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Original Research

Minimum Bactericidal Concentration (MBC) of Extract from *Pouzolzia Zeylanica* against Respiratory Bacteria from Pigs and Chickens

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Abstract

This study aimed to determine the minimum bactericidal concentration (MBC) of the aqueous extract from Pouzolzia zeylanica to 20 bacteria isolates from the respiratory tract of swine and chicken including Pasteurella multocida, Bordetella bronchiseptica, Actinobacillus pleuropneumoniae, Ornithobacterium rhinotracheale (ORT) by agar dilution method. Results showed that that the minimum bactericidal concentration (MBC) for A. pleuropneumoniae, B. bronchiseptica, P. multocida and ORT is 250 - 500 mg/ml, 250 - 500 mg/ml; 500 - 1000 mg/ml; and 500 - 1000 mg/ml, respectively. Thus, the aqueous extract of Pouzolzia zeylanica has the best effect on killing A. pleuropneumoniae, B. bronchiseptica and killing P. multocida and Ornithobacterium rhinotracheale at higher concentrations. The MBC values of control antibiotics including gentamicin were 4 - 8 μg/ml; 8 - 32 μg/ml; 0.25 - 0.5 μg/ml; 8 – 16 μg/ml for the above 4 bacteria, respectively. MBC of tetracycline is 16 - 32 μg/ml, 64 - 128 μg/ml; 64 - 128 μg/ml; 16 – 64 μg/ml for A. pleuropneumoniae, B. bronchiseptica, P. multocida, and Ornithobacterium rhinotracheale, respectively. The aqueous extract from Pouzolzia zeylanica can also kill resistant isolates of these four mentioned bacteria species.

Keywords: *Actinobacillus pleuropneumoniae, Bordetella bronchiseptica,* MBC, *Ornithobacterium rhinotracheale Pasteurella multocida, Pouzolzia zeylanica*

Introduction

According to the World Health Organization (WHO), Vietnam is in the group of countries with the highest rate of antibiotic resistance in the world. One of the causes leading to high bacterial resistance is the inappropriate and overuse of antibiotics in animal husbandry. A recent study shows that the amount of antibiotics used for humans (261.7 mg/kg) and animals (247.3 mg/kg) in Vietnam is much higher than those figures in Europe (122 mg/kg and 151.5 mg/kg, respectively) (Carrique-Mas *et al*., 2020). The situation of antibiotic resistance is increasing rapidly at an alarming rate, which poses an urgent situation to find alternative solutions. The Vietnamese government has implemented several regulations such as National Action Plan to reduce antibiotic use such as the Animal Husbandry Law (2018) to ban antibiotics as growth promoters in animal feed and The Circular 12/2020/TT0BNNPTNT on Management Veterinary Medicines (MARD, 2021) to restrict the use of antibiotic classes in disease prevention. Therefore, scientists and industries must contribute to the process of education and providing alternative measures for farmers to maintain animal health as well as to prevent infections in the new situation. Using herbal medicine to protect animal health is one of the solutions.

Herbs and spices act as antibacterial agents by altering the properties of cell membranes, and causing ion leakage, thereby making bacteria less virulent. Changes in the fatty acid composition may affect microbial viability *(Saha et al.*, 2012). Study and commercialization the products from plants, leaves, by-products of cultivation and processing that will contribute to increased income for farmers, maintain sustainable agriculture, and create organic products, safe food for domestic consumption as well as an export (Mayer *et al.*, 2014)

Pouzolzia zeylanica (L.) Benn. belongs *Urticaceae* family. It has the English name of graceful pouzolzbush and the Vietnamese name of bọ mắm (or thuốc dòi). This herb is extensively grown in Vietnam as well as in Asia. In Vietnam, the plant has been used in traditional remedies to treat respiratory diseases such as cough, sore throat, and other diseases such as dysentery, and dysuria. However, there is limited information on using this plant for animals. Several studies have been published on antibacterial properties of the extract from *Pouzolzia zeylanica* to *Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Shigella dysentariae, Salmonella typhi* (Saha *et al.*, 2012). To our knowledge, there is no data on the bactericidal ability of *Pouzolzia zeylanica* against respiratory bacteria from animals.

Pasteurella multocida, Bordetella bronchiseptica, Actinobacillus pleuropneumoniae are common bacterial agents causing respiratory disease in pigs (van Alstine, 2012). *Pasteurella multocida, Bordetella bronchiseptica* can cause rhinitis and tracheitis. *Actinobacillus pleuropneumoniae* infection can lead to fibrinous bronchopneumonia in swine. Meanwhile, *Ornithobacterium rhinotracheale* (ORT) was considered a primary pathogen in broilers (van Veen *et al.*, 2000).

This study aimed to determine the minimum bactericidal concentration (MBC) of the aqueous extract from *Pouzolzia zeylanica* to bacteria isolates from the respiratory tract of animals including *Pasteurella multocida, Bordetella bronchiseptica, Actinobacillus pleuropneumoniae* isolated from pigs, and *Ornithobacterium rhinotracheale* isolated from chickens by agar dilution assay. The results of the study will contribute to the scientific basis of using herbal extract as an antibiotic alternative for the treatment of respiratory diseases in pigs and chickens with conventinal antibiotics.

Materials and Methods

Preparation of Bacterial Cultures

Isolates of bacteria from previous studies (Nguyen *et al*, 2014; Dang et al, 2019 and Ly *et al*, 2019) were recovered from the -70 Celsius degree freezer using blood agar media. From 3 - 4 fresh colonies, a bacterial concentration of about 10^8 CFU/ml was made by measuring OD at 625 nm which is equivalent to Mc. Farland 0.5 (Wiegand *et al.*, 2008) using sterile saline to dilute the concentration of bacteria to 10^4 CFU/ml (CLSI, 2015).

Extract and Antibiotic Preparation

Crude materials of *Pouzolzia zeylanica* plants were purchased, washed, dried, and cooked according to the method

of extraction in water with heat (2-layer boiler machine). The extracts from three times were collected in a circulating concentrator to evaporate the water until the moisture content is 20% (as described in Pharmacopoeia). From 2.2 kg of dried herbs, 100g of high-density extract as obtained. From the extract, a series of solutions (two-fold) were prepared from 1000 mg/ml to 1.953 mg/ml. According to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) 2015, MHA was used for susceptibility testing. Each petri dish will contain 12 ml of MHA, 0.6 ml of sheep blood (5%), and 1.4 ml of extract solution which was prepared as described previously.

Tetracycline and gentamicin were prepared as a series of solutions from 128 µg/ml to 0,281 µg/ml. In addition, control plates (C1, C2) do not contain any antibiotic or the extract. Plate C1 included bacterial culture to ensure bacteria growth. Plate C2 had no bacteria to ensure the sterilization of the medium used.

Determining MBC

The MBC value is defined as the minimum concentration that can kill 99.9% of bacteria of the substance with antibacterial activity (Xiaoxi, 2011). After preparation of the plates with antibacterial substances (antibiotics or extracts), we have used a cotton swab to spread each bacterial suspension on the surface of the agar except the negative control. The plates were incubated at 37^0C for 24 hours. The results were read with naked eye under natural light, the MBC value was determined to be the lowest concentration of the agar plate at which bacterial growth was absent and no bacteria grow after inoculated in the broth media. Each bacterial isolate was performed in triplicate.

Results and Discussion

Results shown in Table 1 indicated that aqueous extract from *Pouzolzia zeylanica* has bactericidal effect against *A. pleuropneumoniae, P. multocida, B. bronchiseptica* isolated from pigs and *O. rhinotracheale* from chickens. The extract had the maximum bactericidal effect against *A. pleuropneumoniae, B. bronchiseptica* and least against *P. multocida, O. rhinotracheale*. Reviewing from previous studies, we reported that this is the first study of the antibacterial effects of *Pouzolzia zeylanica* aqueous extract against these four bacteria species.

Table 1: Minimum Bactericidal Concentration (MBC) of aqueous extract from *Pouzolzia zeylanica* and antibiotics against bacteria from respiratory tract of animals

Isolates - ID		Minimal Bactericidal Concentration (MBC)		
		Extract *P. z** (mg/ml)	Gentamicin (µg/ml)	Tetracycline (µg/ml)
Actinobacillus pleuropneumoniae	A1	250	4	16
	A2	250	8	32
	A3	500	8	32
	A4	500	8	16
	A5	250	4	16
	A6	500	8	32
Pasteurella multocida	P1	1000	0.5	64
	P2	500	0.25	>128
	P3	1000	0.25	>128
	P4	1000	0.5	128
	P5	500	0.25	>128
Bordetella bronchiseptica	B1	500	32	64
	B2	500	16	64
	B3	250	8	128
	B4	500	16	128
	B5	250	32	64
Ornithobacterium rhinotracheale	O1	1000	16	64
	O2	1000	16	32
	O3	500	8	16
	O4	500	16	16

***** *P.z, Pouzolzia zeylanica*

The resistant breakpoints of tetracycline to *A. pleuropneumoniae*, and *P. multocida*, were ≥ 2 µg/ml, and ≥ 2 µg/ml, respectively and for gentamicin, they were ≥ 8 µg/ml and ≥ 4 µg/ml, respectively. Therefore, *A. pleuropneumoniae* isolates in this study were sensitive to gentamicin but all resistant to tetracycline. Three out of five isolates of *P. multocida* were also resistant to tetracycline. The extract can kill tetracycline-resistant *A. pleuropneumoniae* isolates (A1-A6) at 250 mg/ml to 500mg/ml and tetracycline-resistant *P. multocida* isolates at higher concentration (P2, P3, P5). *B. bronchiseptica* isolates in this study can be considered resistant strains (Kadlec and Schwarz, 2018). It should be noticed that the antibiotic susceptibility of certain bacteria species may differ from the geographic aspects. The sensitivity test with *B. bronchiseptica* isolates in Germany, showed that the bacteria were sensitive to amoxicillin/clavulanic acid, ciprofloxacin, chloramphenicol, colistin, doxycycline, florfenicol, and gentamicin but was resistant to penicillin and cephalosporins (Prüller *et al*., 2015).

The previous study reported that the ethanol extract of *Pouzolzia zeylanica* at 1000 mg/ml using the cup plate method had antibacterial activity against *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Bacillus megaterium, Pseudomonas aeruginosa, Shigella dysentariae, Salmonella typhi* (Saha *et al.*, 2012). The minimum inhibitory concentration of the ethanol extract from *Terminalia catappa* against *B. bronchiseptica* was reported at 10,000 µg/ml, previously (Salares and Balala, 2018). Another study in Pakistan proved the inhibitory activity of *Pinus roxburghi, Centorea calcitropa, Mentha pipereta, Skimmia laureola, Fagonia cretica, Mallotus philippensis, Coleobrookea oppositifolia, Phyllanthus emblica, Datura innoxia, Grewia asiatica and Ficus macrocarpa* against *B. bronchiseptica* (Akhtar and Mirza, 2018). Little data relating to herbal extract against *O. rhinotracheale* (Erfan and Marouf, 2019). However, these authors reported that *O. rhinotracheale* isolates were able to grow in all cinnamon oil dilutions. Our study provides the first information on MBC of *Pouzolzia zeylanica* to *O. rhinotracheale* isolated from chicken.

Conclusion

The aqueous extract of *Pouzolzia zeylanica* has the best bactericidal effect on *A. pleuropneumoniae*, *B. bronchiseptica* and on *P. multocida* and *O. rhinotracheale* at higher concentrations. The extract can kill resistant strains of these four bacterial species from pigs and chicken. Using *Pouzolzia zeylanica* extract as antibiotic alternatives for pig and chicken production should be investigated further in practice.

Conflict of Interests

There is no conflict of interest.

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References

1. Akhtar, N., Mirza, B. (2018). Phytochemical analysis and comprehensive evaluation of antimicrobial and antioxidant properties of 61 medicinal plant species. *Arabian journal of chemistry* 11, 1223–1235.
2. Carrique-Mas, J.J., Choisy, M., Van Cuong, N., Thwaites, G., Baker, S. (2020). An estimation of total antimicrobial usage in humans and animals in Vietnam. *Antimicrobial Resistance & Infection Control* 9, 1–6.
3. Erfan, A.M., Marouf, S. (2019). Cinnamon oil downregulates virulence genes of poultry respiratory bacterial agents and revealed significant bacterial inhibition: An in vitro perspective. *Veterinary World* 12, 1707.
4. Kadlec, K., Schwarz, S. (2018). Antimicrobial resistance in *Bordetella bronchiseptica*. *Microbiol Spectr* 6. https://doi.org/10.1128/microbiolspec.ARBA-0024-2017
5. Mayer, M., Vogl, C.R., Amorena, M., Hamburger, M., Walkenhorst, M. (2014). Treatment of organic livestock with medicinal plants: a systematic review of European ethnoveterinary research. C*omplementary Medicine Research* 21, 375–386.
6. Prüller, S., Rensch, U., Meemken, D., Kaspar, H., Kopp, P.A., Klein, G., Kehrenberg, C. (2015). Antimicrobial susceptibility of *Bordetella bronchiseptica* isolates from swine and companion animals and detection of resistance genes. P*loS one* 10, e0135703.
7. Saha, D., Paul, S., Chowdhury, S. (2012). Antibacterial activity of ethanol extract of *Pouzolzia zeylanica* (L.)

Benn. *International Journal of Pharmaceutical Innovations* 2, 1–5.
8. Salares, E.F., Balala, L. (2018). Phytochemical screening and antimicrobial activity of T*erminalia catappa* L. leaf extract against potential pathogens of animals. J*ournal of Science, Engineering and Technology* 6, 15–26.
9. van Veen, L., van Empel, P., Fabri, T. (200)0. *Ornithobacterium rhinotracheale*, a Primary Pathogen in Broilers. *Avian Diseases* 44, 896–900. https://doi.org/10.2307/1593063
10. Van Alstine W. (2012). Respiratory system. In D*iseases of swine*, edited by Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez, Kent J. Schwartz, Gregory W. Stevenson. Wiley-Blackwell. P349-358
11. Vietnamese Ministry of Agriculture and Rural Development. (2021). Circular No. 12/2020/TT0BNNPTNT. *Regulating the management of veterinary drugs containing narcotics, precursors and veterinary drugs*.
12. Wiegand, I., Hilpert, K., Hancock, R.E. (2008). Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nature protocols* 3, 163–175.
13. Xiaoxi, C. (2011). Determination of the minimum inhibitory concentrations (MICs) and the minimum bactericidal concentrations (MBCs) for a novel antibiotic. *African Journal of Microbiology Research* 5, 572–575.

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