Original Article

Antiviral activity of *Thymus vulgaris* and *Nepeta cataria* hydrosols against porcine reproductive and respiratory syndrome virus

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Abstract

Porcine reproductive and respiratory syndrome (PRRS) is characterized by reproductive failure of sows and respiratory problems of nursery and growing pigs. Current management strategies mainly focus on preventing PRRS virus (PRRSV) infection through vaccination. However, the currently available modified live vaccines are not sufficient to eradicate the virus and do not provide complete immunity from infection. In search of agents that may be proven clinically effective against North American PRRSV (NA-PRRSV or US-PRRSV) infection, 42 natural herb hydrosols were purified and subjected to screening of their antiviral activity against two US-PRRSV strains in MARC-145 cells. Based on results obtained from cell viability test, the highest concentration showing less than 10% cytotoxicity in MARC-145 cells was chosen for each of the plant hydrosols. Antiviral activity assay indicated that both *Thymus vulgaris* and *Nepeta cataria* hydrosols reduced the PRRSV load *in vitro* significantly ($p < 0.05$). Moreover, the anti-PRRSV mechanisms of both *T. vulgaris* and *N. cataria* hydrosols were in both pre-entry and post-entry steps. These results suggest that both *T. vulgaris* and *N. cataria* hydrosols have therapeutic potential in the prophylaxis and treatment of US-PRRSV infection.

Keywords: PRRSV, *Thymus vulgaris*, *Nepeta cataria*, qPCR, hydrosol

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) is characterized by reproductive failure of sows and respiratory problems of nursery and growing pigs in most pig-producing countries. The causative agent, PRRS virus (PRRSV), was identified in the early 1990s as an enveloped, positive-strand RNA virus with a genome of approximately 15 kb. PRRSV belongs to the family *Arteriviridae* and the order *Nidovirales* (Zimmerman et al., 2012). This immunopathogenic disease of pigs is similar to feline infectious peritonitis and dengue virus in cats and humans, respectively. A process known as antibody-dependent enhancement phenomenon characterizes both diseased animals and humans (Yoon et al., 1997).

The control and eradication of PRRSV infection are still questionable since it was identified. Current management strategies mainly focus on the prevention of PRRSV infection using vaccination. Unfortunately, the two available types of PRRSV vaccines, the modified live-attenuated vaccines (MLVs) and inactivated vaccines, have certain drawbacks concerning safety (Scortti et al., 2006) and efficacy (Scortti et al., 2007; Zuckermann et al., 2007). Some cytokines such as IFN-α, IFN-β, IFN-γ and IFN-λ have an antiviral effect against PRRSV (Luo et al., 2011; Overend et al., 2007), but they are still in laboratory research or clinical trial stages. Recently, several anti-PRRSV agents have been identified, including antibiotics, peptides and plant extracts. Tilmicosin is a new semi-synthetic macrolide antibiotic developed from tylosin B. PRRSV-infected piglets treated with tilmicosin exhibited significantly reduced viremia and improved average daily weight gain (Lin et al., 2016). Cheng et al. (2013) reported that chlorogenic acid and scutellarin could inhibit PRRSV infection effectively *in vitro*, and their antiviral activity was most likely directly inactivating and/or disturbing the early stage of PRRSV replication. Karuppannan et al. (2012) identified 4 compounds, which were 12-deoxyphorbol 13-phenylacetate 20-acetate, ouabain, bufalin and valinomycin, which inhibited the PRRSV replication cycle. Pingproa et al. (2014) showed that crude extract of C. dactylon potentially inactivated PRRSV and inhibited replication of PRRSV *in vitro*.

Medicinal herbs have great potential for producing new drugs for human benefit. Medicinal plants used in traditional medicine contain a vast array of substances that can be used to treat chronic and even infectious diseases. According to the World Health Organization (WHO, 2001), 80% of the world population use medicinal plants in the treatment of diseases. The demand for drugs from plant sources is continuously increasing. It is necessary to evaluate traditional herbal medicine for various ailments systematically. Throughout the past decades, natural compounds that consist of a major source of effective pharmacological agents such as artemisinin, alkaloids, salicylates, opiates, etc. have been discovered (Mishra and Tiwari, 2011). Therefore, the purpose of this study was to explore medicinal herbs that might be clinically effective against North American PRRSV (NA-PRRSV or US-PRRSV) infection. A total of 42 natural herb hydrosols were screened for their antiviral activity against two US-PRRSV strains in MARC-145 cells, of which 2 hydrosols showed promising results and their possible mechanisms of anti-viral effects were elucidated.

Materials and Methods

Preparation and purification of natural plant hydrosols: Various herbs (Table 1) were cultivated in the experimental field of Taichung District Agricultural Research and Extension Station. Aerial part of plants was harvest at maturity then subjected to distillation immediately. Hydrosol was collected using an Essential Oil Distiller (Kou-Chou Instrument Co.). In brief, 10 kg of fresh leaves were distilled with 30 L of water, vapor was condensed to collect hydrosol until the volume of hydrosol reached 2 L. Subsequently, the plant hydrosols were stored at the room temperature for future use.

Cell and viruses: MARC-145 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (Gibco®, Life technologies, USA) supplemented with a 10% fetal bovine serum (FBS) (Gibco®, Life technologies, USA), 100 IU/mL penicillin and 50 µg/ml streptomycin in 5% CO_2 at 37°C. Two local PRRSV isolates, MD001 and 763, were used in this study. Both belonged to the US-PRRSV. MD001 is the first Taiwanese PRRSV isolate, discovered in central Taiwan in 1992 (kindly provided by Professor Ling-Ling Chueh, National Taiwan University). Strain 763 was isolated from a nursery pig that suffered during the severe PRRSV outbreak in southern Taiwan in 2010 (kindly provided by Professor Wen-Bin Chung, National Pingtung University of Science and Technology). The sequences of the ORF5 Gene were deposited in GenBank under the access numbers AF035409 and KY073240 for strains MD001 and 763, respectively.

Cell viability: Cell viability was evaluated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Hsieh et al., 2010). Briefly, 2×10^4 MARC-145 cells per well were seeded in 96-well plates. After 24 h incubation at 37°C, 2-fold serially diluted plant hydrosols were added to confluent cell monolayers and incubated for 72 h. MTT was added to each well to a final concentration of 50 mg/mL. After 4 h of incubation at 37°C, the medium containing MTT was removed, and the cells were lysed with 100 µl of DMSO. After gently shaking the plates for 30 min, the absorbance value at 570 nm was measured using an ELISA reader. Cell viability (%) was calculated following the expression: (OD of plant hydrosol - treated cells / OD of untreated cells) × 100.

Screening of antiviral activity: Based on results from the cell viability test, the highest concentration showing less than 10% cytotoxicity in the MARC-145 cells was chosen for each of the test plant hydrosols; these chosen concentrations were premixed with PRRSV at a multiplicity of infection (MOI) of $1TCID_{50}$ and incubated at 37°C for 1 h to monitor the effect in the pre-entry step. The mixture of plant hydrosols and virus was then inoculated into MARC-145 cells in 48-

well plates for 1 h of adsorption, followed by replacement of the mixture by DMEM containing 2% FBS and the same concentration of plant hydrosol to monitor the effect in the entry step. Three replicates were carried out for each plant hydrosol and control group. After 15 h post-infection, the supernatants were harvested to quantify viral load using Real-time PCR assay and cells were fixed with methanol and acetone solution for immunofluorescence assay.

Pre-entry assay: Based on results from the screening of antiviral activity test, the hydrosols showing high inhibition effect were chosen for the mechanism investigation. For pre-entry A step, MARC-145 cells were treated with effective plant hydrosol for 24 h. The MARC-145 cells were washed by PBS three times and were then incubated with PRRSVs at MOI of $1TCID_{50}$ for 1 h at 37°C. After 1 h of adsorption, the mixture was removed and infected cells were washed with PBS three times, then DMEM with 2% FBS was added. The infected cells and cultured supernatant were subjected to thrice frozen-thawed cycles immediately and viral load quantification as previously described. For pre-entry B step, effective plant hydrosols were premixed with PRRSVs at MOI $1TCID_{50}$ for 1 h, then the mixture of plant hydrosol and virus was inoculated into MARC-145 cells in 48-well plates for 1 h of adsorption. After 1 h of incubation, the mixture was removed and infected cells were washed with PBS three times, then DMEM with 2% FBS was added. The procedures of viral load quantification were the same as described in the pre-entry A step. Relative value (%) of antiviral activity was determined compared to the controlled untreated group. Three replicates were carried out for each plant hydrosol.

Table 1 Summary of 42 natural plant extracts used in cytotoxicity test and antiviral activity test

No.	Herbs	Maximum non-cytotoxicity concentration	*P*-value[a]	No.	Herbs	Maximum non-cytotoxicity concentration	*P*-value[a]
1	*Camellia sinensis*	2^{-4}	0.0533	22	*Thymus vulgaris*	2^{-4}	0.000353***
2	*Origanum majoricum*	2^{-3}	0.0189*	23	*Thymus serpyllum*	2^{-5}	0.0118*
3	*Pelargonium fragrans* "Logeei"	2^{-2}	0.0541	24	*Alpinia galanga*	2^{-4}	0.9969
4	*Alpinia officinarum*	2^{-3}	0.0721	25	*Melissa officinalis* (Netherland)	2^{-3}	0.5740
5	*Salvia officinalis* "Purpurascens"	2^{-3}	0.8910	26	*Thymus citriodorus variegata*	2^{-3}	0.9374
6	*Lavandula angustifolia*	2^{-3}	0.0782	27	*Rosmarinus officinalis*	2^{-2}	0.6495
7	*Nepeta cataria*	2^{-3}	0.00007***	28	*Melissa officinalis* "no. 9"	2^{-4}	0.0503
8	*Melissa officinalis* "no. 11"	2^{-2}	0.0395*	29	*Melissa officinalis* "no. 10'"	2^{-3}	0.0465
9	*Vitex negundo*	2^{-3}	0.0530	30	*Mentha arvensis*	2^{-3}	0.9288
10	*Mentha spicata*	2^{-3}	0.0712	31	*Aloysia triphylla*	2^{-3}	0.0172*
11	*Lavendula heterophylla*	2^{-3}	0.0231*	32	*Lavandula × intermetia* "Gray lady"	2^{-4}	0.0143*
12	*Cinnamomum cassia*	2^{-6}	0.00935**	33	*Houttuynia cordata*	2^{-2}	0.1621
13	*Perilla frutescens*	2^{-4}	0.0343*	34	*T. vulgaris x T. pulegioides* "Lemon thyme"	2^{-3}	0.0977
14	*Pogostemon cablin*	2^{-2}	0.8117	35	*Origanum marjorana*	2^{-3}	0.0442
15	*Acorus gramineus* (Stem)	2^{-3}	0.9303	36	*Artemisia argyi*	2^{-2}	0.0488
16	*Acorus gramineus* (Leaf)	2^{-4}	0.2812	37	*Pelargonium graveolens*	2^{-2}	0.0512
17	*Achillea millefolium*	2^{-2}	0.0262*	38	*Lavandula × intermetia* "Provence"	2^{-3}	0.9746
18	*Salvia officinalis* "Berggarten"	2^{-3}	0.6270	39	*Origanum vulgare*	2^{-2}	0.0208*
19	*Salvia officinalis* "Icterina"	2^{-3}	0.1069	40	*Lavendula angustifolia* "Lady"	2^{-3}	0.0509
20	*Salvia officinalis* "Tricolor"	2^{-3}	0.6943	41	*Aloysia citrodora*	2^{-4}	0.9269
21	*Rosa rugosa* "Honey"	2^{-5}	0.0571	42	*Matricaria recutita*	2^{-2}	0.0675

[a]Statistical analyses of antiviral activity were performed using Student's *t*-test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, compared with positive control.

Post-entry assay: The MARC-145 cells were infected with PRRSVs at MOI $1TCID_{50}$ for 1 h of adsorption and then the virus was removed and cells were maintained in fresh DMEM containing 2% FBS and effective plant hydrosols. At 15 h post-infection, the cultured supernatant was harvested and subjected to viral load quantification as described previously. Relative value (%) was determined of antiviral activity compared to the untreated group. Three replicates were carried out for each plant hydrosol.

Real-time PCR and indirect immunofluorescence assay (IFA): The cell culture supernatants were screened using zip nucleic acid probe-based real-time PCR for PRRSV, as described by Lin et al. (2013). Indirect immunofluorescence assay (IFA) was evaluated as previously described (Chiou et al., 2000) with slight modifications. Briefly, at each designated PRRSV 763 strain or MD001 strain infected coverslip preparations in triplicate were fixed in acetone and PRRSV protein was detected by incubation with corresponding specific monoclonal antibody (SDOW17; RTI, LLC, Brookings, SD, USA) (1:500) against PRRSV N protein, and the secondary fluorescein goat anti-mouse IgG (H+L) (Life Technologies, Carlsbad, CA, USA) (1:1000). Positive staining was determined by bright cytoplasmic fluorescence.

Statistical analysis: Data from each plant hydrosols are presented as mean ± standard deviation. The student's *t*-test was used for comparison between the treated and untreated groups. *P* values < 0.05, < 0.01 and 0.001 were considered statistically significant,

highly significant and very highly significant, respectively.

Results

Cell viability and cytotoxicity: Cytotoxicity assays are essential for the initial phases of antiviral drug development. Low moderate toxicity was exhibited by the hydrosols from 42 natural plants ranging from 2^{-2} to 2^{-6} dilutions (Table 1). The cytotoxicity of hydrosols on MARC-145 cells was in a dose-dependent manner, clearly indicating that the cytotoxicity of different hydrosols varied remarkably (data not shown). With the higher concentration of hydrosols, cells underwent more morphological changes such as lyses, granulation, pyknosis, condensation, vacuolization in the cytoplasm, darkening of cell boundaries, and cell detachment (data not shown).

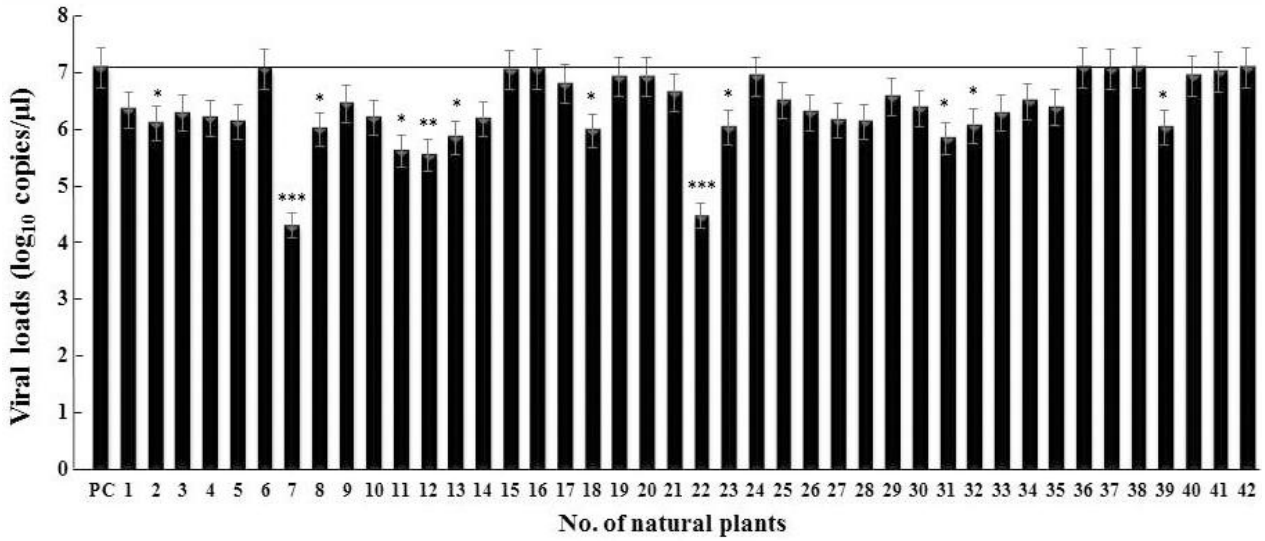

Figure 1 Antiviral activity of 42 natural plant hydrosols. After 15 h post-infection, PRRSV loads were quantified by real-time PCR assay. No. 7 (*Nepeta cataria*) and No. 22 (*Thymus vulgaris*) showed strong inhibitory activity against PRRSV. Statistical analyses of antiviral activity were performed using Student's t-test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, compared with positive control (PC). All samples were analyzed in triplicate.

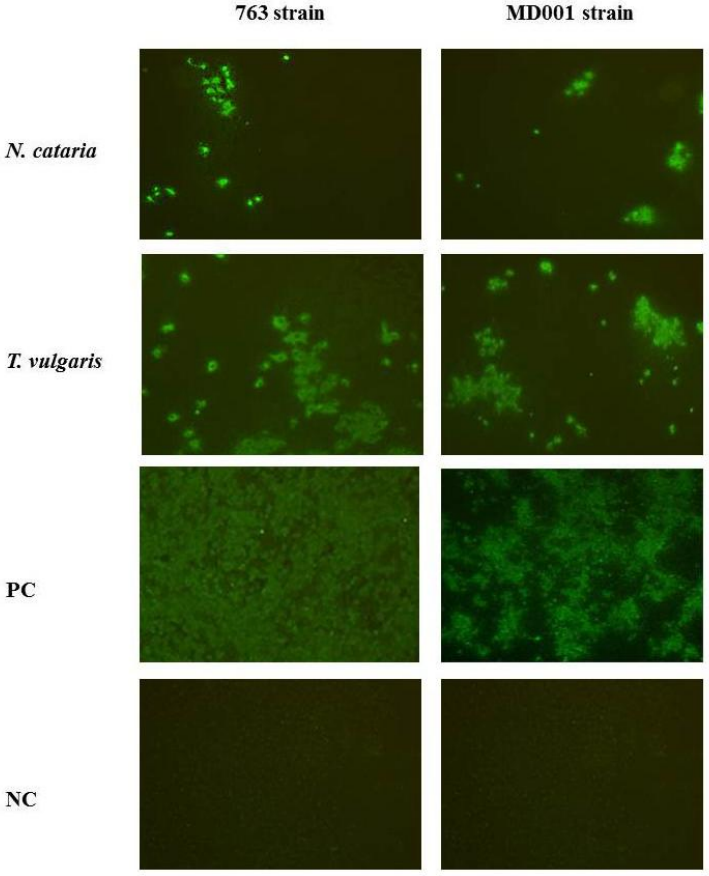

Figure 2 Immunofluorescence assay was evaluated on PRRSV-infected cells under *T. vulgaris* and *N. cataria* hydrosol-treated, untreated and negative groups. After 15 h post-infection, expression of viral antigens was much lower in the infected cells treated with *T. vulgaris* and *N. cataria* hydrosols compared to the untreated group.

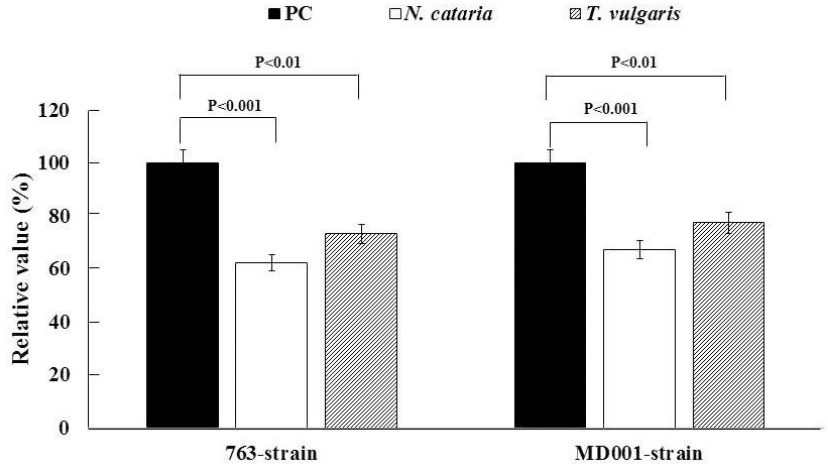

Figure 3 Antiviral activity of *T. vulgaris* and *N. cataria* hydrosols against two PRRSV strains. After 15 h post-infection, PRRSV loads were quantified by real-time PCR assay. *T. vulgaris* and *N. cataria* hydrosols showed strong anti-PRRSVs compared to the untreated group. Statistical analyses of antiviral activity were performed using Student's t-test. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, compared with positive control (PC). All samples were analyzed in triplicate.

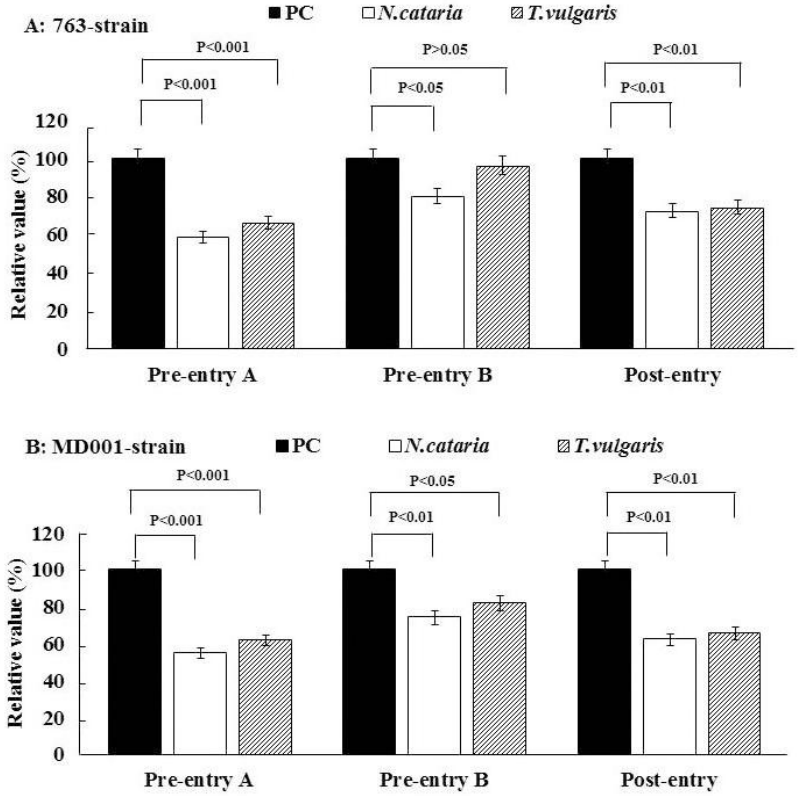

Figure 4 Real-time PCR for PRRSV strain 763 (A) and strain MD001 (B) with various timings of the natural plant hydrosol addition. Both *T. vulgaris* and *N. cataria* hydrosols significantly inhibited PRRSVs compared to the untreated group (positive control, PC). *N. cataria* was more potent than *T. vulgaris* at inhibiting PRRSV replication. Statistical analyses of antiviral activity were performed using Student's t-test. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, compared with PC. All samples were analyzed in triplicate.

Screening of 42 natural plant hydrosols for antiviral activity: Based on the cell viability results, the highest concentration of each tested hydrosol showing less than 10% cytotoxicity was chosen and further tested for antiviral effects. Among the 42 natural plant hydrosols tested, 10 of them significantly inhibited the PRRSV load when compared with the untreated group (Figure 1 and Table 1). No. 7 (*Nepeta cataria*) and No. 22 (*Thymus vulgaris*) showed strong inhibitory activity against PRRSV ($P < 0.001$). Interestingly, the expression of viral antigens was much lower in the infected cells treated with *T. vulgaris* and *N. cataria* than the control (Figure 2). The relative values of *T. vulgaris* and *N. cataria* hydrosols against PRRSV strain 763 were 73.21% ($P < 0.01$) and 62.34% ($P < 0.001$), respectively, compared to the untreated group (Figure 3). Similarly, the relative values of *T. vulgaris* and *N. cataria* hydrosols against PRRSV strain MD001 were 77.44% ($P < 0.01$) and 67.31% ($P < 0.001$), respectively, compared to the untreated group (Figure 3). *N. cataria* was more potent than *T. vulgaris* at inhibiting PRRSV replication (Figure 3). *T. vulgaris* and *N. cataria* were further chosen to investigate the mechanism of anti-PRRSV.

Anti-PRRSV mechanisms of T. vulgaris and N. cataria hydrosols: To disclose the step in which *T. vulgaris* and *N. cataria* hydrosols affect the virus life cycle, time-of-addition experiments were performed on the replication of PRRSVs. *T. vulgaris* showed strong inhibitory activities against both PRRSV strains in the pre-entry A strep ($P < 0.001$) followed by the post-entry step ($P < 0.01$), but did not show any antiviral activities against PRRSV strain 763 in the pre-entry B step ($P > 0.05$) (Figure 4A). Comparing the antiviral activity of *N. cataria* hydrosol among the three steps (pre-entry A, pre-entry B and post-entry) on the PRRSV strain 763 infection, the strongest inhibitory activity was in the pre-entry A step ($P < 0.001$), followed by the post-entry step ($P < 0.01$) and the pre-entry B step ($P < 0.05$) (Figure 4A). Similar anti-PRRSV effects were also observed in the other PRRSV strain, MD001 (Figure 4B). Together, the results suggest that *T. vulgaris* and *N. cataria* hydrosols show strong inhibitory activities against PRRSV strains in both pre-entry and post-entry steps. *N. cataria* was indeed more potent than *T. vulgaris* at inhibiting PRRSV replication.

Discussion

In pig farming, PRRSV infection has led to serious economic loss worldwide. Prevention and control strategies mainly focus on vaccination, for which vaccine strategies have failed to control outbreaks (Scortti et al., 2007; Zuckermann et al., 2007). Drug control is an alternative therapy, but the synthesis of antiviral drugs is time-consuming. Intensive efforts have been made to look for anti-PRRSV agents from natural medicinal herbs *in vitro*. Natural products from pure compounds to plant extract screening can provide unlimited opportunities for new antiviral drugs (Reichling et al., 2009). Infectious viral diseases have remained important global issues for animals and humans. According to the dependency of viruses on host cells, only a few effective antiviral drugs are available to treat viral diseases. Also, only a few reports have described the use of medicinal compounds against PRRSV (Cheng et al., 2013; Gao et al., 2013; Karuppannan et al., 2012; Pringproa et al., 2014; Yang et al., 2015). Thus, this study aimed at searching for natural plant metabolites that might be clinically effective against PRRSV infection. Our antiviral activity assay indicated that both *T. vulgaris* and *N. cataria* hydrosols were able to reduce the PRRSV load *in vitro* significantly. This is the first study to demonstrate that both *T. vulgaris* and *N. cataria* hydrosols can inhibit the replication of PRRSV *in vitro*, which can potentially be clinically applied.

T. vulgaris is a well-known Mediterranean aromatic plant containing essential oils and lipophilic substances (Nabavi et al., 2015; Nikolić et al., 2014). It has been demonstrated to be effective against microorganisms such as fungi and yeasts (Ocana and Reglero, 2012), viruses (Santoyo et al., 2014; Talactac et al., 2015) and bacteria (Golestani et al., 2015; Nezhadali et al., 2014). Our research revealed that *T. vulgaris* hydrosol significantly reduced the PRRSV load. The anti-PRRSV activity occurred in both pre-entry and post-entry steps. The anti-PRRSV mechanisms of *T. vulgaris* remain to be further investigated. However, previous studied actions of these compounds are killing virus and/or interfering with viral replication in ovo (Bakari et al., 2012), interfering with virus envelope and also masking viral components which are necessary for attachment, penetration, or entry into host cells (Reichling et al., 2009). Side effects of long-term use are allergy, gastrointestinal irritation (anecdotal), hypotension, increased risk of bleeding or slow blood clotting, and hormone-sensitive conditions (Dwivedi and Chopra, 2013; Posadzki et al., 2013). However, the side effects above were reported for clinical trials in humans and laboratory animals only; no side effects of *T. vulgaris* have been reported in pigs.

N. cataria is an important medicinal herb, a native of Asia and Southeast Europe (Adiguzei et al., 2009). Its known active ingredients are monoterpenes, sesquiterpenes, diterpenes, triterpenes, flavonoids, phenol, essential oils and others (Formisano et al., 2011). Previous studies have demonstrated that *N. cataria* and its derivatives have been used to relieve gastrointestinal and respiratory disorders (Zenasni et al., 2008), antibacterial (Zomorodian et al., 2012), antifungal (Reichling et al., 2009) and antioxidant activities (Adiguzei et al., 2009). Our results indicated that *N. cataria* hydrosol could reduce the PRRSV load significantly. Furthermore, the antiviral mechanisms of *N. cataria* were investigated and the results indicated that *N. cataria* hydrosol performed its anti-PRRSV activity in the pre-entry A, B and post-entry steps. The *N. cataria*- cell and *N. cataria*-virus interaction remain to be further investigated. However, *N. cataria* compound may cause central nervous system depression and increase susceptibility to seizures. Long-term exposure may induce tolerance to stereotypic behavior, catalepsy and sleep. It may also interact with other medications if used on a daily basis, as previously observed in humans (Galati et al., 2004). These side effects, however, have not been reported in livestock animals or pigs.

Generally, these results suggest that both *T. vulgaris* and *N. cataria* hydrosols can inhibit PRRSV infection effectively *in vitro*, and their antiviral activities are most likely changing cell conformation (pre-entry A step), interfering with viral attachment (pre-entry B step) and inhibiting viral replication and/or virus release (post-entry step). It is encouraged to further explore the antiviral properties and mechanisms of these plant hydrosols *in vitro* and *in vivo*. In our previous study of pigs with high PRRSV loads, it was demonstrated that the high loads correlated with the presence of porcine respiratory disease complex (Lin et al., 2013). Therefore, it was speculated that both *T. vulgaris* and *N. cataria* hydrosols might have great potential to become the candidates not only for preventing pigs from developing clinical signs after PRRSV infection, but also for preventing PRRSV infection.

In conclusion, both *T. vulgaris* and *N. cataria* hydrosols demonstrated their activities against US-PRRSV in MARC-145 cells by blocking viral attachment, adsorption, replication and release. The results of this study suggest that both natural plant hydrosols have therapeutic potential in the prophylaxis and treatment of US-PRRSV infection.

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บทคัดย่อ

การทดสอบคุณสมบัติการต้านไวรัสของสารสกัดบริสุทธิ์จากพืชสมุนไพร *Thymus vulgaris* และ *Nepeta cataria* ต่อเชื้อไวรัส PRRS

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โรค Porcine reproductive and respiratory syndrome หรือ PRRS เป็นโรคที่ทำให้เกิดกลุ่มอาการทางระบบสืบพันธุ์ในแม่สุกรและระบบทางเดินหายใจในลูกสุกรอนุบาลและสุกรขุน ปัจจุบันการจัดการโรคส่วนใหญ่เน้นการป้องกันไวรัสชนิดนี้ด้วยการให้วัคซีน อย่างไรก็ตามวัคซีนชนิดเชื้อเป็นที่มีในปัจจุบัน ไม่มีประสิทธิภาพในการกำจัดไวรัสและส่งเสริมการกระตุ้นการสร้างภูมิคุ้มกันให้กับร่างกายภายหลังจากการติดเชื้อ เพื่อหาสารที่เป็นตัวแทนที่อาจพิสูจน์ว่ามีประสิทธิภาพทางคลินิกต่อการต้านการติดเชื้อไวรัส PRRS การทดลองครั้งนี้จึงได้ศึกษาสารน้ำสกัดบริสุทธิ์จากน้ำมันหอมระเหยของพืชสมุนไพรจากธรรมชาติจำนวน 42 ชนิด และได้ทดสอบคุณสมบัติของสารสกัดสมุนไพรดังกล่าวในการต้านเชื้อไวรัสชนิดนี้จำนวน 2 สายพันธุ์ที่เลี้ยงในเซลล์เพาะเลี้ยง MARC-145 cells การทดสอบเซลล์มีชีวิตพบว่า ความเข้มข้นสูงสุดที่แสดงความเป็นพิษที่ระดับน้อยกว่า 10% ใน MARC-145 cells มีความใกล้เคียงกันในสารสกัดแต่ละชนิด ผลการทดสอบคุณสมบัติในการต้านเชื้อไวรัสชี้ให้เห็นว่า สารสกัดสมุนไพรสองชนิด คือ ***Thymus vulgaris*** และ ***Nepet cataria*** สามารถลดปริมาณไวรัส PRRS ในหลอดทดลองได้อย่างมีนัยสำคัญ ($p < 0.05$) นอกจากนี้ ผลการทดสอบยังแสดงให้เห็นว่ากลไกในการต้านเชื้อไวรัส PRRS ของสารสกัดสมุนไพรทั้งสองชนิดนี้สามารถต้านเชื้อไวรัสนี้ได้ทั้งก่อนและหลังการติดเชื้อ ดังนั้นสารสกัดจากพืชสมุนไพร ***Thymus vulgaris*** และ ***Nepet cataria*** มีศักยภาพในการป้องกันและการรักษาโรค PRRSV

คำสำคัญ: ไวรัส PRRS ไธม์ แคทนิป เทคนิคการเพิ่มปริมาณสารพันธุกรรมในสภาวะจริง สารสกัดด้วยน้ำ

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