Antibacterial activity of phytogenic substances against swine pathogens

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Introduction

Infections in swine with pathogenic bacteria such as *Escherichia coli* can have severe consequences like diarrhea in weaners which subsequently can lead to high mortality. Furthermore, infections with salmonellae, streptococci and clostridia affect the performance of sows, piglets and weaners. Phytogenic substances are promising to counteract these pathogenic bacteria and serve as potential alternative to antibiotics.

Experimental

Nine plant derived substances were tested *in vitro* against four swine pathogenic bacteria. In a broth microdilution assay, conducted in 96-well microplates, bacterial cultures with defined microbial count were incubated together with different concentrations of the test substances (15.6 – 2500 mg/l) for 24 h. The change in optical density (Δ 620 nm) was used to determine bacterial inhibition, compared to the growth control. The MIC_{50} value was defined as the lowest concentration of test substance that inhibited at least 50 % of bacterial growth.

Bacterial strains	Cultivation medium
Escherichia coli F4	Tryptone soy broth
Salmonella typhimurium	Nutrient broth
Streptococcus suis	Brain heart infusion broth
Clostridium perfringens toxin type C	Reinforced clostridial medium

The extracts were prepared using 70 % ethanol (v/v). The plant material was chopped and extraction solvent was added in a ratio 1:5. The extraction process via shaking was conducted at room temperature for 24 h. Afterwards, the ethanol was evaporated and the extract was lyophilized. For the application in the broth microdilution assay, ethanolic stock solutions of all test substances were prepared including sterile filtration (0.2 µm).

Table 1: Test substances

	Type	Component
Thymol	Pure substance	-
Totarol	Pure substance	-
Curcuma longa	Oleoresin	Curcumin
Piper nigrum	Oleoresin	Piperin
Capsicum annum	Oleoresin	Capsaicin
Berberis aristata	Ethanolic extract - root	Berberin
Sophora flavescens	Ethanolic extract - root	Matrine
Holarrhena pubescens	Ethanolic extract - bark	Conessin
Bridelia ferruginea	Ethanolic extract - bark	Quercetin, Myricetin

Results

Table 2: MIC_{50} values of test substances against four swine pathogens.

	MIC_{50} [mg/l]			
	E. coli F4	*S. typhimurium*	*S. suis*	*C. perfringens*
Thymol	125-625	313	625	313-1250
Totarol	>250	>250	15,6	20-39
Curcuma longa	313-625	156-625	78	313->625
Piper nigrum	625	156	156	313
Capsicum annum	625	625	78	625
Berberis aristata	2500	625-2500	625	78-625
Sophora flavescens	>2500	>2500	313	313-625
Holarrhena pubescens	>2500	313	1250->2500	2500->2500
Bridelia ferruginea	>2500	1250	313-625	625-1250

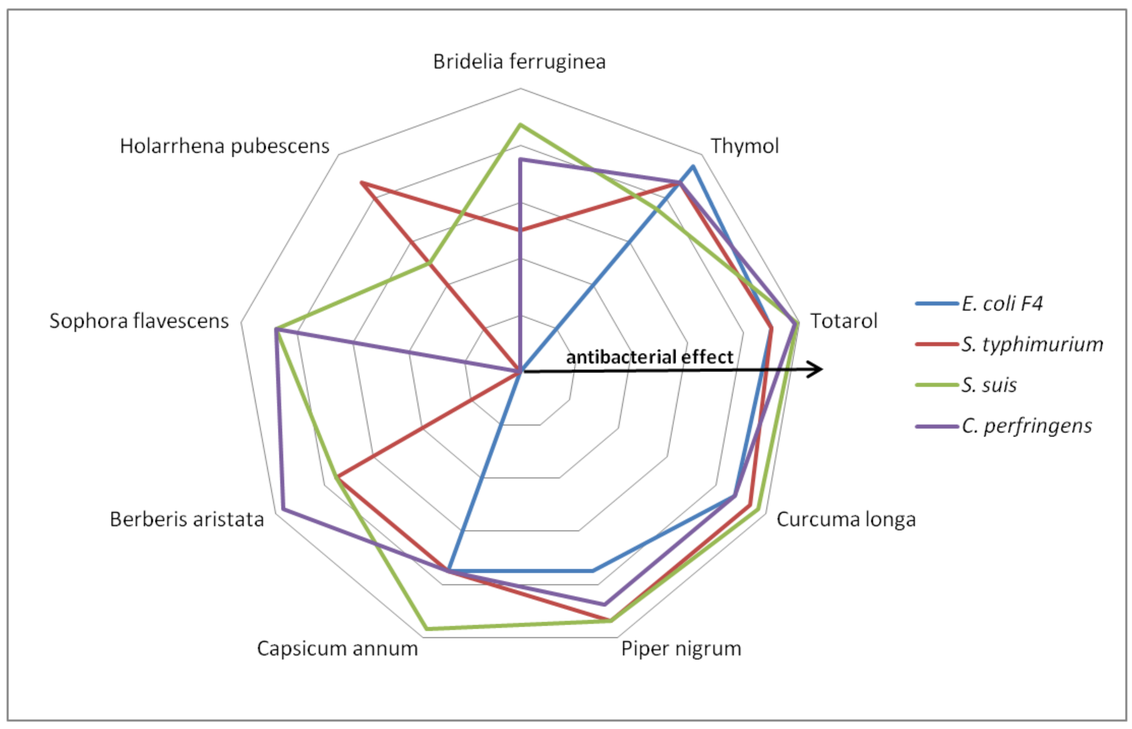

Figure 1: Antibacterial effect of the test substances

The lowest MIC_{50} values were reached for totarol against *S. suis* (15.6 mg/l) and *C. perfringens* (20 - 39 mg/l) as well as the two oleoresins of *Curcuma longa* and *Capsicum annum* against *S. suis* (each 78 mg/l). Additionally, totarol and *Curcuma longa* generally inhibited all four bacterial strains most effective.

The antibacterial effect of *Holarrhena pubescens* only appeared against *S. typhimurium* with a MIC_{50} of 313 mg/l.

Thymol and the ethanolic extract of *Berberis aristata* showed moderate inhibition of all four strains (MIC_{50} 313 - 1250 mg/l and 78 - 2500 mg/l, respectively).

Comparing the four strains, *S. suis* was the most sensitive whereas *E. coli* F4 showed the highest MIC_{50} values.

Discussion

Antibacterial effects of the test substances turned out to be strain specific (see figure 1). Each substance, and particularly the ethanolic extract of *Sophora flavescens,* showed differences between Gram positive and Gram negative bacterial strains. Both Gram negative bacteria, *E. coli* F4 and *S. typhimurium*, seemed to be generally more resistant. As Chao *et al.,* amongst others, concluded the same effects for essential oils, the impact of different cell wall components on the susceptibility to antibacterial substances was again confirmed.

Although four of the tested substances were crude extracts and not purified, low MIC_{50} values were achieved. Hence, the antibacterial potential of their active components could even be higher than determined with this assay, so that further investigations would be required.

Conclusion

In summary, several tested phytogenic substances showed high antibacterial activity and could therefore be used to prevent infections with swine pathogenic bacteria. Regarding the difference between bacterial species which cause infections, phytogenic substances should be chosen appropriately to prevent infections.

Reference Chao SC, Young DG & Oberg CJ (2000). Screening for inhibitory activity of essential oils on selected bacteria, fungi and viruses. *Journal of Essential Oil Research* **12**, 639-649.

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