 2022).

Due to the availability of registered COVID-19 drugs and the continuous emergence of daily COVID-19 cases, alternative treatments that can be easier to obtain for preventing and treating future COVID-19 cases are essential to be developed. Turmeric (Curcuma longa L.) is a WHO-selected medicinal plant that grows throughout tropical areas (WHO, 1999). Turmeric rhizome has been processed into turmeric decoction and turmeric extract, and some has been developed as a standardized medicinal extract. Turmeric extract contains curcuminoid active compounds, with curcumin as its main component (Sharifi-Rad et al., 2020). Clinical studies have demonstrated the effect of nano-encapsulated curcumin in reducing clinical manifestations of COVID-19, such as fever, cough, and dyspnea, compared to the control group (Tahmasebi et al., 2021; Valizadeh et al., 2020).

In silico analysis showed that curcumin has a high affinity for binding to the spike glycoprotein through the formation of six hydrogen bonds (Maurya et al., 2020), thus, curcumin has the potential to prevent the binding of the viral spike protein to the ACE2 receptor and inhibit the initiation of the host cell infection process. Moreover, curcumin forms four hydrophobic interactions via hydrogen bonds with TMPRSS2, which may inhibit cellular entry through the cell fusion mechanism (Motohashi et al., 2020).

A plaque assay study in Vero cells has shown that curcumin inhibits SARS-CoV-2 infection in pre- and post-treatment of the D614 strain and Delta variant (Marin-Palma et al., 2021). A study by Bormann *et al.* (2021) shows that turmeric root juice and curcumin showed inhibition of SARS-CoV-2 infection in Vero and Calu-3 cells determined by plaque assay and in-cell ELISA to detect the signal of SARS-CoV-2 nucleocapsid protein (N). Nonetheless, the effect of turmeric extract (TE) on SARS-CoV-2 infection is less studied than curcumin. Moreover, there is no report about the effects of both curcumin and TE on SARS-CoV-2 cell-to-cell transmission. In the present study, we examined the effects of TE and curcumin on

inhibiting SARS-CoV-2 infection using the pseudovirus (PSV) and syncytia models for targeting viral entry points and cell-to-cell transmission.

## 2. MATERIALS AND METHODS

### 2.1. Cell culture and reagents

The 293T (ECACC 12022001), CHO-K1 (ECACC 85051005), and 293 (ECACC 85120602) cell lines are collections of the Research Center for Genetic Engineering, National Research and Innovation Agency (BRIN, Indonesia). 293T cells were cultured in High-Glucose Dulbecco's-modified Eagle's medium (Gibco, Billings USA) supplemented with 10% (v/v) of heat-inactivated fetal bovine serum (Sigma-Aldrich, St. Louis USA) and antibiotics (100 μg/ml streptomycin and 100 U/ml penicillin). CHO-K1 cells were cultured in F-12 medium (Sigma-Aldrich, St. Louis USA) supplemented with 10% (v/v) of heat-inactivated fetal bovine serum and antibiotics (100 μg/ml streptomycin and 100 U/ml penicillin). 293 cells were cultured in MEM medium (Sigma-Aldrich, St. Louis USA) supplemented with 10% (v/v) of heat-inactivated fetal bovine serum, 1% NEAA (Gibco, Billings USA), and antibiotics (100 μg/ml streptomycin and 100 U/ml penicillin). Cells were grown inside a 37 °C tissue culture incubator maintained at 5% $CO_2$. The pcDNA3.1-SARS2-Spike (#145032), pcDNA3.1-hACE (#145033), and TMPRSS2 (#53887) plasmids were obtained from Addgene. Cells were transfected with expression vectors using polyethylenimine (PEI-Max, Polysiences).

### 2.2. Turmeric extract and curcumin preparation

Turmeric extract (TE) was purchased in the form of soft capsules from PT. Industri Jamu dan Farmasi Sido Muncul Tbk (Semarang, Indonesia). Each capsule was standardised to contain 350 mg concentrated liquid extract, equal to 100 mg of curcuminoids. The TE was dissolved

in DMSO at 50 mg/mL, and the stock was stored at -20 °C. The working solution was freshly prepared by serially diluting the stock in culture media. Curcumin powder was obtained from Sigma (C1386), Sigma-Aldrich, St. Louis, USA), reconstituted in DMSO at 50 mM, and maintained as the stock at -20 °C. For every treatment involving curcumin, the working solution was made at 10 and 100 µM concentrations by diluting the stock with complete culture media with a maximum final concentration of DMSO less than 1%.

### 2.3. MTT assay

MTT cell viability assay was carried out in CHO-K1 and 293T cells. One day prior to the MTT assay, $8x10^4$ 293T or $7x10^4$ CHO-K1 cells per ml were seeded onto a 96-well plate. The next day, the cells were treated and incubated with culture media containing various concentrations of TE or curcumin and incubated for about 24 h. A set of wells containing cells without any treatment and another set of wells were prepared with medium only for background subtraction (blank). Following incubation, the culture media containing TE or curcumin were discarded and cells were washed with 1X PBS. The cells were then incubated for 2 hours in the presence of 0.5 mg/mL MTT solution (3-(4,-5-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide). At the end of incubation, MTT reagents were discarded and 100 µl of DMSO were added to each well and agitated at 100 rpm for 10 minutes to ensure complete formazan crystals solubilisation. Finally, the absorbance of each well was recorded at 570 nm wavelength. Cell viability was calculated according to the formula: $(OD_{treated}-OD_{blank})/(OD_{untreated}-OD_{blank})$ X 100%

### 2.4. Western blot

Cell lysates were prepared using ice-cold RIPA buffer (Abcam, USA) with the addition of a protease inhibitor cocktail, then total protein concentration was determined by BCA assay

(Thermo Scientific). About 10-40 µg protein was subjected for SDS-PAGE and then the resolved protein was transferred onto an activated PVDF membrane. The membrane was then incubated in blocking buffer (5% skim milk in TBS/0.05% tween-20) followed by blotting with primary antibodies (anti-SARS-CoV-2 spike (Abcam, USA) 1:2,000 or anti-α-actin (Sigma, USA) 1:4,000) for 2 h at room temperature or overnight at 4°C. After washing with TBS/T, the membrane was incubated in secondary antibodies (ALP-conjugated antibodies (Abcam or Invitrogen), 1:4,000 or IR-Dye conjugated antibodies (LI-COR), 1:10,000) in blocking buffer for about 2 h at room temperature or overnight at 4°C. Western blot signal was detected by incubating the membrane with NBT-BCIP substrate (Thermo Scientific) or observed by a LI-COR Odyssey CLx instrument.

**2.5. Immunofluorescence staining**

The 293T cells were seeded at a density of $3x10^4$ cells/well on gelatin-coated cover glass placed inside a 24-well plate. After about 3 h incubation, the cells were transfected overnight with the pcDNA3.1-hACE2 expression vector. Transfected cells were washed with 1X PBS and fixed with 4% paraformaldehyde for 10 min. Upon fixation, cells were permeabilised with 0.2 % Triton-X, then incubated for 30 min at RT with blocking buffer (1% Bovine Serum Albumin in 1X PBS) and further incubated for at least 1 hour at RT with rabbit anti-hACE2 antibody (SAB 3500978, Sigma-Aldrich) or rabbit anti-TMPRSS2 antibody (BS-6285R, Bioss) diluted in blocking buffer (1:250). Following primary antibody staining, cells were washed three times with 1X PBS and incubated with goat anti-rabbit Alexa Fluor™ 594 or 488 secondary antibody (1:1,000) for 1 hour at RT. The secondary antibody was then washed with 1X PBS three times and the nuclei were stained with DAPI (4′,6-diamidino-2-phenylindole)-containing mountant (Abcam Ab104139). Samples were imaged with a motorized fluorescence microscope (Olympus IX83, Tokyo, Japan).

## 2.6. Preparation of SARS-CoV-2 pseudovirus

Pseudotyping was performed in 293T cells previously transfected with a plasmid encoding SARS-CoV-2 Spike (293T/Spike). Briefly, 293T/Spike cells were incubated with pseudotyped G*ΔG-GFP rVSV (Kerafast EH1024-PM) at MOI ~3 for 1 h. Then, the medium was replaced with a fresh medium containing anti-VSV-G antibody 1:2,000 to neutralize excess of G*ΔG-GFP rVSV and left overnight in the $CO_2$ incubator. A conditioned medium (CM) containing pseudotyped Spike*ΔG-GFP rVSV (SARS-CoV-2 pseudovirus) was collected and spun the next day to remove cell debris. The supernatant was aliquoted and stored at -80°C prior to being used for PSV entry assay.

## 2.7. Pseudovirus entry assay

PSV entry assay study was performed in the target cells of 293T transiently overexpressing hACE2 and TMPRSS2 (293T/hACE2/TMPRSS2) or 293 cells stably expressing hACE2 (293/hACE2) prepared using lentivirus system. Briefly, a total of $4x10^5$ 293T cells were grown in each well of an 8-well chamber slide (SPL Life Sciences, Pyeongtaek, South Korea) that was previously coated with 2% gelatin. Upon overnight incubation, the cells were transfected with vectors harboring hACE2 and TMPRSS2 by using PEI. On the next day, the medium was aspirated from the cell monolayers and cells were subjected to pretreatment with a complete medium containing TE at 10 and 100 μg/ml or curcumin at 10 and 100 μM for 30 min. Then, the medium was removed and the PSV was added at 1:2 ratios in 300 μl of medium containing TE or curcumin. After 16 h of viral infection, the cells were fixed and mounted. Finally, the images of GFP dots that represented the internalization of pseudovirus were acquired using a motorized fluorescence microscope (Olympus IX83). The GFP dots were counted from 8 different areas and analyzed by Fiji software (National Institute of Health).

293/hACE2 stable cells were generated by transduction of 293 cells using lentivirus produced in 293T cells utilizing plasmids: pCMV-dR8.2-dvpr (Addgene #8455); pCAGGS-G-Kan (Kerafast EH1017); and (RRL.sin.cPPT.SFFV/Ace2.IRES-neo.WPRE(MT129)(Addgene#145840). 293/hACE2 population were then maintained in culture medium containing G418 to obtain 293 cells that stably expressing recombinant hACE2. For the PSV entry assay using 293/hACE2, the cells were grown onto gelatin-coated 8-chamber slides and incubated overnight before PSV treatment. The GFP dots were counted from 5 different areas and analyzed by Fiji software.

**2.8. Syncytia inhibition assay**

293T cells were seeded in a 24-well plate at 1.4x105 cells/ml density in DMEM supplemented with 10% FBS. After overnight incubation, the cells were co-transfected with plasmids bearing SARS-CoV-2 Spike, hACE2, and TMPRSS2 and incubated for about 6 h. Subsequently, 500 µl/well of dilutions of curcumin at 1 and 10 µM or turmeric extract at 1 µg/ml and 10 µg/ml was added to cell monolayers in duplo and incubated for about 16 h. The following day, the syncytia formation was observed using an inverted microscope (Olympus CKX53) and 10 images were acquired to represent each well. The total number of syncytia was calculated with Fiji software and then sorted based on the number of nuclei using 4 categories: (i) <5 nuclei, (ii) 6-10 nuclei, (iii) 11-15 nuclei, and (iv) >15 nuclei.

**2.9. Statistical analysis**

Data were presented as mean ± SD (standard deviation) as indicated in each figure. Student's t-test calculated statistical differences and a *p*-value <0.05 was considered significant.

## 3. RESULTS

### 3.1. Cell viability assay of curcumin and TE on CHO-K1 and 293T cells

Curcumin and TE have been known to show cytotoxic effects in various cancer cells for instance breast cancer cells, colorectal cancer cells, and brain cancer cells (Tomeh et al., 2019). In addition, even though curcumin and TE show less cytotoxicity against normal cells at high concentrations, the toxic effects could still be observed (Li et al., 2022). Therefore, before we investigated their antiviral activities, we evaluated the cytotoxicity of curcumin and TE on CHO-K1 and 293T cells to determine the non-toxic concentration to be used in PSV entry and syncytia fusion assays.

We applied 1, 5, 10, 25, and 50 µM curcumin serial concentrations and 1, 5, 10, 25, and 50 µg/ml TE for MTT assay in CHO-K1 cells. The results showed that following a 24 h treatment, CHO-K1 cell viability declined according to increase in curcumin concentrations which were 95.82, 91.08, 72.61, 41.66, and 26.27 %, respectively. While after 24 h of TE treatment, the cell viabilities were 91.70, 96.09, 92.64, 39.06, and 35.19 % as the concentration increased. Moreover, we tested the effect of curcumin and TE in 293T cells using serial concentrations of 1, 10, and 100 µM curcumin or 1, 10, and 100 µg/ml TE for 24 h. The results indicated that the cell viabilities of 293 cells after curcumin treatment were 86.52, 74.18, and 11.09 %. In addition, the cell viabilities after TE treatment were 81.52, 76.54, and 5.33 %, respectively (**Fig 1**). Higher concentrations of tested samples significantly reduced cell viability, with the 293T cells showing more susceptibility to curcumin and TE treatment than CHO-K1 cells. Therefore, to minimize the cytotoxic effect of tested samples in further assays, we applied lower concentrations of curcumin (1 and 10 uM) and TE (1 and 10 ug/ml) and treated the cells for only about 16-18 h.

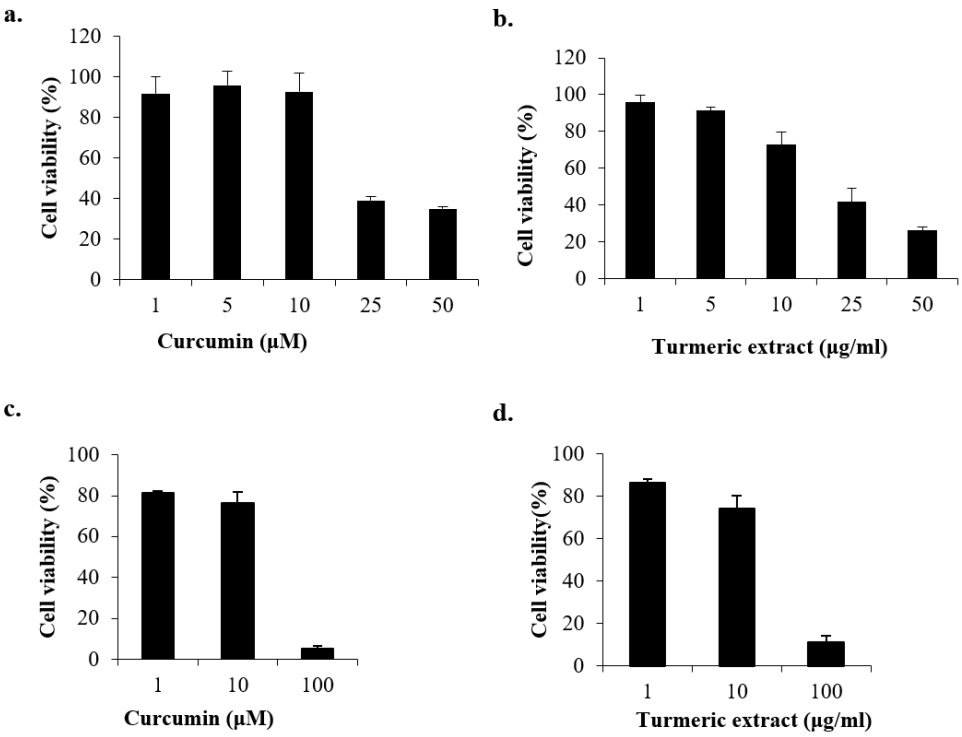



**Fig 1. CHO-K1 (a-b) cell viability profiles and 293T cells (c-d) after treatment with curcumin and turmeric extract.** Cells were plated onto a 96-well plate and treated with the tested samples in triplicate for 24 sh. The medium was then replaced with MTT-containing medium and incubated for 2 h. At the end of incubation, the formazan generated was dissolved with DMSO and the optical density was measured at 570 nm.

### 3.2. Pseudovirus entry assay

We prepared SARS-CoV-2 PSV with a VSV backbone and GFP reporter in Spike-transfected 293T cells (pseudotyped Spike*ΔG-GFP rVSV). Spike expression in 293T/Spike cells used for pseudotyping was confirmed by Western blot. Moreover, we also detected Spike in the CM obtained after pseudotyping, indicating the formation of SARS-CoV-2 pseudovirus and its release into the culture medium (**Fig 2**).

Furthermore, we used the CM to perform PSV entry assay in 293T overexpressing hACE2/TMPRSS2 and in 293/ACE2 stable cells. The 293T/hACE2/TMPRSS2 cells were pretreated with curcumin or TE for 30 min, then treated with PSV/curcumin or PSV/TE and followed by 16-18 h incubation in a $CO_2$ incubator. GFP expression indicating PSV internalization and viral genome release was observed by fluorescence microscopy with 60x magnification to expose the GFP dots. To confirm that GFP dots were formed within the cells, we also observed the cells in bright field mode and used DAPI to stain the nuclei. As the results showed, nontreated cells showed a higher number of GFP dots compared to the treated cells with fluorescence focus units (FFU) 296±2. The FFU of cells treated with curcumin 1 and 10 µM were 141±8.76 and 56±6.52. Whereas, FFU of cells treated with TE 1 and 10 µg/ml were 81+5.93 and 49±3.31. Cells treated with curcumin and TE significantly reduced the FFU number, especially at higher concentrations, indicating the potential inhibitory effect on SARS-CoV-2 viral cell entry (**Fig 3**).

Next, we clarified the inhibitory effect of curcumin as the active compound to inhibit SARS-CoV-2 PSV entry by performing an entry assay in 293 cells that stably expressed ACE2 (293/ACE2). Besides treating the 293/ACE2 cells with curcumin prior to PSV addition, the curcumin was also combined with PSV after incubation for 30 min. The number of GFP dots/cell for non-treated cells was 1.45. The number of GFP dots per cell in curcumin and PSV/curcumin-treated cells was 0.99, while the number of GFP dots per cell in curcumin-treated cells was 0.887. The results indicated that curcumin reduced PSV entry, especially for curcumin pretreatment before the addition of PSV ($P = 0.035$) (**Fig 4**).

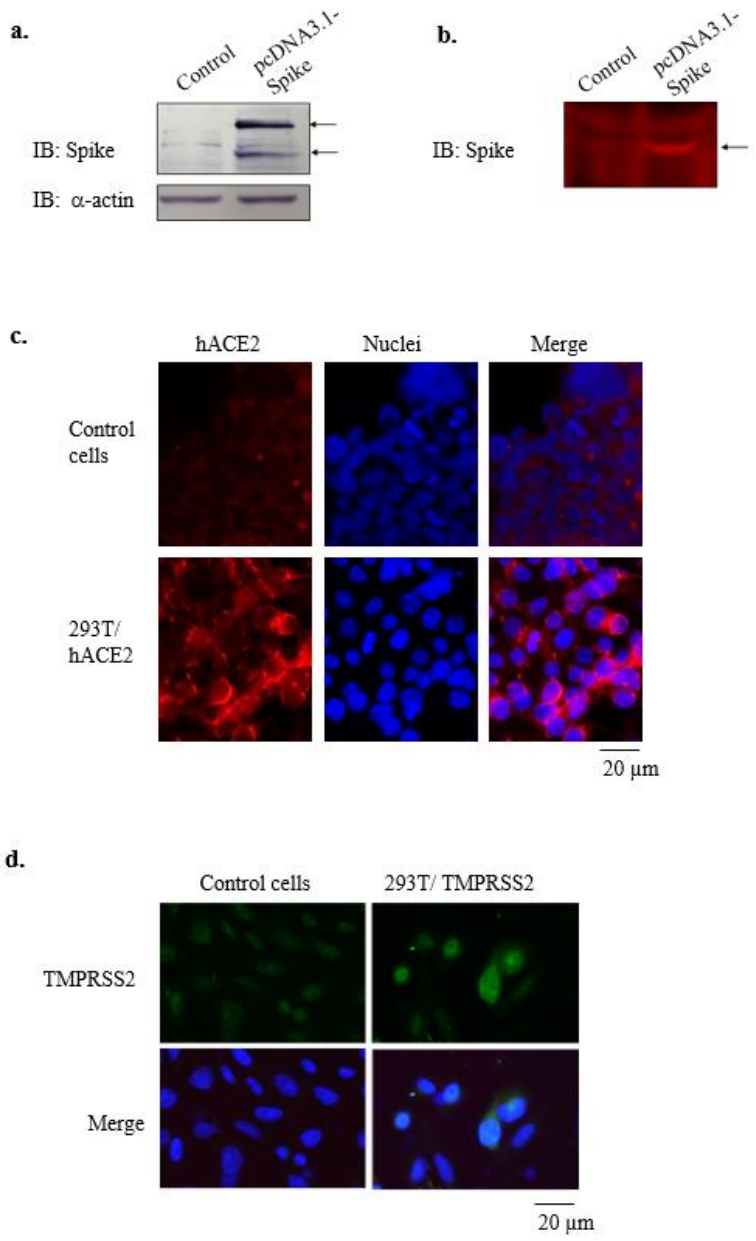



**Fig 2. Observation of Spike, hACE2, and TMPRSS2 expression.** a. Detection of Spike expression in 293T cells transfected with Spike plasmid. b. Detection of Spike in the conditioned medium, which represented SARS-CoV-2 PSV. c-d. Detection of hACE2 and TMPRSS2 expression by immunofluorescence staining.

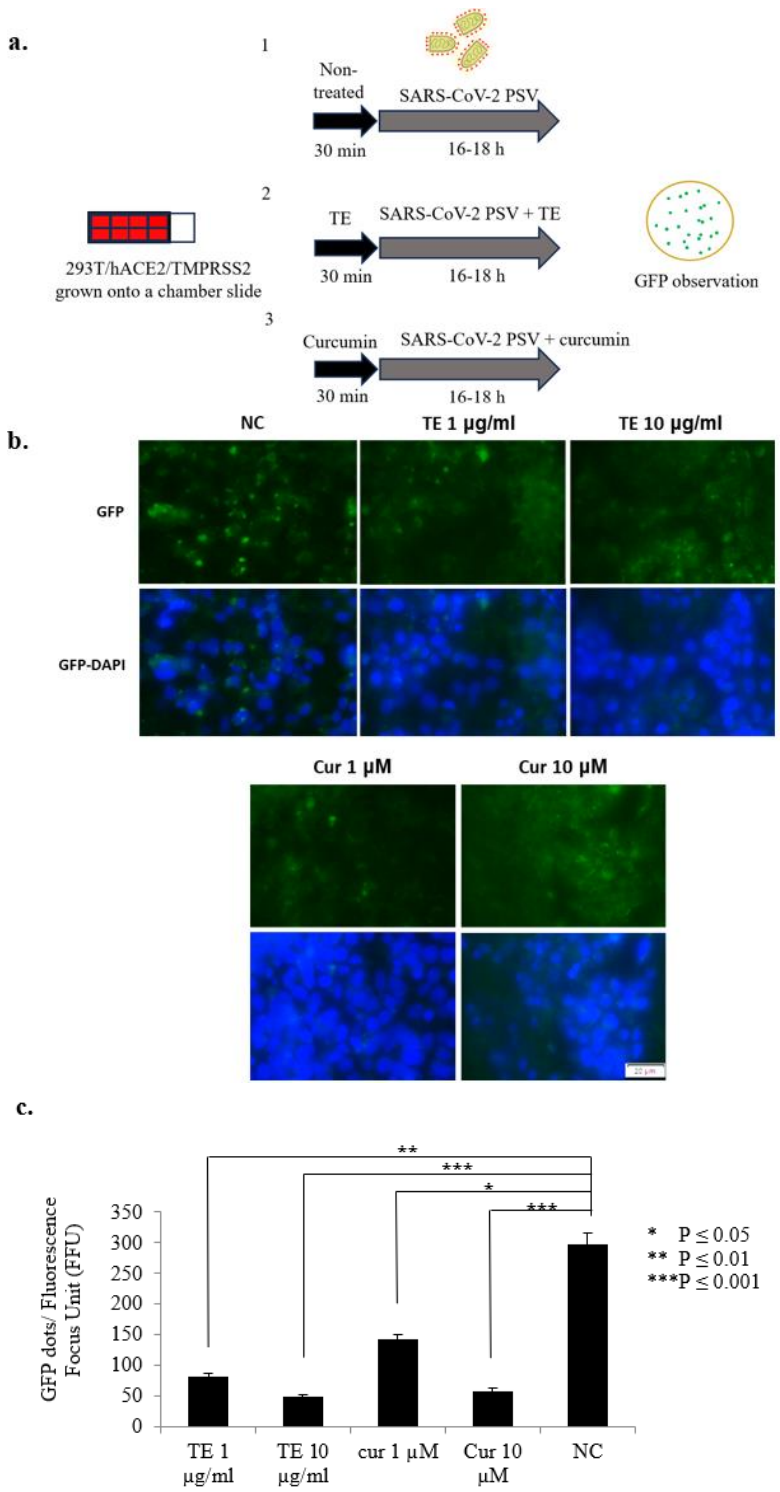



**Fig 3. Effect of turmeric extract and curcumin on PSV entry in 293T/hACE2/TMPRSS2 cells.** a. Schematic representation of PSV entry assay in 293T/hACE2/TMPRSS2 cells. b. Representative image showing GFP dots to represent PSV internalization. c. Graph representing quantification of GFP dots (n = 8 microscope fields). TE: turmeric extract; Cur: curcumin; NC: nontreated cells.

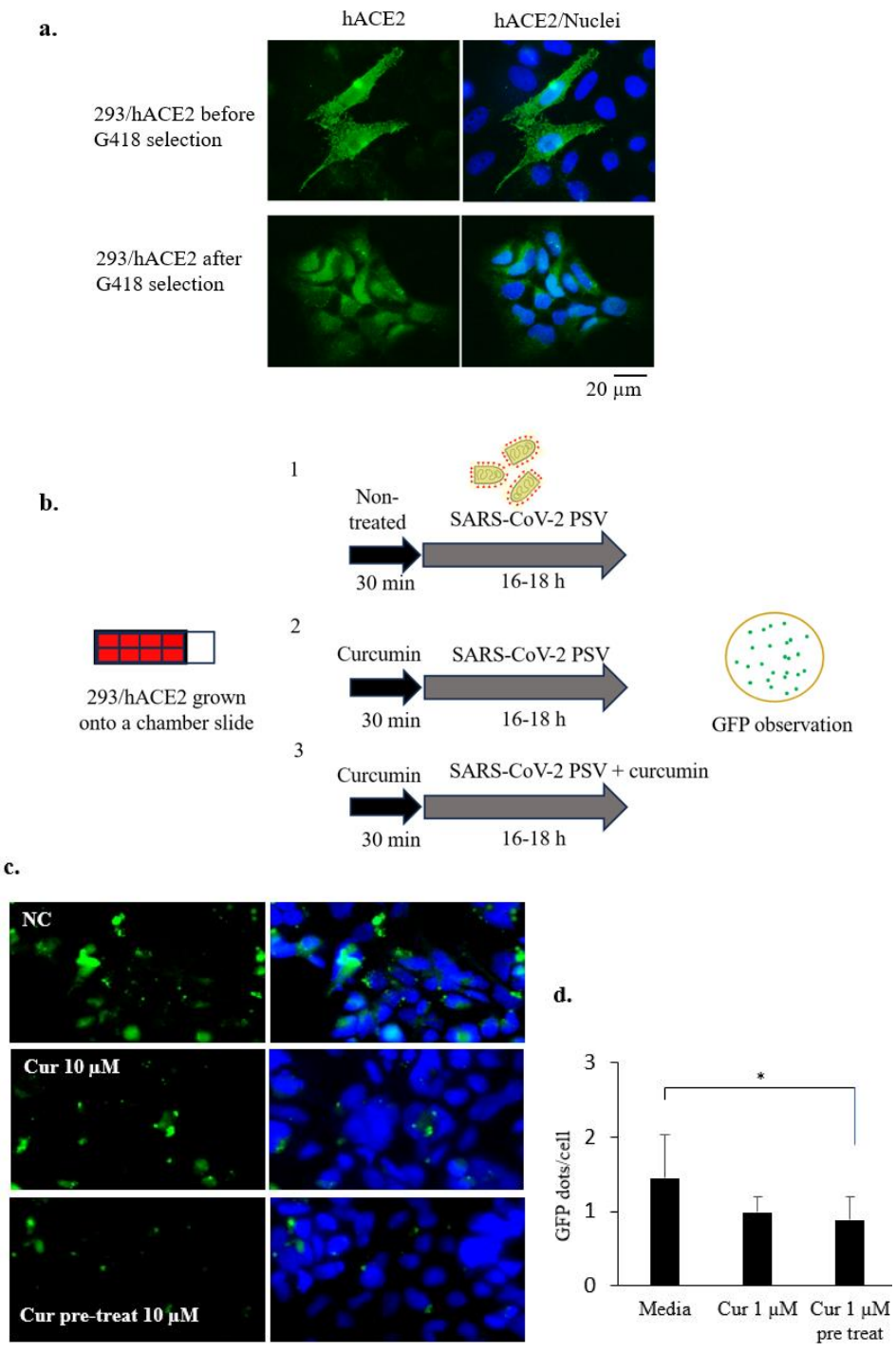



**Fig 4. Effect of turmeric extract and curcumin on PSV entry in 293/ACE2 cells.** a. Observation of hACE2 expression in 293/hACE2 cells after transduction, before and after selection with antibiotic G418. b. Schematic representation of PSV entry assay in 293/hACE2 stable cells. c. Representative image showing GFP dots to represent PSV internalization. d. Graph representing quantification of GFP dots (n = 5 microscope fields). $P<0.05$ = *. NC: nontreated cells.

### 3.3. Syncytia formation assay

This assay represents the cells infected with SARS-CoV-2 and expressing its membrane protein Spike which has the capability to bind hACE2 receptor of the adjacent cell. Instead of using the original virus, we transfected the cells directly with Spike plasmid. We used 293T cells transfected with plasmids encoding SARS-CoV-2 Spike, hACE2, and TMPRSS2 to perform the syncytia assay. The cells expressing Spike will locate the Spike in the transmembrane region, enabling the interaction between Spike with the hACE2 receptor and TMPRSS2 of the adjacent cells. As a result, the two interacting cells will form an intercellular bridge that eventually will fuse and form multinucleated cells (Buchrieser et al., 2020). The more cells interact and fuse, the more multinucleated cells or syncytia will be generated.

Here we show that 293T cells after cotransfection and treatment with DMSO 0.1% and 1% formed syncytia as much as 157.5±5.9 and 314±11.32 per field. In addition, the treatment of cells with curcumin and TE reduced the number of syncytia. The number of syncytia formed after cell treatment with curcumin 1 and 10 µM was 158.5 ($P<0.0001$ vs DMSO 0.1%) and 55 ($P<0.0001$ vs DMSO 1%), while the number of syncytia formed after cell treatment with TE 1 and 10 µg/ml was 140.5 ($P<0.0001$ vs DMSO 0.1%) and 66.5 ($P<0.0001$ vs DMSO 0.1%) (**Fig 5a-b**).

Furthermore, we analyzed the distribution of nuclei numbers present within the syncytia and categorised it into nuclei number <5, 5-10, 11-15, and <15. The syncytia with nuclei number <5 for DMSO 0.1, curcumin 1 µM and TE 1 µg/ml were 36.5, 20, 42.5, while nuclei number 5-10 were 98, 35.5, 45, nuclei no 11-15 were 59.5, 43.5, 33, and nuclei number <15 were 117.5, 42.5, 28. The syncytia with nuclei number <5 for DMSO 1, curcumin 10 µM and TE 10 µg/ml were 20, 18, 20, while nuclei number 5-10 were 60.5, 23, 17.5, nuclei no 11-15 were 38.5, 18.5, 9, and nuclei no <15 were 38.5 ,18.5, 4. These data indicated that treatment of

the cells with curcumin and TE reduced the nuclei number within syncytia, representing lower fusion events compared to the DMSO treatment (**Fig 5c**).

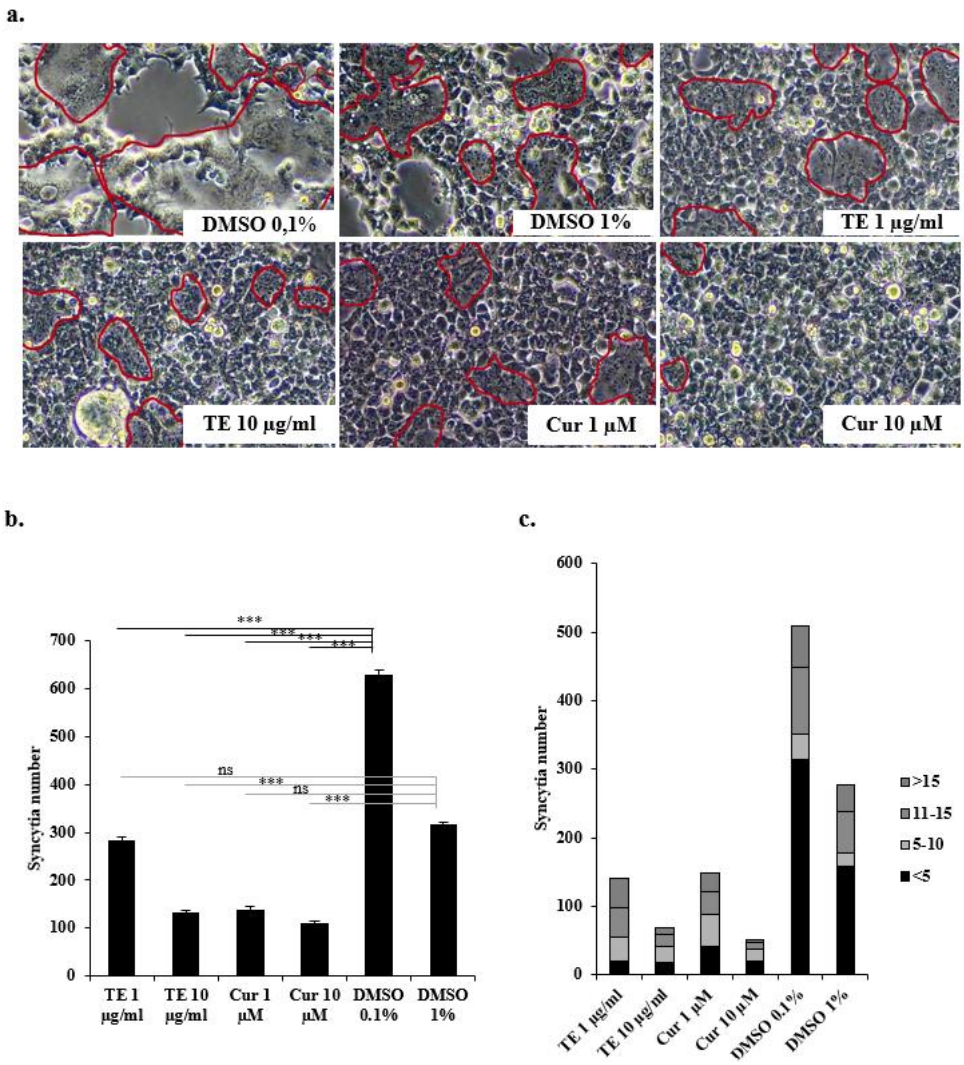



**Fig 5. Effect of curcumin and turmeric extract on syncytia formation.** a. Representative image showing the syncytia formed after curcumin, TE, and DMSO treatment. b. Graph representing quantification of total syncytia (n = 10 microscopic fields). c. Graph representing quantification of nuclei number per categories of syncytia (n = 10 microscopic fields). Red area: syncytia. P<0.05 = *, P<0.01 = **, P<0.001 = ***, ns = not significant.

## 4. DISCUSSION

Curcumin has been tested for its anti-SARS-CoV-2 activities by plaque assay in Vero cells. Using the original virus, curcumin inhibits SARS-CoV-2 infection (Marin-Palma et al., 2021). However, surface receptors in Vero cells are different from human cells, and the study may not correlate well with actual events (Pandamooz et al., 2022). Thus, antiviral study of curcumin has also been carried out using human Calu-3 cells, which endogenously express hACE2 and TMPRSS2. A study using these cells demonstrated that curcumin may inhibit SARS-CoV-2 viral replication as indicated by reduced N protein expression following viral infection (Bormann et al., 2021). Besides investigating curcumin's antiviral effect, Bormann et al. (2021) also tested the effect of turmeric juice (water extract). However, antiviral study of turmeric extract (TE) has not been widely reported compared to its active compound, curcumin.

In this manuscript, we reported the SARS-CoV-2 antiviral activities of curcumin and TE especially at the entry point, by utilising the pseudovirus model, and also the syncytia model to represent the cell-to-cell transmission. Here we showed that curcumin and TE reduced PSV entry in 293T/hACE2/TMPRSS2 cells in which 10 µM curcumin and 10 µg/ml TE significantly affected the number of GFP dots. The effect of TE was comparable to the curcumin effect on PSV cell entry in which 10 µg/ml TE was equal to 2.86 µg/ml curcuminoid with the majority of curcumin content and 10 µM curcumin was equal to 3.68 µg/ml of curcumin.

From the previous studies, Marin-Palma et al. (2021) reported that 10 µM curcumin is capable of inhibiting SARS-CoV-2 infection in Vero E6 cells. Wen et al. (2007) also reported that curcumin inhibits SARS-CoV-2 infection at concentrations of 3-10 µM. Furthermore, in A549 cells, curcumin shows SARS-CoV-2 antiviral concentration of 5 µM (Goc et al., 2021). We also showed that curcumin inhibited PSV entry in 293/hACE2 cells. These results

corroborate curcumin effects against SARS-CoV-2 infection with our data representing curcumin inhibition at PSV cell entry point. Previous reports by Mounce et al. (2017). Chen et al. (2010) and Chen et al. (2013) suggest that curcumin affects the early stages of viral replication cycles including viral-receptor attachment, internalization and fusion that have been studied against several types of viruses which involve influenza, dengue, zika, chikungunya, pseudorabies, and VSV.

Moreover, both curcumin and TE inhibit secondary infection via cell-to-cell transmission in a syncytia formation model mediated by SARS-CoV-2 Spike expression. Cells treated with curcumin and TE showed smaller syncytia with fewer nuclei than control cells. The more cells fused to generate syncytia, the larger syncytia with more nuclei will be formed and vice versa.

Based on in silico data, curcumin can interact with SARS-CoV-2 Spike RBD (Shanmugarajan et al., 2020), hACE2 (Subbaiyan et al., 2020), and TMPRSS2 as well (Motohashi et al., 2020). These data align with our results that curcumin inhibited PSV entry and syncytia formation. Curcumin is capable of interfering with the Spike-receptor binding either during direct viral infection or intercellular transmission, which hinders viral infection and cell fusion (Marin-Palma et al., 2021). In addition, TE as the crude extract which contains curcumin also has the potential to inhibit SARS-CoV-2 infection and potentially to be developed as an independent herbal-derived product for prevention of viral infection with curcuminoids used as identity compounds for TE standardization.

## 5. CONCLUSION

Our in vitro study using PSV and syncytia models revealed that both curcumin and TE are potential inhibitors of SARS-CoV-2 infection, especially at the entry points either by direct infection or cell-to-cell transmission mediated by Spike induced cell fusion.

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## ABBREVIATIONS

**ACE2** : angiotensin converting enzyme-2

**COVID-19** : coronavirus disease-2019

**GFP** : green fluorescence protein

**PSV** : pseudovirus

**RBD** : receptor binding domain

**SARS-CoV-2** : severe acute respiratory syndrome coronavirus-2

**TE** : turmeric extract

**VSV** : vesicular stomatitis virus