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(54) **Title:** MIXTURES OF ACIDS FOR USE AS ANTIVIRAL AGENTS

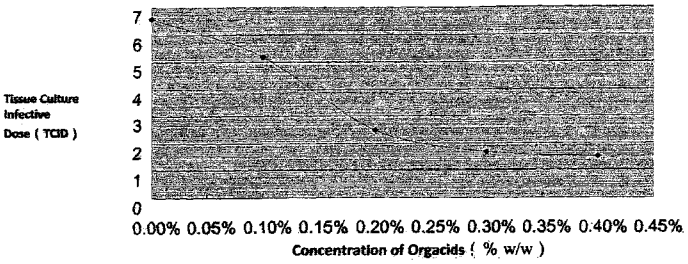

Figure 1

(57) **Abstract:** The present invention provides mixtures of acids containing lactic acid, formic acid, tartaric acid, malic acid, citric acid and orthophosphoric acid for use in prophylaxis and/or treatment of viral diseases, particularly the foot and mouth disease in animals.

Title

Mixtures of acids for use as antiviral agents

Field of Art

The present invention relates to mixtures of acids and their use as antiviral agents, particularly in animals.

Background Art

Fopt and mouth disease is an infectious viral disease affecting cloven-hoofed animals, including bovids. Susceptible animals include cattle, water buffalo, sheep, goats, pigs, antelopes, deers, bisons, elephants, hedgehogs. It can spread very easily, e.g., by aerosol or by feed. At present, the animals are protected against the virus by vaccination, but it takes at least 3 weeks to become active. The animal feed cart be protected by disinfection, which brings potentiaHy undesirable components into the feed, and also the disinfectants cannot be applied under freezing environment.

The use of mixtures of acids as animal food additive is known. For example, products containing mixture of lactic acid, formic acid, tartaric acid, malic acid$_s$ citric acid and orthophosphoric acid are known, which promote growth of animals, such as swine or poultry, and improve feed efficiency. These products also kill harmful bacteria, such as Salmonella. Their use improves the growth of lactobacillus and other beneficial organisms in the gastrointestinal tract of the animals. These products were never used or tested against viruses.

Within the framework of the present invention, it was found that a mixture of acids is surprisingly active against viruses.

Summary of invention

The present invention relates to mixture of acids containing lactic acid, formic acid, tartaric acid, malic acid, citric acid and orthophosphoric acid for use in prophylaxis and/or treatment of viral diseases. The mixture of acids contains at least 50 % of orthophosphoric acid^ up to 10 % of lactic acid, up to 10 % of formic acid, up to 10 % of tartaric acid, up to 10 % of malic acid, and up to 10 % of citric acid; Also, the mixture of acids contains 75 to 95 % of orthophosphoric acid, 1 to 5 % of lactic acid, 1 to 5 % of formic acid, 1 to 5 % of tartaric acid, 1 to 5 % of malic acid, 1 to 5 % of citric acid.

More particulartly, the viral disease is a foot and mouth disease.

Yet another aspect of the present invention discloses a method of treatment and/or prophylaxis of a viral disease (preferably a foot and mouth disease) in an animal, characterized in that a mixture of acids containing lactic acid, formic acid, tartaric acid, malic acid, citric acid and orthophosphoric acid is administered to the animal.

Finnaly, the present invention discloses an antiviral composition, characterized in that it contains a mixture of acids containing lactic acid, formic acid, tartaric acid, malic acid, citric acid and orthophosphoric acid, and optionally auxiliary substances, such as fillers or carriers.

Disclosure of the Invention

Object of the present invention is a mixture of acids containing lactic acid, formic acid, tartaric acid, malic acid, citric acid and orthophosphoric acid for use in prophylaxis and/or treatment of viral diseases.

Preferably, the mixture of acids contains at least 50 % of orthophosphoric acid, up to 10 % of lactic acid, up to 10 % of formic acid, up to 10 % of tartaric acid, up to 10 % of malic acid, and up to 10 % of citric acid.

More preferably the mixture of acids contains 75 to 95 % of orthophosphoric acid, 1 to 5 % of lactic acid, 1 to 5 % of formic acid, 1 to 5 % of tartaric acid, 1 to 5 % of malic acid, 1 to 5 % of citric acid.

In a preferred embodiment, the viral disease is a foot and mouth disease.

This invention further includes a method of treatment and/or prophylaxis of a viral disease in an animal, in which a mixture of acids containing lactic acid, formic acid, tartaric acid, malic acid, citric acid and orthophosphoric acid is administered to the animal. The viral disease is preferably a foot and mouth disease.

Another object of the present invention is an antiviral composition containing a mixture of acids containing lactic acid, formic acid, tartaric acid, malic acid, citric acid and orthophosphoric acid, and optionally auxiliary substances, such as fillers or carriers. The antiviral composition can be administered to the animals for example together with their feed.

The fillers and carriers used in the antiviral composition can be any suitable liquid or solid fillers or carriers, such as, for example, water, cereal-based carriers, cellulose-based carriers, polymers and co-polymers based on acrylic acid or its derivatives, hyaluronic acid, aiginic acid, chitosane, silica, talc, dextrane, etc.

Brief Description of Figures

Figure! shows the effect of the mixture of acids oh FMD virus titer.

Example

Evaluation of the inhibition activity of the mixture of acids according to the present invention against foot and mouth disease virus (FMD virus)

In the testing, pandemic foot and mouth disease virus serotype O (OR/83), grown in BHK-21 cell line (Syrian hamster fibroblast cell line) was used. The product containing the mixture of acids used was a commercially available product Orgacids®, supplied by Sunzen Lifesciences Sdn Bhd, which contains lactic acid, formic acid, tartaric acid, malic acid, citric acid and orthophosphoric acid.

The toxicity test against BHK-21 cell line was first perform to evaluate the cytotoxic effect. Different concentration of Orgacids with 0.025 % (w/w), 0.05 % (w/w), 0.1 % (w/w),

0.2 % (w/w), 0.3 % (w/w), 0.4 % (w/w) and 0.5 % (w/w) were added into cell line medium. The cytopathic effect (CPE) on the cell line was recorded to determine the maximum non-toxic dose of Orgacids against the cell line.

Concentration	Number of tests	CPE	Cell damage (%)
0.5 %	8	6	75.0
0.4 %	8	1	12.5
0.3 %	8	0	0
0.2 %	8	0	0
0.1 %	8	0	0
0.05 %	8	0	0
0.025 %	8	0	0

Table 1: Toxicity test against BHK-21 cell line

Table 1 shows that 0.3 % of the mixture of acids do not have any toxic effect on BHK-21 cells line; 0.4 % of the mixture of acids caused minor damage on BHK-21 cells line, the cell injury rate Was 12.5%; 0.5 % of the mixture of acids cause serious damage on BHK-2 1 cells line, cell injury rate was 75.0%.

In order to assess the in-vitro activity of the mixture of acids against foot and mouth disease virus, the BHK-21 cell line was cultured using conventional methods. The trial is divided into 3 groups, which are the positive control group (no virus and additives were added), negative control group (added with virus and without additives) and the treatment group. In the treatment group, 5 different concentrations of Orgacids which are 0.05 % (w/w), 0.1 % (w/w), 0.2 % (w/w), 0.3 % (w/w) and 0.4 % (w/w) were added into the medium, using 96-well cell culture plates, each concentration has ***6*** replicates, the whole treatment was repeated 3 times (8 well for each concentration with 3 replicates). Tests were carried out in three different ways:

a) direct inactivation effect of the mixture of acids on FMD virus: 50 micro liter of FMD virus and 50 micro liter of medium contain different concentration of Orgacids were mixed well, incubate in 37"C, then infect the cell line, the infected cell line were observed on 24h and 48h for cell damage. Cell damage rate was calculated as below:

Cell damage rate= number of CPE affected well ÷ number of well tested XI00%

Concentration	24h cell damage rate (%)				48h cell damage rate (%)			
	1	2	3	Average	1	2	3	Average
(+)control	0	0	0	0.0±0	0	0	0	0.0±0
0.4%	0	0	0	0.0±0	0	0	0	0.0±0
0.3%	0	0	0	0.0±0	0	12.5	0	4.2±7.2
0.2%	75	75	87.5	79.2±7.2	100	100	100	100±0
0.1%	100	100	100	100±0	100	100	100	100±0
0.05%	100	100	100	100±0	100	100	100	100±0
(-)control	100	100	100	100±0	100	100	100	100±0

Table 2: Direct inactivation effect of the mixture of acids on FMD virus

Table 2 shows 0.3 % and 0.4 % of the mixture of acids have directly effect in inactivation of FMD virus, even though after 48 hours of incubation period, it significantly reduces the cellular damage caused by FMD virus. In between, 0.2 % of the mixture of acids have minor effect in inactivation of FMD virus, it significantly reduce the cellular damage caused by FMD virus after 24 hours of incubation.

b) inhibitory effect of the mixture of acids on FMD virus to infect cell line: 100 micro liter of medium with cell line were added with 50 micro liter of medium contain different concentration of Orgacids, after lh, those mixed cell line were experimental infected with FMD virus, the infected cell lines were observed on 24h and 48h for cell damage. Cell damage rate was calculated as below:

Cell damage rate= number of CPE affected well ÷ number of well tested X100%

concentration	24h cell damage rate (%)				48h cell damage rate (%)			
	1	2	3	Average	1	2	3	Average
(+)control	0	0	0	0.0±0	0	0	0	0.0±0
0.4%	0	25	12.5	12.5±12.5	50	37.5	50	45.8±7.2
0.3%	75	62.5	87.5	75±12.5	100	100	87.5	95.8±7.2
0.2%	100	100	100	100±0	100	100	100	100±0
0.1%	100	100	100	100±0	100	100	100	100±0
0.05%	100	100	100	100±0	100	100	100	100±0
(-)control	87.5	100	100	95.8±7.2	100	100	100	100±0

Table 3: Inhibitory effect of the mixture of acids on FMD virus to infect cell line

Table 3 shows 0.4 % of the mixture of acids prevent FMD virus infect the cell line, even though after 48 hours of incubation period, it significantly reduces the cellular injury caused by FMD virus. In between, 0.3 % of the mixture of acids has minor effect in inhibiting FMD virus to infect the cell line, it significantly reduce the cellular injury caused by FMD virus after 24 hours of incubation.

c) Inhibitory effect of the mixture of acids on FMD virus infected cell line: Infect the cell line with FMD virus, after lh, 50 micro liter of medium contain different concentration of Orgacids were added into the FMD virus infected cell lines. Incubate the flask in 37°C, 5% $C0_2$ incubator, the infected cell line were observed on 24h and 48h for cell damage. Cell damage rate was calculated as below:

Cell damage rate= number of CPE affected well ÷ number of well tested XI00%

concentration	24h cell damage rate (%)				48h cell damage rate (%)			
	1	2	3	Average	1	2	3	Average
(+) control	0	0	0	0.0±0	0	0	0	0.0±0
0.4%	75	62.5	87.5	75±12.5	100	100	100	100±0
0.3%	100	100	100	100±0	100	100	100	100±0
0.2%	100	100	100	100±0	100	100	100	100±0
0.1%	100	100	100	100±0	100	100	100	100±0
0.05%	100	100	100	100±0	100	100	100	100±0
(-)control	87.5	100	100	95.8±7.2	100	100	100	100±0

Table 4: Inhibitory effect of the mixture of acids on FMD virus infected cell line

Table 4 shows 0.4 % of the mixture of acids lias minor inhibitory effect on FMD infected cell, it significantly reduce the cellular damage on BHK-21 cells line caused by FMD virus after 24 hours of incubation. However, others concentration do not have inhibitory effect on the FMD virus infected cell.

Effect of the mixture of acids on FMD virus titer

Firstly, determine the OR/83 virus titer, then added 10^7 TCID 50 FMD viruses into flask with single layer of BHK-21 cell line, and then added in medium with different concentration of Orgacids. Incubate the flasks in 37°C, 5% **CO2** incubator, until 95% cytopathic effect (CPE) were observed, then cell venom was harvested (When CPE

reached 95%, harvest the viral supernatant), and it was diluted with the cell culture medium for 10 times at the dilution titer of 8 (10-1 ~ 10-8). Each titrate was transferred to 96-wells cell plates, each titer plus 8 holes (8 well for each titer/concentration), each hole 50µl. At the same times prepare the normal cell control, virus control. Observe after 24h for CPE. 50% Tissue Culture Infective Dose (TCID50) was calculated using Reed-Muench's method.

Groups	FMDV virus titre (titration level)								$TCID_{50}$
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	
0.4 %	8/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8	1.5
0.3 %	8/8	2/8	0/8	0/8	0/8	0/8	0/8	0/8	1.67
0.2 %	8/8	8/8	0/8	0/8	0/8	0/8	0/8	0/8	2.5
0.1 %	8/8	8/8	8/8	8/8	3/8	2/8	0/8	0/8	5.18
0.0 %	8/8	8/8	8/8	8/8	8/8	7/8	2/8	0/8	6.59
Virus	8/8	8/8	8/8	8/8	8/8	4/8	0/8	0/8	
Master fluid	8/8	8/8	8/8	8/8	8/8	3/8	0/8	0/8	5.9

Table 5 Effect of the mixture of acids on FMD virus titer.

Table 5 and Figure 1 show that the serotype OR/83 TCID 50 is 5.9, after 15 hours of infection on BHK-21 cell line, infected cell was harvested when the cell line shown 95% of cytophatic effect (CPE), TCID50 (50% tissue culture infective dose) is 6.59. However, by including different concentration of Orgacids significantly reduce the TCID50. By increasing the concentration of Orgacids from 0%-0.4%, the TCID50 reduce from 6.59 to 1.5. From the result, it proven Orgacids significantly reduce the infectivity of FMD virus serotype OR/83.The results show that Orgacids reduce the infectivity of FMD virus in-vitfo. By increasing the inclusion rate from 0.1% to 0.4 %, TCID 50 of FMD virus reduces from 5.18 to 1.5.

Orgacids also play an important role in inactivation of FMD virus, with inclusion rate of 0.2% or above, it can directly inactivate the FMD virus. In addition, in higher inclusion rate (0.3% or higher), Orgacids prevent FMD virus infecting the cell and inhibit the FMD infected cell.

CLAIMS

1. A mixture of acids containing lactic acid, formic acid, tartaric acid, malic acid, citric acid and orthophosphoric acid for use in prophylaxis and/or treatment of viral diseases.

2. The mixture of acids according to claim 1, wherein it contains at least 50 % of orthophosphoric acid, up to 10 % of lactic acid, up to 10 % of formic acid, up to 10 % of tartaric acid, up to 10 % of malic acid, and up to 10 % of citric acid.

3. The mixture of acids according to claim 2, wherein it contains 75 to 95 % of orthophosphoric acid, 1 to 5 % of lactic acid, 1 to 5 % of formic acid, 1 to 5 % of tartaric acid, 1 to 5 % of malic acid, 1 to 5 % of citric acid.

4. The mixture of acids according to claim 1, wherein the viral disease is a foot and mouth disease.

5. A method of treatment and/or prophylaxis of a viral disease in an animal, characterized in that a mixture of acids containing lactic acid, formic acid, tartaric acid, malic acid, citric acid and orthophosphoric acid is administered to the animal.

6. The method of claim 5, wherein the viral disease is preferably a foot and mouth disease.

7. An antiviral composition, characterized in that it contains a mixture of acids containing lactic acid, formic acid, tartaric acid, malic acid, citric acid and orthophosphoric acid, and optionally auxiliary substances, such as fillers or carriers,

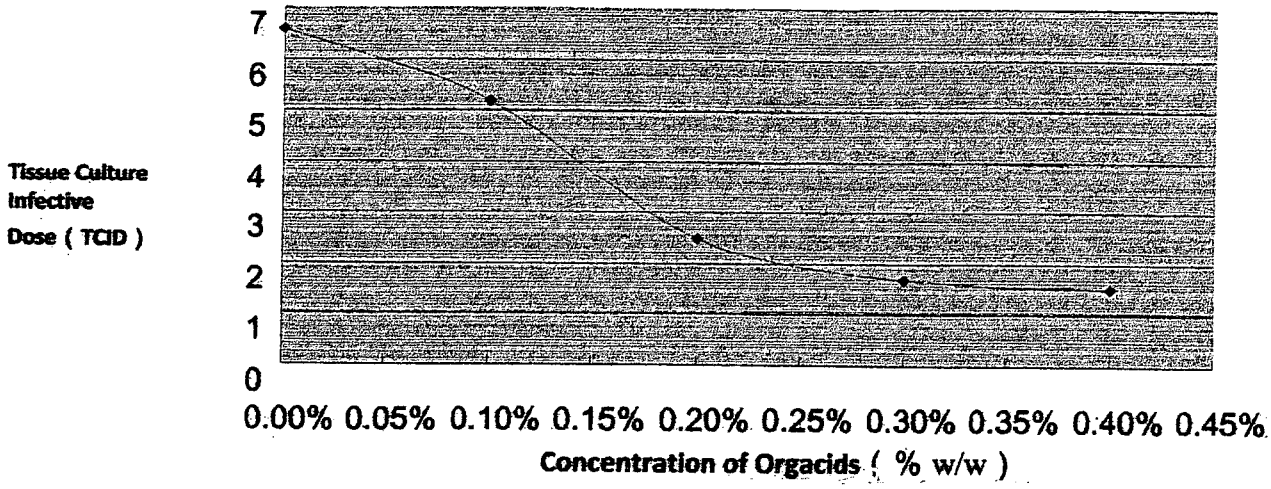
Tissue Culture Infective Dose (TCID)
7
6
5
4
3
2
1
0
0.00% 0.05% 0.10% 0.15% 0.20% 0.25% 0.30% 0.35% 0.40% 0.45%
Concentration of Orgacids (% w/w)

Figure 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/MY2011/000057

A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/194(2006.01)i, A61K 31/185(2006.01)i, A61K 31/19(2006.01)i, A61P 31/12(2006.01)i, A61P 31/00(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K 31/194; A01N 59/06; A01N 59/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(KIPO internal)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6350474 B1 (DZNELADZE DAVID et al.) 26 February 2002 See claim 1	1-7
A	S. ESWARANANDAM et al, 'Antimicrobial Activity of Citric, Lactic, Malic, or Tartaric Acids and Nisin-incorporated Soy Protein Film Against Listeria mono cytogenes, Escherichia coli O157:H7, and Salmonella gaminara', JOURNAL OF OOOD SCIENCE, Vol 69, Nr.3, pp. 81-84, 2004. See abstract	1-7

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
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"P" document published prior to the international filing date but later than the priority date claimed
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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 FEBRUARY 2012 (28.02.2012)	**29 FEBRUARY 2012 (29.02.2012)**

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Authorized officer
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Telephone No. 82-42-481-8270

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/MY2011/000057

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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