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THE EFFECT OF GARLIC (ALLIUM SATIVUM L.) BULBS AND GINGER (ZINGIBER OFFICINALE) RHIZOMES EXTRACTS ON ESCHERICHIA COLI AND SALMONELLA SPP ISOLATED FROM DICK DIARRHEA SYNDROME

Nguyen Van Thanh[*1], Nguyen Thanh Hai[2], Bui Thi Tho[3], Miyamoto Atsushi[4]

[*1]Department of Veterinary Surgery and Reproduction, Faculty of Veterinary Medicine, Vietnam National University of Agriculture, Trau Quy crossing, Gia Lam district, Hanoi, Vietnam.
[2]Department of Plant Bio-technology, Faculty of Biotechnology, Vietnam National University of Agriculture, Trau Quy crossing, Gia Lam district, Hanoi, Vietnam.
[3]Department of Veterinary internal medicine and pharmacology, Faculty of Veterinary Medicine, Vietnam National University of Agriculture, Trau Quy crossing, Gia Lam district, Hanoi, Vietnam.
[4]Department of Veterinary Pharmacology, Joint Faculty of Veterinary Medicine, Kagoshima University, 1-21-24 Korimoto, Kagoshima 890-0065, Japan.
[*1]ThanhHaiKagoshimaJapan@yahoo.com , [2]nthaicnsh@vnua.edu.vn , [3]bttho@vnua.edu.vn , [4]k1330977@kadai.jp

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Abstract

The odor inhibition and diffusion inhibition properties of garlic (*Allium sativum* L.) bulbs and ginger (*Zingiber officinale*) rhizomes were investigated with multi-antibiotic *Salmonella*. spp and *Escherichia coli* (*E. coli*) isolated from duck diarrhea. The results showed that both of the 2 plants had antibacterial ability on tested *Salmonella*. spp and *E. coli*. Among 3 tested solvents, distilled water, 5 % ethanol and 5 % acid acetic, acid acetic showed the best efficacy. Garlic had stronger antibacterial effect than ginger in both of odor inhibition and diffusion inhibition tests, suggesting that garlic have more potential and therefore should be recommended for the application in duck diarrhea treatment.

INTRODUCTION

The diarrhea syndrome of duck has been known as one of the diseases which cause much lost in duck husbandry in Vietnam (Kim Ngoc Hung, 2012). The long-term reliance on a small range of antibiotics led to the development of drug resistance and this increment in resistance has made the treatment of duck diarrhea become much more difficult recently (Nguyen The Hung, 2013). In addition, the increased public awareness for synthesized drug residues in consumed products which possessed potential to hazard human also enforces the search for alternatives therapy, and medicine plants have been considered as one of the most potential candidates to alternate synthesized drugs (Nguyen Thanh Hai et al., 2014). This therapy is also known to possess many advantageous features, such as easily degraded in natural environments, do not residue in animal products, less side-effect with animals and lower costs (Nguyen Thanh Hai et al., 2014; Nguyen Van Thanh et al., 2014; Nguyen Thanh Hai et al., 2013). *E. coli* and *Salmonella.* spp played a key role in the diarrhea syndrome of duck in Vietnam (Kim Ngoc Hung, 2012). The significant increment in antibiotic resistance of pathogen *E. coli* and *Salmonella*. spp involved in animal diarrhea has been recognized widely in Vietnam and therefore promote many researches on plants which possesses antibacterial properties to cure diarrhea disease (Nguyen Van Thanh et al., 2014; Nguyen Thanh Hai et al., 2013; Pham Ngoc Thach, 2009; Bui Thi Tho, 1996). However, the search for effective medicine plants to deal with high antibiotic-resistant *Salmonella* spp and *E. coli* in duck diarrhea has not yet performed. Therefore, it is necessary to investigate the effect of herbs on this disease, firstly on the pathogen bacteria. In our study, we chose garlic and ginger, which have long history of application in diarrhea prevention and treatment in Vietnam for the test with multi-antibiotic resistant *Salmonella* spp and *E. coli* isolated from duck diarrhea to evaluate their potential of applying as alternative therapy.

MATERIALS AND METHODS

The bacterial collection: *E.coli* and *salmonella* spp isolated from feces of duck affected with diarrhea was collected in the period from 1987 to 2012 and kept in Laboratory of Veterinary Internal Medicine and Pharmacology Department, Faculty of Veterinary, Vietnam National University of Agriculture, Hanoi, Vietnam. Fourteen *E.coli* and *Salmonella*. spp strains, which have been identified to be resistant to 5 tested antibiotics, including: colistin, amoxillin, oxacillin, streptomycin, gentamycin were selected to test with herb extracts.

The collection of medicine plants: The garlic bulbs and ginger rhizomes were collected in Garden of Traditional Medicine Plants, Department of Veterinary Pharmacology, Faculty of Veterinary Medicine, Vietnam National University of Agriculture, Hanoi, Vietnam. The authentication was performed in laboratory under the supervision of Associate Professor Bui Thi Tho, lecturer in subject of Traditional Medicine Plants, Vietnam National University of Agriculture, Hanoi, Vietnam. The fresh garlic and ginger were washed, peeled and squashed in the ceramic mortar. The crushed materials were then squeezed through 2 layers of gauzes. The obtained juices were used in maximum 2 hours after producing.

The odor antibacterial effect evaluation: the odor antibacterial effect evaluation was performed following the method described by Bui Thi Tho (2012). The crushed juices were spread evenly on the surfaces of experimental Petri dish lids, at the concentration of 100 µl, 200 µl and 300 µl crude extracted juices per dish. These lids were then kept in the Bio-clean Bench from 2 to 4 minutes, until the applied extracts on the lids were dried. The Petri dishes with Mueller-Hinton agar were prepared and then the bacteria at concentration of 10^8 cfu/ ml were smeared on the dished. The bacterial solution used for inoculation was prepared in Mueller-Hinton broth at a density adjusted to a 0.5 McFarland turbidity standard for disc diffusion. These inoculated dishes were then covered by the lids which had been applied with herbal extracts. These dishes were sealed and upside down incubated for 24 hours at 37°C in the ambient condition before taken to observe the bacterial development

The diffusion inhibition antibacterial effect evaluation: the diffusion inhibition antibacterial effect was evaluated through the diameter of inhibition zones induced by the infusion of the extracts on the inoculated agar. The tests were conducted following the Norman Heatley methods with some modification described by Bui Thi Tho (1996) and Nguyen Nhu Vien (1975). Crude juice of garlic and ginger was diluted with distilled water, 5 % ethanol and 5 % acid acetic to make the concentration of 1g/ ml, 0.5 g/ ml and 0.25 g/ ml. Those solvents were chosen because water, diluted wine (ethanol) and vinegar (5 % acid acetic) are usually applied to process traditional medicine plants in Vietnam (Bui Thi Tho et al., 2009). Petri Dishes are poured to a depth of 5mm with Mueller-Hinton agar and then the bacteria at concentration of 10^8 cfu/ ml were smeared on the dished. The glass cylinders with the 5.0 ± 0.2 mm internal diameter were then carefully placed the on the surface of the agar. 0.1 ml of extracts at different concentrations was pour into cylinders by the syringes. The controls were made by alternate the extracts with correspondent solvents, including distilled water, 5 % ethanol and 5 % acid acetic. After 24 hours of incubation, the dishes were taken out to measure the diameters of inhibition zones around the cylinders. The antibacterial effect of the extracts was evaluated through the diameter of inhibition zones following the Vietnamese standard for medicine plants extracts (Bui Thi Tho, 2012), in which the efficacy was determined as followed:

Good efficacy: the diameter >= 30mm
Intermediate efficacy: 15 <= diameter < 30mm
Weak efficacy: < 15mm

RESULTS

The odor antibacterial effect evaluation

The odor antibacterial effect of garlic and ginger extracts is shown in Table 1

Table 1. The odor inhibition effect of garlic and ginger extracted juice on tested Salmonella spp and E. coli

	Garlic			Ginger		
	100 µl /dish	200 µl /dish	300 µl /dish	100 µl /dish	200 µl /dish	300 µl /dish
Salmonella. spp	0	5*	9*#O	0	1	1
E. coli	0	7*	13*#O	0	2	5*

* indicates the case in which the number of inhibited dishes in concentration of 300 µl /dish is significantly higher than that of 100 µl /dish of the same extract ($p < 0.05$) by Chi-square test. # indicates the case in which the number of inhibited dishes in concentration of 300 µl /dish is significantly higher than that of 200 µl /dish of the same

extract ($p < 0.05$) by Chi-square test. ^{O} indicates the case in which the number of inhibited dishes of garlic is significantly higher than that of ginger at the same concentration ($p < 0.05$) by Chi-square test.

From Table 1, we see that both garlic and ginger have odor antibacterial effect in the dose-dependent manner. At 100 µl /dish, both of these extracts had no inhibition efficacy. Garlic at 300 µl /dish inhibited the development of 13/ 14 *E. coli* strains, which is significantly stronger than the effect of 200 µl /dish (inhibited 7/ 14 strains). Similarly, 300 µl /dish of garlic inhibited 9/ 14 strains of *Salmonella*. spp, significantly higher than that of 200 µl /dish (inhibited 5/ 14 strains). In case of ginger extract, 300 µl /dish inhibited the development of 5/ 14 *E. coli* strains and 1/ 14 *Salmonella*. spp, which is significantly stronger than the effect of 100 µl /dish (inhibited 0/ 14 strains *E. coli* and 0/ 14 strains of *Salmonella*. spp). However, the significance was not observed between the concentrations of 300 µl /dish and 200 µl /dish of ginger extract. In addition, at the 300 µl /dish, garlic extract had significantly stronger effect than ginger extract, to both of *E. coli* (13/ 14 strains vs. 5/ 14 strains) and *Salmonella*. spp (9/ 14 strains vs. 1/ 14 strains).

The diffusion inhibition antibacterial effect evaluation:

The diffusion inhibition antibacterial effect evaluation with Salmonella. spp:

The results on inhibition zone diameters of extracts with *Salmonella.* spp are shown in Table 2.

Table 2. The inhibition zone diameters of garlic and ginger extracts with Salmonella. spp

Conc	Garlic			Ginger		
	Distilled water	5 % ethanol	5 % Acid acetic	Distilled water	5 % Alcohol	5 % Acid acetic
0.25 g/ ml	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
0.5 g/ ml	$13.31^{bC} \pm 4.34$	$16.43^{abB} \pm 7.34$	**$20.38^{bA*} \pm 3.75$**	$11.22^{bA} \pm 2.10$	$12.25^{bA} \pm 3.01$	$14.35^{bA} \pm 4.28$
1 g/ ml	$19.70^{aB} \pm 5.02$	$20.37^{aAB} \pm 4.20$	**$33.53^{aA*} \pm 5.96$**	$17.09^{aA} \pm 3.92$	$17.33^{aA} \pm 5.34$	$19.43^{aA} \pm 5.34$

$^{a, b,c}$ Values with different superscripts on the same column are significantly different ($p < 0.05$) by One way ANOVA followed with Bonferroni *post-hoc* test . $^{A, B, C}$ Values with different superscripts on the same rows of each plant extract are significantly different ($p < 0.05$) by One way ANOVA followed with Bonferroni *post-hoc* test. * and bold letter indicates the case in which garlic extract has significantly higher inhibition effect than that of ginger extract .

From Table 2, we observed that both garlic and ginger extracts had dose-dependent antibacterial effect to *Salmonella*. spp, showing by significantly higher inhibition along with the increment of concentration in all of 3 tested solvent, including distilled water, 5 % ethanol and 5 % acid acetic. 5% Acid acetic solvent extract of garlic showed significantly higher effect than 5 % ethanol and distilled water extracts at 0.5 g/ ml (20.38± 3.75 vs 16.43 ± 7.34 and 13.31 ± 4.34, respectively). 5 % ethanol garlic extracts showed significantly larger inhibition zones at 0.5 g/ ml and 1.0 g/ ml (16.43 ± 7.34 vs. 13.31 ± 4.34 and 20.37 ± 4.20 vs. 19.70 ± 5.02, respectively). However, the different effect between extracted solvents was not observed in case of ginger. The good inhibition effect (inhibition zone diameter >= 30mm) to *Salmonella.* spp was only detected in 1g/ ml of 5 % acid acetic garlic extract. In our study, the garlic showed significantly stronger effect than ginger extract in the case of 5 % acid acetic solvent at 0.5 g/ ml and 1.0 g/ ml (20.38 ± 3.75 vs. 14.35 ± 4.28 and 33.53 ± 5.96 vs. 19.43 ± 5.34, respectively)

The diffusion inhibition antibacterial effect evaluation with E. coli:

The results on inhibition zone diameters of extracts with *E. coli* are shown in Table 3.

Table 3. The inhibition zone diameters of garlic and ginger extracts with E. Coli

Conc	Garlic			Ginger		
	Distilled water	5 % ethanol	5 % Acid acetic	Distilled water	5 % Alcohol	5 % Acid acetic
0.25 g/ ml	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
0.5 g/ ml	$11.83^{bB} \pm 5.23$	$12.69^{abAB} \pm 3.48$	$16.77^{bA} \pm 2.55$	$10.62^{bA} \pm 3.90$	$10.75^{bA} \pm 1.81$	$11.65^{bA} \pm 5.98$
1 g/ ml	$16.55^{aB} \pm 5.32$	$18.78^{aAB} \pm 2.34$	**$23.93^{aA*} \pm 7.56$**	$12.79^{aA} \pm 4.98$	$12.93^{aA} \pm 4.94$	$16.95^{aA} \pm 3.64$

$^{a, b,c}$ Values with different superscripts on the same column are significantly different ($p < 0.05$) by One way ANOVA followed with Bonferroni *post-hoc* test . $^{A, B, C}$ Values with different superscripts on the same rows of each plant extract are significantly different ($p < 0.05$) by One way ANOVA followed with Bonferroni *post-hoc* test. * and bold letter indicates the case in which garlic extract has significantly higher inhibition effect than that of ginger extract.

From Table 3, we observed that both garlic and ginger extracts had dose-dependent antibacterial effect to *E. coli*, showing by significantly higher inhibition along with the increment of concentration in all of 3 tested solvent, including distilled water, 5 % alcohol and 5 % acid acetic. 5% Acid acetic solvent extract of garlic showed significantly higher effect than distilled water extracts at 0.5 g/ ml and 1.0 g/ ml(16.77± 2.55 vs. 11.83 ± 5.23 and 23.93± 7.56 vs. 16.55 ± 5.32, respectively). However, the different effect between extracted solvents was not observed in case of ginger. There is no good inhibition effect (inhibition zone diameter >= 30mm) detected in test with *E.coli*. In our study, the garlic showed significantly stronger effect than ginger extract in the case of 5 % acid acetic solvent at 1.0 g/ ml (23.93 ± 7.56 vs. 16.95 ± 3.64)

DISCUSSION

Our study is the first study which investigated and proved the effect of garlic and ginger on multi-antibiotic resistant *Salmonella.* spp and *E.coli* isolated from clinical diarrhea duck, and therefore propose those plants as the therapy for the treatment of duck diarrhea in Vietnam. The antibacterial properties of garlic and ginger have been known for a long time (Hiba Ali Hasan et al., 2013; A. Sebiomo et al., 2011; Miri Park et al., 2008; K. I. Auta et al., 2001; Serge Ankri et al., 1999; Bui Thi Tho, 1996). In our study, garlic and ginger possessed inhibition effects on multi-antibiotics bacteria, and proved the advantage of herb application when the antibiotic treatment is ineffective due to the resistance. The results are similar with several previous results. Various bacterial strains resistant to antibiotics were all found to be sensitive to medicine plants compounds (Nguyen Van Thanh et al., 2014; Nguyen Thanh Hai et al., 2011; Serge Ankri et al., 1999; Bui Thi Tho, 1996). In addition, the antibacterial activity of plant compounds is the apparent inability of most bacteria to develop resistance to it because the mode of action is completely different from that of other antibiotic substances (Serge Ankri et al., 1999; Bui Thi Tho, 1996; Gupta K.C. et al., 1955). Our results further emphasized the advantages of garlic and ginger usage as antibiotic replacement in duck diarrhea treatment. In addition, in our study, garlic was found to have significantly *in vitro* stronger effect than ginger, suggested that garlic might be the better candidate. However, the *in vivo* and clinical experiments are still required to strengthen the advantage of garlic.

The inhibition effect of the extracts with 5 % acid acetic was significantly higher than distilled water and 5 % ethanol for both of garlic and ginger, therefore recommended the application of this solvents in extraction of those herbs for antibacterial purpose. The dependence of inhibition effect of medicine plants compounds on the extracted solvents was usually reported (Nguyen Thanh Hai et al., 2014; Nguyen Van Thanh et al., 2014; Hiba Ali Hasan et al., 2013). In our study, the mechanisms by which acid acetic induced higher inhibition effect was not determined, and the follow-up research is therefore necessary to investigate this point.

CONCLUSION

Both garlic and ginger are able to apply as the replacement for antibiotic in duck diarrhea, especially the disease caused by multi-antibiotic resistant *E. coli* and *Salmonella.* spp, in which 5% acid acetic extract of garlic is the best candidate.

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