Antimicrobial susceptibility of selected Essential Oils and their compounds against Streptococcus suis

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Abstract

Background: Streptococcus suis is an emerging zoonotic pathogen causing different diseases, in both humans and pigs. Generally, the control of diseases caused by this pathogen is based on antimicrobial therapy, but the development of bacterial resistance has led to look for new options. In this sense, the Essential Oils (EOs) constitute an alternative to the use of conventional antimicrobials. The activity of oregano, cinnamon, common thyme and red thyme EOs and their main active compounds (carvacrol, cinnamaldehyde and thymol) against 56 S. suis isolates from pigs (n=50) and human (n=6) was determined by broth microdilution. MIC50-90, MBC50-90 and the bactericidal index (MBC/MIC) were calculated. Also, the time-kill curve of each product against the S. suis P1/7 European reference strain was determined.

Results: No differences in the MIC or MBC values were observed between all the tested products, which suggest a homogenous behaviour of S. suis, independently of their origin, organ of isolation or resistance profile. All the products showed a concentration-dependent and time-dependent killing activity and achieved the virtual eradication of S. suis (reduction of ≥ 4 log10) at suprainhibitory (2x-4x MIC) concentrations within the first 5 minutes of exposure, except cinnamaldehyde, that showed only bacteriostatic effect (reduction of < 3 log10).

Conclusions: While all the tested products demonstrated an antimicrobial activity, red thyme and cinnamon followed by thymol showed the best results. It suggests that these products would be recommended as antimicrobials in veterinary medicine for the control of this zoonotic pathogen, although more pharmacology, toxicology, tolerability and formulation studies are necessary.

Background

Streptococcus suis is an emerging zoonotic pathogen causing diseases, such as meningitis, arthritis, polyserositis and septicaemia in humans and pigs, and bronchopneumonia in pigs (1). Although large outbreaks of human diseases have been recorded in Asia, sporadic cases occur in Western countries, being personal in contact with infected pigs or pork-derived products at risk (2). The main reservoirs of the disease are the healthy carrier pigs, harbouring the bacteria in the upper respiratory, intestinal and genital tract (1). Based on the capsular polysaccharide, 29 serotypes of this pathogen are now recognized (3), being serotypes 1 to 10 (except 6) and 14, 15, 16, 1/2 and 1/14 the most important for both human and pigs(1, 4).

The control of these diseases should focus on the correct use of antimicrobials and sanitary measures implemented on the affected farms (5). However, the increasing cases of antimicrobial resistance in S. suis to many classes of antimicrobial agents, such as lincosamides, macrolides, sulphonamides and tetracycline, have been a global problem in recent years (6, 7). It has been suggested that S. suis may be responsible for the spread of resistance genes to important human pathogens like Streptococcus pyogenes, S. pneumoniae and S. agalactiae (5, 8).

Nowadays, an important pressure to reduce the use of antimicrobials in pig farming worldwide, to avoid the development of bacterial resistance, does exit (9). Different studies have proposed the use of Essential Oils (EOs) as natural antimicrobial agents alone or in combination with conventional antimicrobials (10, 11). EOs are natural bioactive compounds derived from plants obtained by steam distillation and composed by terpenes, aldehydes and alcohols (12). The EOs activity is the result of the effects of all components and their interactions. Nevertheless, just a limited number of compounds account for up to 85 per cent of the total mixture compared with the minors (10, 13–15) and their mechanisms of action include enzymatic systems inactivation, membrane proteins alteration and increased membrane permeability (16).

Previous studies have shown the in vitro antibacterial activity of cinnamon, oregano and common and red thyme oils against Streptococcus spp. (10, 11, 17). However, the effect of their main compounds (cinnamaldehyde, carvacrol and thymol) has not been studied yet in S. suis. For this, the antimicrobial activity of these essential oils and their main active components against S. suis isolates obtained from human and pigs was analysed in this study. Furthermore, the time required to get a bactericidal effect of each product against the S. suis P1/7 European reference strain was determined.

Methods

Bacterial strains

A total of 56 S. suis isolates belonging to pigs (n = 49) and humans (n = 6) were analysed. The European reference S. suis strain P1/7 was also included (Table 1). All strains were maintained at − 80 °C in Microbank® beads (Pro-Lab Diagnostics Inc. Merseyside, UK) since analysed. The antimicrobial susceptibility of the isolates was determined by broth microdilution method performed as outlined by the Clinical and Laboratory Standards Institute for fastidious organisms (Clinical and Laboratory Standards Institute CLSI, 2013). The following antimicrobial agents (Sigma Aldrich Co., USA) were used: penicillin G; enrofloxacin; ceftiofur; sulfamethoxazole/trimethoprim (19/1); gentamicin; oxytetracycline.

Table 1

Serotype, origin, pathology, organ and resistance profile of the 56 Streptococcus suis isolates analysed in this study.

Reference	Serotype	Origin	Pathology	Organ	Resistance profile
638/03	1	swine	Arthritis	Joint	SXT; CN; OT
P1/7*	2	swine	Meningitis	Brain	CN
235/02	2	swine	Septicaemia	Kidney	SXT; CN; OT
365/03	2	swine	Meningitis	Brain	SXT; CN; OT
682/06	2	swine	Septicaemia	Liver	CN; OT
123/11	2	swine	Arthritis	Joint	SXT; CN; OT; P
93/05	2	swine	Bronchopneumonia	Lung	SXT; OT
203/05	2	swine	Meningitis	Brain	OT
225/00	3	swine	Arthritis	Joint	SXT; CN; OT
14/03	4	swine	Septicaemia	Kidney	CN; OT
636/03	4	swine	Bronchopneumonia	Lung	SXT; CN; OT
196/05	4	swine	Septicaemia	Spleen	SXT; OT
792/02	4	swine	Bronchopneumonia	Lung	CN; OT
633/99	5	swine	Abortions	Foetus	SXT; CN; OT; ENR
5215	6	swine	Unknown	Unknown	SXT; CN; OT; ENR
316/12	6	swine	Unknown	Lymph node	OT
22/02	7	swine	Septicaemia	Lung	-
204/03	7	swine	Meningitis	Brain	SXT; CN; OT
26/03	7	swine	Meningitis	Brain	SXT; OT
40/03	8	swine	Arthritis	Joint	CN; OT
160/03	8	swine	Bronchopneumonia	Lung	CN; OT
553/05	8	swine	Septicaemia	Liver	CN; OT
3144	9	swine	Meningitis	Brain	SXT; CN; OT; P
8010*	9	swine	Unknown	Unknown	SXT; CN; OT
233/01	9	swine	Arthritis	Joint	SXT; CN; OT
10/06	9	swine	Meningitis	Brain	SXT; OT; ENR
228/06	9	swine	Meningitis	Brain	CN; OT; CEF; P
34/11	9	swine	Meningitis	Brain	SXT; CN; OT
746/02	9	swine	Meningitis	Cerebellum	SXT; OT
340/05	10	swine	Bronchopneumonia	Lung	CEF
546/05	14	swine	Meningitis	Brain	CN; OT
232/06	14	swine	Arthritis	Joint	CN; OT
5225*	15	swine	Unknown	Unknown	SXT; CN; OT
609/02	15	swine	Bronchopneumonia	Lung	SXT; OT; P
668/02	15	swine	Meningitis	Brain	SXT; CN; OT

*Pig and human strains kindly provided by Dr. Henk Wisselink (Central Veterinary Institute of Wageningen, Lelystad, the Netherlands) and Dr. Juan A. Saez Nieto (National Health Institute of Carlos III, Madrid, Spain). Isolates without resistance profile were sensitive to all the antimicrobials. Serotype was determined by agglutination method, the origin is referred to the organ of the isolation, resistance profile was performed by the broth microdilution test (CLSI 2013).

Abbreviations: P, Penicillin; ENR, Enrofloxacin; CEF, Ceftiofur; SXT, Sulfamethoxazole/Trimethoprim (19/1); GEN: Gentamicin; OTC: Oxytetracycline.

Reference	Serotype	Origin	Pathology	Organ	Resistance profile
724/02	16	swine	Septicaemia	Kidney	OT
226/03	16	swine	Meningitis	Brain	SXT; CN; OT; P
592/06	16	swine	Meningitis	Brain	OT
261/12	16	swine	Bronchopneumonia	Lung	SXT; OT
20/17	16	swine	Meningitis	Brain	SXT; CN; OT; P
273/12	21	swine	Septicaemia	Liver	SXT; P
6217	24	swine	Unknown	Unknown	SXT; OT; P
338/12	24	swine	Non-sick	Lymph node	SXT; OT
6218*	25	swine	Unknown	Unknown	OT
10/17	25	swine	Non-sick	Heart	ENR
6221*	28	swine	Unknown	Unknown	SXT; OT; P
36/12	31	swine	Non-sick	Lymph node	-
635/03	1/2	swine	Meningitis	Brain	SXT; OT
658/02	1/14	swine	Meningitis	Brain	SXT; CN; OT
699/02	1/14	swine	Meningitis	Brain	SXT; CN; OT
857/06*	2	human	Meningitis	Cerebrospinal fluid	-
1299/06*	2	human	Meningitis	Cerebrospinal fluid	OT
34/11*	2	human	Meningitis	Cerebrospinal fluid	SXT
1086/11*	2	human	Meningitis	Blood	OT
117/12*	2	human	Meningitis	Blood	CN
41/14*	2	human	Meningitis	Blood	SXT; OT

*Pig and human strains kindly provided by Dr. Henk Wisselink (Central Veterinary Institute of Wageningen, Lelystad, the Netherlands) and Dr. Juan A. Saez Nieto (National Health Institute of Carlos III, Madrid, Spain). Isolates without resistance profile were sensitive to all the antimicrobials. Serotype was determined by agglutination method, the origin is referred to the organ of the isolation, resistance profile was performed by the broth microdilution test (CLSI 2013).

Abbreviations: P, Penicillin; ENR, Enrofloxacin; CEF, Ceftiofur; SXT, Sulfamethoxazole/Trimethoprim (19/1); GEN: Gentamicin; OTC: Oxytetracycline.

Essential oils and their main components

Cinnamon, oregano, common thyme and red thyme EOs (purity ≥ 95%) were purchased from Aromium™ (Barcelona, Spain) and analysed by Gas Chromatography/Quadrupole Mass Spectroscopy to chemotype (data provided by manufacturer). Cinnamaldehyde, carvacrol and thymol were supplied by Sigma-Aldrich, Inc. (Madrid, Spain) with a purity of ≥ 95% (Table 2). All the products were stored following the manufacturer instructions.

Table 2
Botanical classification and chemotype of the studied essential oils.

Essential oil	Common name	Family	Chemotype*
Cinnammomum zeylanicum (bark)	Cinnamon	Lauraceae	Cinnamaldehyde (69.18%), linalool (3.19%), eugenol (3.03%)
Origanum vulgare	Oregano	Lamiaceae	Carvacrol (63.01%), thymol (10.56%), γ- terpinene (8.11%)
Thymus vulgaris	Common thyme	Lamiaceae	Thymol ^{NA}, carvacrol ^{NA}, p-cymene ^{NA}, linalool ^{NA}
Thymus zygis	Red thyme	Lamiaceae	Thymol (46.9%), p-cymene (21.72%), γ-terpinene (9.32%), linalool (4.8%)

* Data provided by the manufacturer. ^{NA} Not available.

In vitro susceptibility test

The broth microdilution method (18) was carried out with slight modifications. Mueller-Hinton broth was replaced with Brain-Heart Infusion (BHI) and supplemented with 0.15% agar (Oxoid Ltd., Cheshire, UK) to improve the dilution of the products (17, 19). Double serial dilutions of natural products

(ranging from 39.0625 µg/ml to 5000 µg/ml) were prepared and mixed with an equal volume (100 µL) of bacterial inoculum up to a final concentration of 5×10^5 CFU/ml. Then, the 96-well plates were incubated at 37 °C for 20–24 h under aerobic conditions. Positive (antimicrobial-free broth with bacterial inoculum) and negative (antimicrobial-free broth without bacterial inoculum) growth controls were included. As quality control, Penicillin G was used. The Minimum Inhibitory Concentration (MIC) was defined as the lowest concentration that avoided the visible growth of S. suis.

For the Minimum Bactericidal Concentration (MBC), 10 µl from the last four wells without visible bacterial growth were subcultured onto Blood Mueller-Hinton agar plates (BMHA) at 37 °C/20–24 h in 5% CO_2-enriched atmosphere. The MBC was defined as the lowest concentration resulting in a negative subculture or a single colony growth after incubation. All the tests were conducted in triplicate, taking as final value the highest concentrations of MIC and MBC.

Time-kill curve assays

The S. suis reference strain P1/7 was inoculated at a final concentration of 5×10^5 CFU/ml in 20 mL of BHI, supplemented with 0.15% agar mixed with each one of the tested products at concentrations of 0x (growth control), 0.5x, 1x, 2x and 4x MIC. The cultures were incubated in shaking (80 rev min-1) at 37 °C in a water bath for 24 h. Bacterial growth was monitored at 0, 1, 5, 15, 30, 60, 120, 240, 480 minutes and 24 h by plating serial 10-fold dilutions onto Blood Agar plates in duplicate (20, 21). Penicillin G (Sigma-Aldrich, USA) was used as a positive control with the same concentrations used with products, but with bacterial viable counts at 0, 60, 120, 240 minutes and 24 h of exposure. The experiment was performed twice independently.

After the incubation at 37 °C for 24 h, colonies were counted, and the number of CFU/ml was determined using an average count of the duplicated plates and multiplying by the corresponding dilution factor.

Data analysis

The statistical SPSS Software v18.8 (IBM Company, Nueva York, USA) and Microsoft Excel 2010 (Microsoft Corporation, USA) were used for the data processing. Basing on the results of the susceptibility tests, the MIC and MBC frequency distribution of the different products against S. suis isolates were determined. The MIC_{50} and MIC_{90}, MBC_{50} and MBC_{90} were estimated as the product concentration that inhibited and killed the 50% and 90% of the tested strains, respectively. The bactericidal index (BI) was calculated by the MBC_{90}/MIC_{90} ratio to characterize the product as bactericidal ($BI < 4$) or as bacteriostatic ($BI \geq 4$) (22, 23). To compare the antimicrobial activity of different products, the MIC and MBC were treated as ordinal numerical variables. The normality of the distributions obtained was checked by means of the Kolmogorov-Smirnov test ($p < 0.05$) and the non-parametric tests of Friedman and Wilcoxon, with the Bonferroni correction for multiple testing (p-value of 0.05), were carried out.

The time-kill curve was analysed by plotting the average concentration of viable bacteria ($\log_{10}$ CFU/ml) versus time for each product concentration. The effectiveness (E) of the product during the assay was quantified as the difference between the viable bacteria count ($\log_{10}$ CFU/ml) at each time (Tn) and at the beginning (T0). Following the criteria of Sidhu et al. (2010) (24), three cut-off points were established: bacteriostatic effect, when a reduction of $< 3 \log_{10}$; bactericidal effect, when a reduction of $\geq 3 \log_{10}$ (99.90% reduction of the original inoculum count was detected) was detected; virtual eradication effect, in cases of reduction of $\geq 4 \log_{10}$ (99.99% reduction of the original inoculum count was detected).

Results

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC and MBC values of the seven products tested (EOs and their main compounds) against all the S. suis isolates are showed in Table 3. In all cases, a non-normal unimodal distribution of MIC and MBC, with a narrow range of values (2–3 dilutions), was obtained. In general, for the main compounds (cinnamaldehyde, carvacrol and thymol), lower MIC and MBC values were obtained ($p < 0.05$) in comparison with their essential oil (cinnamon, oregano and common and red thyme).

Table 3

Minimum Inhibitory Concentration (MIC), MIC_{50} and MIC_{90}, Minimum Bactericidal Concentration (MBC), MBC_{50} and MBC_{90}, and Bactericidal Index 90 (BI_{90}) of the four Essential Oils and their main compounds against 56 Streptococcus suis isolates.

Products	Nº of isolates with MIC (µg/mL)										MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	
	19,531	39.062	78.125	156.25	312.5	625	1250	2500	5000	10000			
Cinnamon		0	0	0	13	29	14	0	0		625	1250	
Oregano		0	0	20	36	0	0	0	0		312.5	312.5	
Common thyme		0	0	16	39	1	0	0	0		312.5	312.5	
Red thyme		0	0	10	44	2	0	0	0		312.5	312.5	
Cinnamaldehyde		0	0	14	38	4	0	0	0		312.5	312.5	
Carvacrol		0	2	52	2	0	0	0	0		156.25	156.25	
Thymol		0	16	34	6	0	0	0	0		156.25	312.5	
Products	Nº of isolates with MBC (µg/mL)										MBC_{50} (µg/mL)	MBC_{90} (µg/mL)	BI_{90}
	19,513	39.062	78.125	156.25	312.5	625	1250	2500	5000	10000			
Cinnamon		0	0	0	11	22	21	2	0		625	1250	1
Oregano		0	0	14	42	0	0	0	0		312.5	312.5	1
Common thyme		0	0	14	39	3	0	0	0		312.5	312.5	1
Red thyme		0	0	9	39	8	0	0	0		312.5	625	2
Cinnamaldehyde		0	0	6	35	13	2	0	0		312.5	625	2
Carvacrol		0	0	50	5	1	0	0	0		156.25	312.5	2
Thymol		0	14	36	6	0	0	0	0		156.25	312.5	1

MIC_{50} and MBC_{50}: concentration (µg/ml) obtained for 50% of the strains (28/56); MIC_{90} and MBC_{90}: concentration (µg/ml) obtained for 90% of the strains (51/56); BI_{90}: bactericidal index estimated with MBC_{90}/MIC_{90} ratio.

Regarding to the MIC_{90} values, carvacrol showed the highest inhibitory activity (156.25 µg/mL), followed by thymol, cinnamaldehyde, oregano, common and red thyme (312.5 µg/mL) and cinnamon (1250 µg/mL). All tested products presented the same values for MICs and MBCs (Table 3), except for red thyme, cinnamaldehyde and carvacrol for MBC_{90}. Apparently, all the tested S. suis isolates showed a homogenous behaviour against to all the products, independently of their origin, organ of isolation or resistance profile.

The BI showed the bactericidal or bacteriostatic character of all the tested products (BI < 4) (Table 3). The four products that showed the highest BI (BI = 1) were cinnamon, oregano, common thyme and thymol.

Time-kill assay

This assay was carried out to determine the bactericidal power of different concentrations of EOs and their main compounds throughout time. In general, concentration-dependent and time-dependent killing activities were observed in all the products tested against the European reference strain P1/7 (Figs. 1a-1c).

Cinnamon and cinnamaldehyde showed marked differences in their bactericidal activities (Fig. 1a). At 1x MIC, cinnamon showed a bactericidal effect (reduction of ≥ 3 $\log_{10}$) after 15 minutes of exposure, and the virtual eradication (reduction of ≥ 4 $\log_{10}$) was obtained after 30 minutes. At supra-inhibitory concentration (2x MIC and 4x MIC), this EO achieved the virtual eradication of the S. suis P1/7 within the first 5 minutes. Cinnamaldehyde at 1x MIC and 2x MIC showed only a bacteriostatic effect (reduction of < 3 $\log_{10}$), while at 4x MIC reached the inhibitory virtual eradication within 3 h post-exposure.

Oregano and carvacrol showed similar kinetics for all tested concentrations (Fig. 1b). Both products were bacteriostatic at 1x MIC, but the effectiveness of oregano decreased significantly at the end of evaluated period, while the carvacrol reached values close to the bactericidal limit. At supra-inhibitory concentrations, both products achieved the virtual eradication of the bacteria within 5 minutes of exposure.

Differences between common thyme, red thyme and thymol were observed (Fig. 1c). At 1x MIC, common thyme was bacteriostatic and reached the virtual eradication at supra-inhibitory concentrations (2x MIC and 4x MIC) after the first minute. However, the red thyme showed virtual eradication of

S. suis P1/7 at 1x MIC, 2x MIC and 4x MIC at the same time. Regarding thymol, it demonstrated at 2x and 4x MIC, the virtual eradication of S. suis P1/7 after the first minute of exposure whereas at 1x MIC just showed bactericidal effect from 2 h of exposure.

Discussion

The extensive use of antimicrobials in veterinary medicine is considered one of the main causes of the emergence and diffusion of resistant microorganisms (5, 25). Recent studies about S. suis report a remarkable decrease in the susceptibility of this zoonotic pathogen to antimicrobials commonly used in livestock, such as lincosamides, macrolides, sulphonamides and tetracyclines (25, 26). Furthermore, this microorganism has been identified as reservoir for antibiotic resistance genes which can be transferred horizontally to streptococcal human pathogens such as S. pyogenes, S. pneumoniae and S. agalactiae (8).

Essential oils are natural products with hydrophobic character and a complex chemical composition (13), which could be used for the treatment of diseases caused by resistant bacteria (27–29). In previous studies, we showed that cinnamon, oregano, thyme and their main compounds (cinnamaladehyde, carvacrol and thymol) could be used in combination with gentamicin and tetracycline for the control of resistant S. suis isolates from swine (19). However, the efficacy of these products alone against porcine and human S. suis isolates is poorly known. This study includes not only an considerable number of S. suis strains (n = 56), but also human isolates.

According to our results, values of MIC_{90} of these EOs against all the S. suis isolates analysed in this study ranged from 1250 µg/ml (cinnamon) to 312.5 µg/ml (oregano and common and red thyme), like those previously obtained against 20 porcine isolates (11). Regarding the main compounds, the MIC_{90} ranged from 321,5 µg/ml (cinnamaldehyde and thymol) to 156,25 µg/ml (carvacrol). Moreover, in this work, all the products showed a bactericidal character (BI < 4).

Time-kill curves that monitor bacterial growth and death have been frequently used to evaluate the effect of antimicrobials over time (21, 30). In our work, these assays showed a time-dependent and concentration-dependent activity for all the tested products. In general, all of them have shown an antimicrobial potential against S. suis as described for other Streptococcus species (15, 17), although a huge variability was observed regarding concentration and time among them. EOs showed better results than their main compounds, showing stronger antimicrobial activity at the same concentration (1x MIC) at relatively earlier times. It has already been suggested that the antimicrobial activity of the EOs is better, which can be attributed to the wide variety of components, which may act in synergy against different targets (19, 31–33).

The best results were obtained for red thyme and cinnamon, that reached the virtual eradication after 1 minute and 30 minutes of exposure, respectively, coinciding with previous results which demonstrated the effectiveness of both products against S. suis isolated from pigs (21, 34, 35). Regarding the main compounds, thymol demonstrated a bactericidal activity after 2 hours of exposure against the porcine and human S. suis isolates, which are in accordance with previous studies with Streptococcus species (15, 36). Nevertheless, the seven products achieved the virtual eradication at 2x MIC and 4x MIC within 1–5 minutes, except the cinnamaldehyde that reached this effect at 4 h.

Previous studies with S. pyogenes ATCC 19615 and S. aureus ATCC 25923 have also highlighted the strong and quick bactericide effect of oregano (5–10 min) at doses equal to or higher than the MIC (21, 34). However, our study showed slightly different results, decreasing its effectiveness at the end of the evaluated period. Variations in the EOs composition (extraction methods, geographic region, plant part, botanical species) and the susceptibility of studied strains would explain these differences.

A slight regrowth of the bacteria was observed with some of the products (cinnamon, oregano, red thyme and thymol) at concentrations 1x MIC, which could be associated with the persistence phenomena described for other concentration-dependent antimicrobials such as fluoroquinolones (30).

One of the main controversies is the cytotoxic effect of EOs when used at high doses. Some studies have shown a cytotoxic effect of these EOs at concentrations like our MIC_{90} and the MBC_{90} results (34.55–500 µg/ml) (36–40). However, recent studies on pig tracheal epithelial cell lines showed that cinnamon or thyme had a minimal effect on cell viability following a 2 h exposure at concentrations effective against this pathogen (35). Moreover, in a study carried out on vero cell lines, the thyme showed a marked inhibitory activity against many bacteria, despite a low cytotoxicity (38). Furthermore, the toxicity tests carried out in rats, showed a low oral toxicity with the oregano used in this work (LD50 < 2000 mg/kg and NOAEL < 200 mg/kg/day) (39, 41).

The knowledge of EOs is scarce, therefore, research efforts must be intensified to clarify the role that these products play as antimicrobials. In this context, time-kill assays and other dynamics studies together with MIC or MBC, are of interest to monitor the behavior of different bacterial species against the EOs.

The lack of the standardized method, the differences on the EOs composition (geographical origin, growth climatic and environmental conditions, part of the plant and the extraction method) and the variability of the botanical names, difficult the comparison of the results between different studies (29, 32, 35, 42). In our study, EOs showed better antimicrobial activity than their main compounds, results that can be explained if consider that the molecules contained in very low proportion can also play an important role (32). However, the use of these main compounds of the EOs

represents an interesting research line, since the standardisation is easier and, thus, the comparison of the results and their subsequent application in the diseases control (43, 44).

We found a homogenous behaviour of all the S. suis isolates against all the tested products, independently of their origin, organ of isolation or resistance profile, which encourage further studies to favor the use of these EOs in S. suis infections control. To do so, a complete assessment (e.g. in vitro cell and in vivo animal model) on the toxicity and safety of these products is strongly advisable.

Conclusion

Cinnamon, oregano and Thyme (red and common thyme) and their main compounds showed an antimicrobial activity against strains of S. suis but thyme, its main compound (thymol) and cinnamon were revealed with the best results. These findings suggest that these products can be an alternative to conventional antimicrobials to reduce outbreaks of diseases caused by this zoonotic pathogen, although further studies are recommended.

Declarations

Ethics approval and consent to participate
Not applicable

Consent to publish
Not applicable

Availability of data and materials
The dataset analyzed during the current study is available from the corresponding author on reasonable request.

Competing interests
None of the authors of this manuscript has a financial or personal relationship with other people or organizations that could inappropriately influence the content of this work.

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Author Contributions
All authors designed the study, performed the experiments and analyzed and interpreted the results. All of them helped in the written and the revision of the manuscript and approved the presented version of that.

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Figures

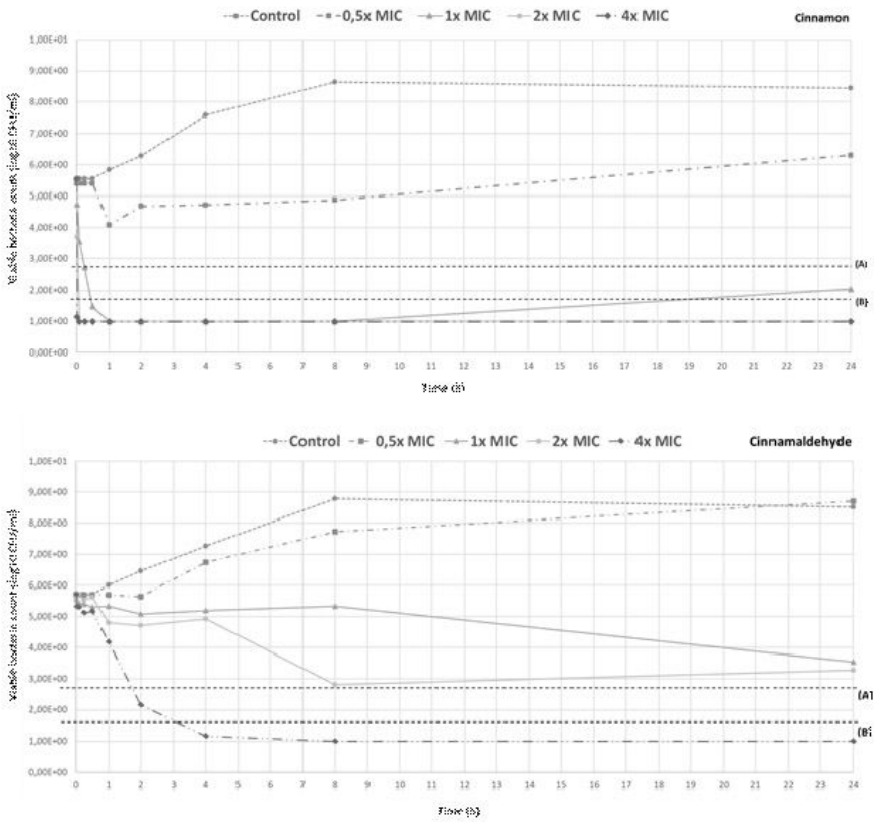

Figure 1

Time-kill curve of S. suis P1/7 for cinnamon and cinnamaldehyde. The horizontal dotted lines mark the theoretical cut-off points and the areas to evaluate the effectiveness: (A) bactericidal effect (99.90%) and (B) virtual eradication of the bacteria (99.99%).

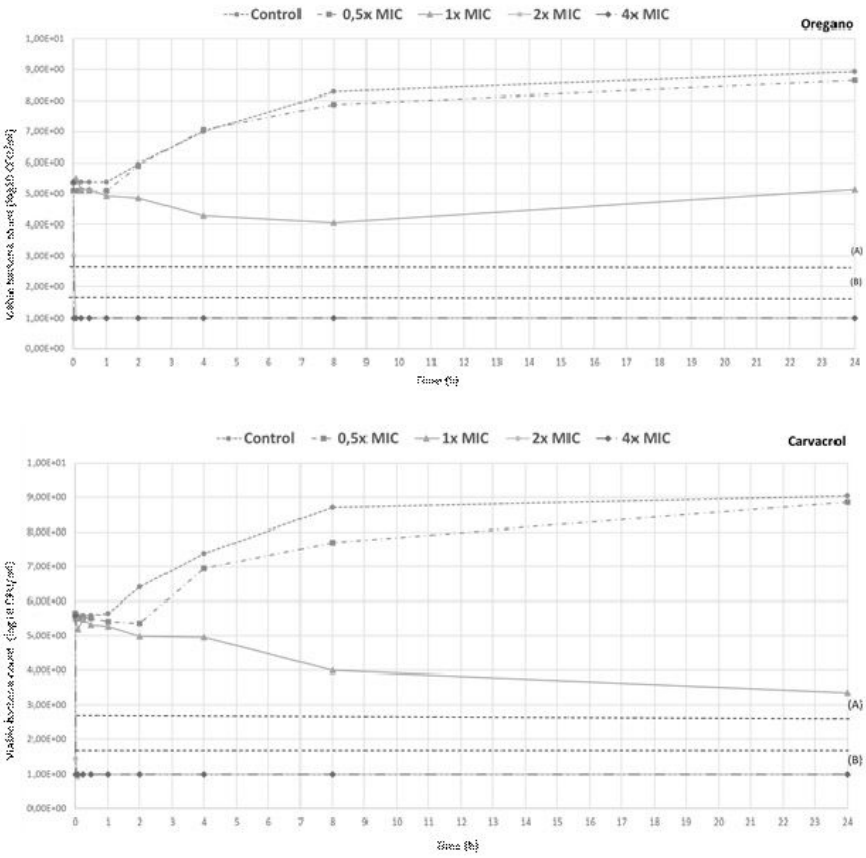

Figure 2

Time-kill curve of S. suis P1/7 for oregano essential oil and carvacrol. The horizontal dotted lines mark the theoretical cut-off points and the areas to evaluate the effectiveness: (A) bactericidal effect (99.90%) and (B) virtual eradication of the bacteria (99.99%).

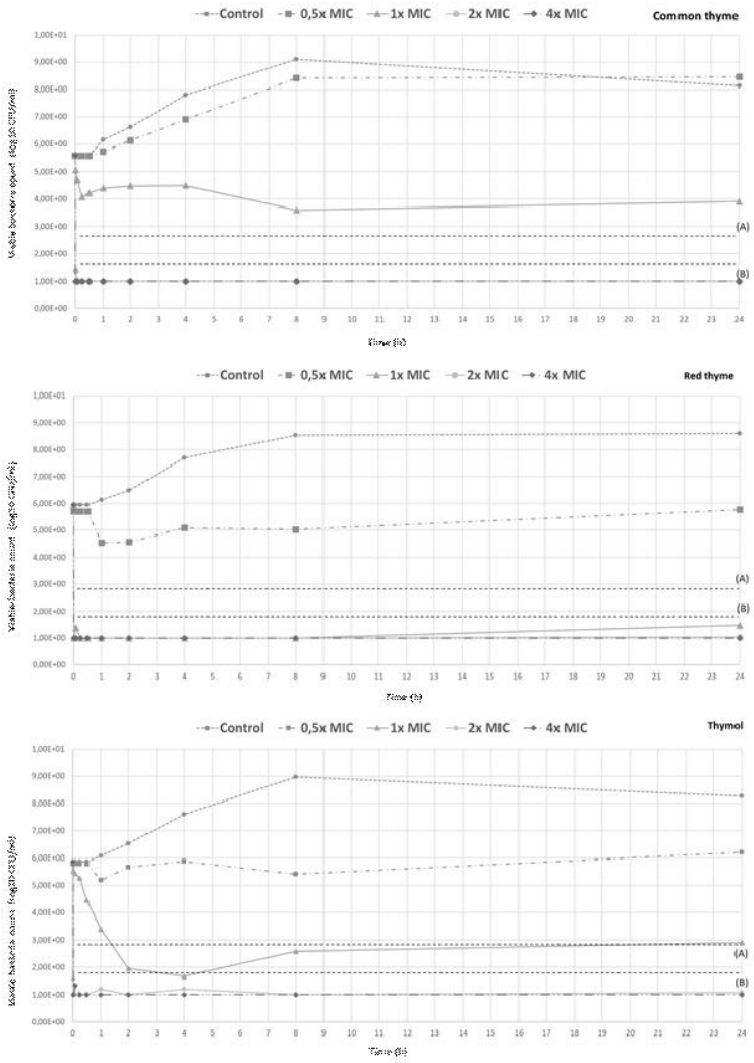

Figure 3

Time-kill curve of S. suis P1/7 for common thyme and red thyme essential oil and thymol. The horizontal dotted lines mark the theoretical cut-off points and the areas to evaluate the effectiveness: (A) bactericidal effect (99.90%) and (B) virtual eradication of the bacteria (99.99%).