In vitro Screening Antiviral Activity of Thai Medicinal Plants Against Porcine Reproductive and Respiratory Syndrome Virus

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Abstract

Background

Porcine reproductive and respiratory syndrome (PRRS) caused by the PRRS virus (PRRSV) results in economic losses in the swine industry globally. Several studies have investigated the use of plant extracts for the prevention and control of PRRS outbreaks. Thai medicinal plants may be useful for treating PRRSV infection in pigs. Therefore, we investigated the in vitro anti-PRRSV and antioxidant properties of seven Thai medicinal plants: *Caesalpinia sappan* Linn., *Garcinia mangostana* Linn., *Houttuynia cordata*, *Perilla frutescens*, *Clinacanthus nutans*, *Phyllanthus emblica*, and *Tiliacora triandra*.

Results

In the antiviral screening test, we observed that the *T. triandra* extract strongly inhibited the infectivity of PRRSV into MARC-145 cells [virus titer 3.5 median tissue culture infectious doses (TCID 50 /ml (log 10 ))] at 24 h post-infection, whereas the *C. sappan* Linn. extract strongly inhibited PRRSV replication [virus titer 2.5 TCID 50 /ml (log 10 )] at 72 h postinfection. *C. sappan* Linn. extract had the highest total phenol content [220.52 mM gallic acid equivalents (GAE)/g] and the lowest half-maximal inhibitory concentration [IC 50 ; 1.17 mg/ml in 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2.58 mg/ml in 2,2-azino-bis (3-ethylbenzothiazole-6-sulfonic acid) diammonium salt (ABTS)].

Conclusion

Our study showed that *T. triandra* could inhibit the infectivity of PRRSV, whereas *C. sappan* Linn was the most effective for inhibiting PRRSV replication in MARC-145 cells. This study elucidates the antiviral activities of Thai medicinal plant extracts in vivo. The result promising that the extracts of Thai medicinal plants, especially that of *T. triandra* and *C. sappan* Linn, can be developed into pharmaceutical drugs for the prevention of PRRS in pigs.

Background

The porcine reproductive and respiratory syndrome virus (PRRSV) is endemic in most pig-producing countries, and it results in enormous economic losses to the swine industry globally [1]. This enveloped, positive-sense, single-stranded RNA virus belongs to the family Arteriviridae (order
Nidovirales), which also includes the equine arteritis virus, mouse lactate dehydrogenase-elevating virus, and simian hemorrhagic fever virus [2]. In general, infection with PRRSV causes a disease that is characterized by reproductive failure in sows and respiratory infections in growing pigs [3] and this disease also predisposes the pigs to infection by bacterial and other viral pathogens [4, 5]. This disease is known as the porcine reproductive and respiratory syndrome (PRRS) and has become endemic in many countries throughout the world following an epidemic phase [6, 7]. The incidence of PRRS was first reported in Thailand in 1989, since then several outbreaks have been reported [8], and it has become a major infectious disease that causes high mortality in swine and production losses in the swine industry in this country.

Although preventative measures such as the acclimatization of gilts, vigilant biosecurity, and vaccination have been shown to be useful in controlling the outbreaks of PRRS, and supportive treatments are available for alleviating its severity; no specific treatment for PRRS is currently available [9, 10]. Antiviral therapeutics are a critical tool for combating viral infections, particularly in cases wherein no vaccines are available against the circulating virus. Thus, pharmacological intervention may represent as an alternative approach in controlling PRRSV. A number of natural compounds and compositions have been shown to possess antiviral activities against PRRSV. Gao et al. [11] showed that the Cryptoporus volvatus extract exhibited antiviral activity against PRRSV infection and replication. Pringproa et al. [12] reported that the crude extract of Cynodon dactylon significantly inhibited the replication of PRRSV as early as 24 h postinfection (hpi). Therefore, the antiviral activities of other Thai medicinal plants against PRRSV should also be investigated. Thai medicinal plants such as Caesalpinia sappan Linn., Garcinia mangostana Linn., Houttuynia cordata, Perilla frutescens, Clinacanthus nutans, Phyllanthus emblica, and Tiliacora triandra are known to have antioxidant activities and antiviral activities. These plants have already been promoted for use in primary health care and have been classified according to their pharmacological actions [13–18]. Therefore, the aim of this study was to determine the antiviral activities of Thai medicinal plant extracts against PRRSV infection in vitro and to measure their phytochemical contents to develop an alternative anti-PRRSV therapy for use in veterinary medicine.
Results
Cytotoxic activities of the plant extracts
Prior to determining the antiviral activity, we evaluated the cytotoxicity of the seven Thai medicinal plant extracts on the viability of MARC-145 cells, and the viability was shown as 50% cytotoxic concentrations (CC₅₀). The results showed that the cytotoxic concentration CC₅₀ of the seven Thai medicinal plant extracts ranged from 78 µg/ml to 2,500 µg/ml, and the effect of the concentration of the Thai medicinal plant extracts on the tested cells increased in a dose-dependent manner (Fig. 1). P. emblica had the lowest CC₅₀ concentration of 78 µg/ml. The CC₅₀ of G. mangostana Linn. was the second lowest concentration (312.5 µg/ml) and that of C. sappan Linn. was 625 µg/ml. In this study, we found that T. triandra and H. cordata had CC₅₀ of 1,250 µg/ml, whereas C. nutans and P. frutescens showed the highest CC₅₀ concentration (2,500 µg/ml).

Inhibition of the PRRSSV infection by the extracts of the Thai medicinal plants
We treated the virus with different concentrations of Thai medicinal plant extracts by using a range of concentrations that were determined based on their CC₅₀ values so that these plant extracts did not affect the proliferative activity of MARC-145 cells. The screening results of the inhibition of virus infection activity showed the potential of the Thai medicinal plant extracts to inhibit the infectivity of PRRSV (Fig. 2). T. triandra significantly inhibited the infectivity of PRRSV into MARC-145 cells at 24 hpi when supplied at a concentration of 1,250 µg/ml (P < 0.05), and the observed virus titer at this concentration was 3.5 TCID₅₀/ml (log₁₀). Interestingly, P. emblica at a low concentration of 78 µg/ml could inhibit the infectivity of PRRSV [virus titer = 4.5 TCID₅₀/ml(log₁₀)]. As shown in Fig. 3, the immunoperoxidase monolayer assay (IPMA) indicated that T. triandra and P. emblica blocked the infectivity of PRRSV in MARC-145 cells, as shown by the slight brown staining in the cells.

Thai medicinal plant extracts inhibit PRRSV replication
Different Thai medicinal plant extracts were tested in an in vitro inhibition screening assay to determine their inhibition of PRRSV replication at three time intervals (24, 48, and 72 hpi). At various time points after the infection, PRRSVs in the supernatants were quantified for determining the virus titer by IPMA. The screening results were the same as that of the inhibition test of virus infectivity,
i.e., the PRRSV replication was inhibited in a dose-dependent manner (Fig. 4). Interestingly, as shown in Fig. 5, we found that C. sappan Linn. had significant potential to inhibit PRRSV replication in vitro. As shown in Fig. 5, few cells that were stained brown showed the efficiency of C. sappan Linn. at a concentration of 625 µg/ml, and the inhibition of the replication activity of PRRSV by C. sappan Linn. was significantly stronger than that by the other plant extracts at 72 hpi [2.7 TCID<sub>50</sub>/ml (log<sub>10</sub>)].

**Phytochemical contents of Thai medicinal plant extracts**

The total phenol contents of the seven Thai medicinal plant extracts were determined using the Folin-Ciocalteu assay by constructing a standard curve of gallic acid. The total phenol content was the highest in C. sappan Linn. [mean ± standard error: 220.52 ± 4.47 mM gallic acid equivalents (GAE)/g sample], followed by G. mangostana Linn. (91.16 ± 4.62 mM GAE/g sample), and the lowest phenol content was observed in H. cordata (8.51 ± 0.04 mM GAE/g sample) (Table 1).

| Plant Name             | Total Phenol (mM GAE/g) | DPPH (IC<sub>50</sub>, mg/ml) | ABTS (IC<sub>50</sub>, mg/ml) | FRAP (mM Fe<sup>2+</sup>/g) |
|------------------------|-------------------------|--------------------------------|-------------------------------|----------------------------|
| Caesalpinia sappan     | 220.52 ± 4.47           | 1.17 ± 0.06                    | 2.57 ± 0.16                   | 334.78 ± 13.15             |
| Garcinia mangostana    | 91.16 ± 4.62            | 4.82 ± 0.58                    | 4.98 ± 0.10                   | 46.12 ± 1.27               |
| Houttuynia cordata     | 14.25 ± 0.20            | 97.79 ± 4.14                   | 72.02 ± 4.01                  | 8.55 ± 0.18                |
| Perilla frutescens     | 29.86 ± 0.41            | 11.68 ± 0.51                   | 21.37 ± 1.28                  | 43.32 ± 0.92               |
| Clinacanthus nutans    | 25.52 ± 0.22            | 50.34 ± 5.60                   | 37.82 ± 1.25                  | 18.39 ± 0.54               |
| Phyllanthus emblica    | 44.35 ± 0.24            | 3.49 ± 0.17                    | 4.95 ± 0.11                   | 94.17 ± 0.62               |
| Tiliacora triandra     | 30.45 ± 1.51            | 17.77 ± 0.22                   | 21.16 ± 1.06                  | 30.58 ± 1.13               |

**Antioxidant activities**

The C. sappan Linn. extract had the highest antioxidant activity, with IC<sub>50</sub> values of 1.17 ± 0.06 mg/ml in 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2.57 ± 0.16 mg/ml IC<sub>50</sub> in 2,2-azino-bis (3-ethylbenzothiazole-6-sulfonic acid) diammonium salt (ABTS) and a reducing power of 334.78 ± 13.15 mM Fe<sup>2+</sup>/g in the ferric reducing antioxidant power (FRAP) assay (Table 1). The P. emblica extract had the second strongest antioxidant activity against free radicals, with IC<sub>50</sub> values of 3.49 ± 0.17 mg/ml in DPPH and 4.95 ± 0.11 mg/ml in ABTS and a reducing power of 94.17 ± 0.62 mM Fe<sup>2+</sup>/g sample in the FRAP assay.

**Discussion**
PRRS virus outbreaks causes significant economic loss in the pig production industry worldwide. The current commercial PRRSV vaccines are inadequate to protect pigs from virus infections [19].

Medicinal plants have progressively been explored as suitable alternative sources of antiviral agents [20]. Thai medicinal plants have widely been used as a source of herbal medicines because of their high bioactive compound contents that are effective against various diseases. In this study, seven Thai medicinal plant extracts were screened for their antiviral activity against PRRSV.

Before determining the antiviral properties of a compound, it is essential that a cytotoxicity assay is performed to determine the concentrations that can be used to avoid cell damage and ensure selectivity for the virus in vitro. In this study, we report the cytotoxicity as CC$_{50}$, which indicates the concentration of a substance that can inhibit viral activity by 50%. We found that *P. emblica* showed the highest cell toxicity (78.1 µg/ml). The high potential plant extracts in this study were found to be *C. sappan* Linn. and *T. triandra*, with CC$_{50}$ concentrations of 625 and 1,250 µg/ml, respectively.

Antiviral compounds should be highly effective while showing minimal toxicity to normal cells and tissues [21].

In this study, we investigated the antiviral activity of seven Thai medicinal plant extracts against PRRSV by checking the inhibition of virus infection and virus replication in MARC-145 cells. The range of concentrations of the plant extracts were determined based on their CC$_{50}$ values. The *P. emblica* extract inhibited PRRSV infection in MARC-145 cells, as well as in vitro. *P. emblica* at a concentration of 78 µg/ml inhibited the infectivity of PRRSV at a virus titer of 4.5 TCID$_{50}$/ml (log$_{10}$). In this study, *P. emblica* showed the highest cytotoxicity toward MARC-145 cells with CC$_{50}$ of <100 µg/ml. Therefore, the antiviral activity of other plant extracts were investigated in this study. We found that *T. triandra* at a concentration of 1,250 µg/ml significantly inhibited PRRSV infectivity at a virus titer 3.5 TCID$_{50}$ (log10). While *T. triandra* has been used as an anti-inflammatory agent [22], anticancer agent [23], and antimicrobial agent against *Mycobacterium tuberculosis* [24], its antiviral activity, particularly against PRRSV, has not been investigated previously. Therefore, this is the first report to indicate that *T. triandra* could significantly prevent the entry of PRRSV into MARC-145 cells. However, *T. triandra*
was not found to be effective in inhibiting PRRSV replication. All the plant extracts used in this study could inhibit PRRSV replication when applied at high concentrations, as shown by the linear regression model from 24 to 72 hpi after incubation with PRRSV. *C. sappan* Linn. at 625 µg/ml could inhibit PRRSV replication as 72 hpi [virus titer 2.7 TCID<sub>50</sub> (log10)]. Although the antiviral activity of *C. sappan* Linn. against the influenza virus [13] and the antimicrobial properties of *C. sappan* Linn. [25] have previously been investigated, this is the first study to reveal the inhibitory activity of *C. sappan* Linn. on PRRSV replication in MARC-145 cells.

In terms of the phytochemical contents, *C. sappan* Linn. extract had the highest total phenol content (220.52 ± 4.47 mM GAE/g sample). The total phenol content of a plant is considered to be an indicator of its antioxidant capacity because the redox properties of the phenolic compounds allow them to act as reducing agents, hydrogen donors, and radical scavengers [20]. Previously, Lee et al. [21] reported that ethanolic *C. sappan* Linn. extract had a total phenol content of 723.67 µg GAE/mg. The values of the total phenol content in this study varied slightly compared with those reported previously in the literature. This may be because of the different durations, geographical variations, or methods of extraction, which may have altered the amounts of the phenol content. Ethanolic plant extracts can be used for the investigation of the antiviral activity in a cell line. Abu-Jafar and Huleihel [26] reported that ethanolic extracts of *Eucalyptus camaldulensis* leaves had strong antiviral activity against different members of the herpes virus family (HSV-1, HSV-2 and VZV). Ramalingam et al. [27] reported that the ethanolic extracts of *Andrographis paniculata* have the highest antiviral inhibitory effects against dengue virus in Vero cells.

The screening of plants as possible sources of antiviral agents has led to the discovery of potent inhibitors of *in vitro* viral replication, thereby increasing the probability of identifying new bioactive plant compounds [28]. These findings suggest an appropriate species and the concentration of the plant extract that could effectively inhibit PRRSV replication with both *T. triandra* and *C. sappan* Linn. being highly effective in inhibiting PRRSV infection *in vitro* by interfering with viral attachment and inhibiting viral replication and/or virus release, respectively. The modes of action of *T. triandra* and *C. sappan* Linn. against PRRSV require further investigation but are likely to be related to the natural
compounds they contain. Therefore, it was speculated that both *T. triandra* and *C. sappan* Linn. might have the potential to become candidates for preventing PRRSV infection in pigs. However, the plant extracts used for testing the antiviral activity was a crude extract. In future, we would like to purify the most effective Thai medicinal plant extract (*T. triandra* and *C. sappan* Linn.) for screening the active compound that is highly effective against PRRSV.

**Conclusion**

The findings of this study show that Thai medicinal plant extracts exhibit antiviral activity against PRRSV, and *T. triandra* effectively inhibiting PRRSV infection and *C. sappan* Linn. having the strongest antiviral activity against PRRSV replication. These activities can be presumably attributed to the total phenol contents and the antioxidant activities of these plant extracts. Although several previous studies have shown the antiviral activity of plant extracts against PRRSV, but there are no reports on the antiviral activities of *T. triandra* and *C. sappan* Linn. extracts against PRRSV. To the best of our knowledge, this study is the first to report the inhibitory activity of *T. triandra* and *C. sappan* Linn. against PRRSV activity *in vitro*. Further studies are required to elucidate the mechanisms of action of these plant extracts on PRRSV.

**Methods**

**Chemicals**

All of the chemicals that were used in this study were of analytical grade or higher. Ethanol and methanol were obtained from Merck (Darmstadt, Germany). 2,2-Azino-bis(3-ethylbenzothiazole-6-sulfonic acid) diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picryl hydrazyl (DPPH), Folin & Ciocalteu phenol reagent, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), sodium carbonate, and 2,4,6-tri-pyridyl-s-triazine (TPTZ) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Ferric chloride hexahydrate and potassium persulfate were procured from LOBA CHEMIE PVT (Mumbai, India). Gallic acid was procured from Fluka Chemical Co. (Buchs, Switzerland). Dulbecco’s modified Eagle’s medium (DMEM) was procured from Gibco (Massachusetts, USA).

**Plant extracts, cells, and viruses**

Ethanolic extracts from the Thai medicinal plants *C. sappan* Linn., *G. mangostana* Linn., *H. cordata*, *P.*
frutescens, C. nutans, P. emblica, and T. triandra were purchased from Specialty Natural Product Co. Ltd. (Thailand).

MARC-145 tissue culture cells were grown in Dulbecco’s modified Engle medium (DMEM) containing 10% fetal bovine serum (Gibco) and 1% Penicillin/Streptomycin and incubated at 37°C in a 5% CO₂ atmosphere. To produce inoculated cells, PRRSV (VR2332 North American genotype) was propagated in the MARC-145 cells and the virus titer was quantified using the virus titer by immunoperoxidase monolayer assay (IPMA).

Cytotoxicity assay
The cytotoxicity of the seven Thai medicinal plant extracts was determined using the MTT assay. Briefly, MARC-145 cells were plated at a density of 5,000 cells/well in 96-well plates and incubated in a 5% CO₂ atmosphere at 37°C for 24 h. When the cells were at least 90% confluent, the medium was removed and replaced with medium containing two-fold serial dilutions of the plant extracts. In addition, medium without plant extract was used as a positive control. Incubation was then continued in a 5% CO₂ atmosphere at 37°C for 72 h. After this time, the medium was removed, 20 μl of freshly prepared MTT solution (5 mg/ml) was added to each well, and the plates were incubated at 37°C for 4 h. The medium was then replaced with 150 μl DMSO to dissolve the crystals and the plates were incubated at 37°C for 5 min to dissolve any air bubbles before measuring the MTT signal at an absorbance of 550 nm. The results were reported as 50% cytotoxic concentrations (CC₅₀).

Inhibition of virus infection assay
The inhibition of virus infection test was evaluated as previously described [12]. Briefly, the plant extracts at the concentration that was determined in the cytotoxicity test outlined above, as well as two lower level concentrations in two-fold dilution were mixed with PRRSV at 10⁸ TCID₅₀/ml in the ratio of 1:1 and incubated for 1 h at 37°C. DMSO (1%) containing medium mixed with PRRSV served as the control. Thereafter, the mixture of virus and plant extracts, as well as controls were inoculated into MARC-145 cells as 5,000 cells/well in a 96-well plate and incubated at 37°C for 1 h. Subsequently, the medium was removed and replaced with a fresh medium containing 10% FBS. The plates with MARC-145 cells were cultured under standard conditions for 24 h post-infection (hpi) and then the
supernatants were collected to quantify the virus titer.

Inhibition of virus replication assay
The inhibition of virus replication test was performed as previously described [12]. Briefly, MARC-145 cells were plated at a density of 5,000 cells/well in 96-well plates and infected with PRRSV at a multiplicity of infection (MOI) of 1 for 1 h at 37°C. The virus was then removed from each of the wells and replaced with the diluted plant extracts at the concentration that was determined in the cytotoxicity test, as well as two lower levels concentrations in two-fold dilution. Also, 1% DMSO was mixed to medium as a control. The plates were cultured under standard conditions, and the supernatants were collected at 24, 48, and 72 hpi and quantified the virus titer.

Virus Titer
The virus titer was further assessed by Immunoperoxidase Monolayer Assay (IPMA) was adapted from [29]. Briefly, cells were fixed with 100 μl cold 4% formalin for 15 min at room temperature (RT), washed once with 100 μl phosphate-buffered saline (PBS) and twice with 100 μl of 0.5% PBS Tween-20 (PBST), and then blocked with 100 μl of 1% BSA in 0.5% PBST for 30 min at RT. After blocking, the cells were stained with 70 μl anti-PRRSV NC protein monoclonal antibody (Median Diagnostics, Gangwon-do, Korea) diluted 1:400 for 60 min at RT and then washed and incubated with Peroxidase-conjugated AffiniPure Goat Anti-Mouse IgG (H+L) (Jackson ImmunoResearch, Pennsylvania, USA) diluted 1:1,200 for 60 min at RT. After washing three times in PBS, the cells were counter-stained with 1,5-diaminopentane (DAP) substrate and examined under a microscope. The virus titer was shown at the viral median tissue culture infectious dose (TCID50) was then determined using the Reed–Muench method.

Phytochemical analysis
The total phenolic contents of the plant extracts were determined using the Folin–Ciocalteu method [30], and their free radical scavenging activities were determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay and 2,2-azino-bis (3-ethylbenzothiazole-line-6-sulfonic acid) diammonium salt (ABTS) scavenging assay, as previously reported [31,32]. The antioxidant activities were determined using the ferric reducing antioxidant power (FRAP) assay, according to the Benzie and Strain method [33].

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Statistical analyses
Differences in the antiviral activities among different concentrations of each plant extract were tested using one-way analysis of variance (ANOVA) with Tukey’s post hoc test for a comparison of the means. The CC$_{50}$ was calculated through regression analysis of the dose-response curves for the MTT assay. All statistical analyzes were performed using the SPSS 23.0 software (SPSS Inc., Chicago, IL, USA) with a significance level of $P \leq 0.05$.

Declarations

Confict of interest statement
We declare that we have no conflict of interest.

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Figures

![Graph showing cytotoxic activity of Thai medicinal plant extracts](image)

**Figure 1**

The cytotoxic activity of the seven Thai medicinal plant extracts in MARC-145 cells determined by the MTT assay. MARC-145 cells were incubated with various concentrations of the seven Thai medicinal plant extracts or the control without plant extract for 72 h prior to the MTT assay. Values are shown as means ± standard errors. CN, C. nutans; PF, P. frutescens; HC, H. cordata; TT, T. triandra; CS, C. sappan Linn.; GM, G. mangostana Linn. and PE, P. emblica; CC50, 50% cytotoxic concentration.
Figure 2

The virus titer of the inhibition of the viral infection activity of seven Thai medicinal plant extracts against PRRSV at 24-h postinfection (hpi). MARC-145 cells were infected with PRRSV at a multiplicity of infection of 1. Plant extracts were incubated with PRRSV for 1 h at 37°C before inoculation onto a monolayer of MARC-145 cells. At 24 hpi, the supernatants were collected to quantify the virus titer by the IPMA assay. The control was infected and virus titer was quantified as TCID50 (log10) = 8.0. The statistical analyses of the antiviral activity were performed using ANOVA. A, C. nutans; B, P. frutescens; C, H. cordata; D, T.
trandra; E. C. sappan Linn.; F. G. mangostana Linn. and G. P. emblica. a, b, and c; P < 0.05 compared with different concentrations of the plant extracts. All samples were analyzed in triplicate.

Figure 3
The immunoperoxidase monolayer assay (IPMA) showing the inhibition of PRRSV infection in MARC-145 cells by T. triandra (TT) at 312.5, 625, and 1,250 µg/ml (A-D) and P. emblica (PE) at 19.5, 39, and 78 µg/ml (E-H). T. triandra and P. emblica were incubated with PRRSV for 1 h at 37°C before inoculation onto a monolayer of MARC-145 cells. After 24 hpi, the supernatant was collected for quantification of the virus titer. The MARC-145 cells that were infected with PRRSV show brown staining. In the control, large numbers of PRRSV-infected cells were observed in the supernatants of the MARC-145 cells (A and E), while only few PRRSV-infected cells were observed in T. triandra and P. emblica.
Figure 4

The virus titer in the inhibition of viral replication activity of Thai medicinal plant extracts against PRRSV at 24, 48, and 72-h post-infection (hpi). The MARC-145 cells were infected with PRRSV for 1 h and then placed in a medium containing Thai medicinal plant extracts at various concentrations according to CC50 and incubated at 24, 48, and 72 h, respectively. After various times of infection, the supernatant was collected for quantifying the virus titer by the IPMA assay. The control was infected and the virus titer was quantified as TCID50 (log10) = 8.0. The statistical analyses of the antiviral activity were performed using ANOVA. A, C. nutans; B, P. frutescens; C, H. cordata; D, T. triandra; E, G. mangostana Linn. and F, P. emblica. a, b, and c; P < 0.05 compared with the different concentrations of the plant extracts. All samples were analyzed in triplicate.
The IPMA assay of Caesalpinia sappan Linn. inhibiting the PRRSV replication of MARC-145 cells at 24 (A-D), 48 (E-F), and 72-h post-infection (hpi) (I-L). The MARC-145 cells were infected with PRRSV for 1 h and then placed in a medium containing C. sappan Linn. at various concentrations according to CC50 and incubated at 24, 48, and 72 h, respectively. After various times of infection, the supernatant was collected for quantifying the virus titer by IPMA that was used to detect the infectivity of PRRSV in MARC-145 cells. The MARC-145
cells that were infected with PRRSV showed brown staining. In the control, large number of PRRSV-infected cells were observed in the supernatants, while only a few PRRSV-infected cells were observed in C. sappan Linn.-treated supernatants. The bar chart represents the virus titer of C. sappan Linn. inhibiting PRRSV replication at 24, 48, and 72 hpi. The control was infected and virus titer was quantified as TCID50 (log10) = 8.0. At C. sappan Linn. concentration of 625 µg/ml, the virus titer at 72 hpi was lower than that at other concentrations. Statistical analyses of the antiviral activity were performed using ANOVA. a, b, and c: P < 0.05 compared with different concentrations of C. sappan Linn. All samples were analyzed in triplicate.