

## THE EFFECT OF GARLIC (*ALLIUM SATIVUM* L.) BULBS AND PEPPER (*PIPER NIGRUM* L.) SEEDS ETHANOL EXTRACTS ON PIGLET DIARRHEA

Nguyen Van Thanh<sup>\*1</sup>, Nguyen Thanh Hai<sup>2</sup>, Dao Thi Huong<sup>3</sup> and Atsushi Miyamoto<sup>4</sup>

<sup>1</sup>Department of Veterinary Surgery and Reproduction, Faculty of Veterinary Medicine, Vietnam National University of Agriculture, Trau Quy crossing, Gia Lam district, Hanoi, Vietnam.

<sup>2</sup>Department of Plant Bio-technology, Faculty of Biotechnology, Vietnam National University of Agriculture, Trau Quy crossing, Gia Lam district, Hanoi, Vietnam.

<sup>3</sup>PDS Dog Professional Research Center, Faculty of Veterinary Medicine, Vietnam National University of Agriculture, Trau Quy crossing, Gia Lam district, Hanoi, Vietnam.

<sup>4</sup>Department of Veterinary Pharmacology, Joint Faculty of Veterinary Medicine, Kagoshima University, 1-21-24 Korimoto, Kagoshima 890-0065, Japan.

**Correspondence Author:** Dr. Nguyen Van Thanh, DVM, PhD, Department of Veterinary Surgery and Reproduction, Faculty of Veterinary Medicine, Vietnam National University of Agriculture, Trau Quy crossing, Gia Lam district, Hanoi, Vietnam.

### *Abstract*

#### **Keywords:**

*Garlic, pepper, medicinal plants, piglet diarrhea.*

Ethanol extracts of garlic (*Allium sativum* L.) and pepper (*Piper nigrum* L.) at different concentrations were applied on piglets affected with diarrhea to investigate the *in vivo* treatment effect. The results showed that both of the plants showed significant treatment on this disease. Garlic extract at dose of 2 ml/ kg/ d showed the best efficacy, because it cured 100% of infected piglets and there was no re-infection occurred. In the comparison with a synthetic drug, the garlic extract showed the similar treatment results but was significant lower in the cost spent for each treatment. Because this economic advantage of the medicinal plant is certainly important in the condition of developing countries like Vietnam, we suggested that garlic should be considered as a promissory candidate to alternate antibiotic in the treatment of piglet diarrhea.

## INTRODUCTION

Garlic (*Allium Sativum* L.) and pepper (*Piper nigrum* L.) has a long history of being applied as traditional therapies to treat many digestive disorders, including diarrhea, for both of human and livestock in Vietnam (Do Tat Loi, 2005 a, b). Researchers have also reported the strong *in vitro* antimicrobial activity of garlic (Xiaonan Lu et al., 2011; Ponmurugan Karuppiah et al., 2012; V. Viswanathan et al., 2014) and pepper (Maryam Zarringhalam et al., 2013). Our group has previously demonstrated the advantage of garlic to show the strong *in vitro* inhibition on multi-antibiotic resistant bacteria, including those that were pathogenic and induced diarrhea on animals (Nguyen Van Thanh et al., 2015; Nguyen Thanh Hai and Bui Thi Tho, 2013; Bui Thi Tho, 1996). However, we have not yet investigated the *in vivo* activity of garlic or other high potential medicinal plants on livestock diarrhea. This study therefore aimed to test *in vivo* effect of garlic and pepper by applying them on domestic animals affected with diarrhea. We chose to investigate the effect on the piglet diarrhea, because this

syndrome has been known to cause significant losses for pig husbandry and usually affects about 70% to 90% of piglets raised in Vietnam (Bui Thi Tho, 1996). In addition, the long-term and the over-use of antibiotics to treat this syndrome have led to the development of bacterial resistance with many common antibiotics and therefore made the treatment of this disease become difficult (Bui Thi Tho, 1996). We investigated the *in vivo* effect of garlic and ginger with the view of applying them as alternative therapies to treat the disease in the condition of Vietnamese husbandry, where the prevalence of bacteria with multi-antibiotic resistance is very high (Ha Viet Le et al., 2015; An T. T. Vo et al., 2010).

## MATERIALS AND METHODS

### The collection of medicine plants:

The garlic bulbs and pepper seeds were collected in Garden of Traditional Medicine Plants, Department of Veterinary Pharmacology, Faculty of Veterinary Medicine, Vietnam National University of Agriculture, Hanoi, Vietnam. The identity of plant materials was confirmed by Dr. Tho Thi Bui, lecturer in subject of pharmacology and medicinal plants, Department of Internal Medicine and Pharmacology, Faculty of Veterinary Medicine, Vietnam National University of Agriculture, and based on voucher specimens that have been deposited at Vuon Duoc Lieu Thu Y Herbarium, Vietnam National University of Agriculture in Vietnam. The fresh materials were washed, peeled and dried at 40°C for 7 d in the drying machine, and then squashed into powder by using the ceramic mortars and kitchen blender. The powders were then dried for another in 40°C for 5 h before being sealed in plastic bags and kept in refrigerator for preservation. The process of producing ethanol extract was followed the method described by Andrew Chevallier (1996) with some modification. Briefly, 300 g dried material was stirred with 1 liter of 40% ethanol for 1 h, and then the container was seal and kept for 14 d for infusion. During the steeping time, the container was shaken once a day to homogenize the extraction. After 14 d, the extract was filtered through the 2 layers of gauzes with the aid of juicer to obtain the maximum volume of filtrate. Filtrate was then sealed and used for experiments within the period of 1 week from the collection day.

### The treatment of ethanol extract with piglets affected with diarrhea:

In the first experiment, we compared the treatment effect of different doses of extracts. Piglets from 1 to 21 d age which affected with diarrhea was divided to different groups, each group contained 9 individuals. Piglets in control group received no treatment, piglets in 1 ml-extract group received treatment at dose of 1 ml extract per 1 kg per day and piglets in 2 ml-extract group received treatment at dose of 2 ml extract per kg per day. All extracts were given in the morning at the same time (7 a.m). After 5 d of treatment if the piglets were not cured from the diarrhea, the treatment was recorded as ineffective. In the 2<sup>nd</sup> experiment, we evaluated the effect of good extract with a larger number of piglets and in comparison with one common commercial drug that are usually used to treat piglet diarrhea in Vietnam: Flocidin (component: enrofloxacin, Intervet Company, Ho Chi Minh city, Vietnam). The purpose is to evaluate the potential of medicinal plants to alternate antibiotics in the treatment of this disease.

## RESULTS

### The treatment effects of different doses of garlic and pepper ethanol extracts:

The treatment effects of different doses of garlic and pepper ethanol extracts are shown in Table 1. From Table 1, we see that both garlic and pepper showed significant treatment effect on piglet diarrhea, shown by significantly higher number in piglets cured from the disease when comparing with the control group. We observed that garlic at the dose of 2 ml/ kg/ d showed the best effect, because only this treatment resulted in 100 % of piglets cured from diseases and no re-infection. It also showed the remarkable short necessary treatment

time:  $2.13 \pm 0.05$  d. Lower dose of garlic ethanol extract (1ml/ kg/ d) resulted in only 88.9% of piglets cured from the disease, and of which 12.5% was re-infected. None of treatment with pepper ethanol extracts showed 100% of disease clearance, and the re-affected also occurred in both of tested doses. In addition, the treatment with pepper ethanol extracts required significant longer treatment time than the treatment with garlic ethanol extract.

**Table 1: The treatment effects of different doses of garlic and pepper ethanol extracts**

Group	Dose (ml/ kg/ d)	Piglet Number	Treatment results					Re-infected	
			Piglet cured from diarrhea (Number)	(%)	Time of necessary treatment (d)	Ineffective treatment (Number)	(%)	Piglet (Number)	(%)
Control	0	9	0	0		9	100		
Garlic	1	9	8 <sup>***</sup>	88.9	$2.60 \pm 0.06^b$	1 <sup>***</sup>	11.1	2	12.5
	2	9	9 <sup>***</sup>	100	<b><math>2.13 \pm 0.05^a</math></b>	0 <sup>***</sup>	0	0	0
Pepper	1	9	7 <sup>***</sup>	77.8	$2.83 \pm 0.06^b$	2 <sup>***</sup>	22.2	2	28.8
	2	9	8 <sup>***</sup>	88.9	$2.50 \pm 0.08^b$	1 <sup>***</sup>	11.1	1	12.5

The maximum time for treatment was 5 d, and if the piglets were not cured from diarrhea, the treatments were recorded as ineffective. \*\*\* indicates that the number of piglets cured from diarrhea in the treatment group was significantly higher than that number in the control group ( $p < 0.001$ ) by Chi-square test. † † † indicates that the number of piglets still affected with diarrhea after 5 d of treatment was significantly lower than that number in the control group ( $p < 0.001$ ) by Chi-square test. Time of necessary treatment (d) was expressed as Mean  $\pm$  S.E.M. Means with different superscripts in this column are significantly different ( $p < 0.05$ ) by one-way ANOVA followed by Bonferroni post hoc analysis, and number with bold letter indicates the shortest value of the column. The piglets after being cured from the diseases were continuously observed for 10 d, and if any piglets were again showed the symptoms of diarrhea, they were recorded as re-infected.

#### The treatment of garlic ethanol extract in the comparison with synthesized drug:

Because treatment of garlic ethanol extract at the dose of 2ml/ kg/ d showed the best effect among all of treatments by extracts, we selected this extract at this dose to investigate with a larger number of piglets and in the comparison with a common commercial drug (Flocidin, Intervet Company). The results showed that this extract showed similar results with Flocidin in all of parameters indicated treatment effect, including of number/ percentage of piglet cured from diarrhea, time of necessary treatment and re-infected number/ percentage. However, the economic cost when using garlic was significant lower than that when using Flocidin ( $500 \pm 10$  Vietnam dong/ cured piglet vs.  $1300 \pm 20$  Vietnam dong/ cured piglet,  $p < 0.01$ ).

**Table 2: The treatment effects of ethanol garlic extract in the comparison with Flocidin**

Group	Piglet Number	Treatment results					Re-infected		Economic cost (Vietnam dong/ cured piglet)
		Piglet cured from diarrhea (Number)	(%)	Time of necessary treatment (d)	Ineffective treatment (Number)	(%)	Piglet (Number)	(%)	
Garlic	45	45	100	$2.55 \pm 0.12$	0	0	2	4.44	<b><math>500^{**} \pm 10</math></b>
Flocidin	45	45	100	$2.45 \pm 0.21$	0	0	2	4.44	$1300 \pm 20$

Time of necessary treatment (d) was expressed as Mean  $\pm$  S.E.M. The piglets after being cured from the diseases were continuously observed for 10 d, and if piglets showed no symptoms of diarrhea, they were recorded as not be re-infected. \*\* indicates that the economic cost (Vietnam dong/ cured piglet) of garlic was significantly lower than that of Flocidin ( $p < 0.01$ ) by Student's t test.

---

## DISCUSSION

The treatment potential of garlic and pepper in many digestive disorders, including diarrhea, has been well recorded in the Vietnamese herbalism (Do Tat Loi 2005 a, b), and researches have also reported their inhibition on pathogenic bacteria (Nguyen Van Thanh et al., 2015; Maryam Zarringhalam et al., 2013). However, these studies have just limited on investigating the *in vitro* effects, and have not yet performed the *in vivo* experiments. Therefore, in this study, we examined the effects of garlic and pepper extracts on livestock that had already been affected with diarrhea, with the view of evaluating their *in vivo* effect. Our study showed that both of garlic and pepper have treatment effect on piglets affected with diarrhea, indicated by a high percentage of piglets being cured from the disease after receiving extract treatment. These *in vivo* evidences further added pharmacological explanations for the therapeutic use of these plants to treat diarrhea in traditional herbalism. In our study, garlic showed better effect than pepper. In addition, the best dose to apply this extract was also identified as 2ml/ kg/ d. In the comparison experiments with a commercial drugs, this extract showed similar results in the treatment effect, but the economic cost was much lower. Many researches have highlighted the importance of cheap price of medicine plants in developing countries, but mainly from the view of application for human (Nivo H. Rakotoarivelo et al., 2015; CN Fokunang et al., 2011). However, considering the current conditions of Vietnamese husbandry, where medicine plants play an important role in treatment of livestock diseases in rural area and price is still one of the main concerns for farmers to select the drug (Bui Thi Tho and Nguyen Thi Thanh Ha, 2009), we suggested that lower treatment cost is certainly an important advantage of garlic. In addition, comparing to synthesized drugs, medicinal plants have also been known to have many other favorable features, such as easily degraded in natural environments, do not residue in animal products and less side-effect with animals (Nguyen Thanh Hai et al., 2014; Nguyen Van Thanh and Nguyen Thanh Hai 2014; Nguyen Thanh Hai and Bui Thi Tho, 2013). However, our study has just demonstrated the advantage of garlic in lower cost and has not yet investigated other factors. Follow-up research is therefore necessary and will be carried on to further clarify advantageous characteristics of garlic, with the expectation to widen its application and to alternate antibiotic use in piglet diarrhea.

## CONCLUSION

Both ethanol extracts of garlic and pepper showed significant treatment effect on piglets affected with diarrhea. Garlic showed more potency than pepper. In the comparison with a synthesized drug for the treatment of piglet diarrhea, garlic extracts showed the same treatment effect but were significant lower in the cost of treatment. Our results therefore suggested that garlic might be considered as a candidate to alternate synthetic drugs in piglet diarrhea treatment, because it showed similar effect but was cheaper. However, further research is necessary to clarify garlic potential.

## ACKNOWLEDGEMENT

The author gratefully acknowledge the assistance of Mr Nguyen Manh Ha, chief director of Professional Dog Research Center PDS, Faculty of Veterinary Medicine, Vietnam National University of Agriculture in Vietnam for the use of the services and facilities in the center. The finance for this study was partly supported by a grant for young researcher of PDS center (number PDS-GFYR-2010).

---

**REFERENCES**

1. An T. T. Vo, Engeline van Duijkeren, Wim Gaastra, Ad C. Fluit. Antimicrobial Resistance, Class I Integrons, and Genomic Island 1 in Salmonella Isolates from Vietnam. *PLoS One*. 2010; 5: e9440. (doi: 10.1371/journal.pone.0009440).
2. Andrew Chevallier. *Duoc Thao Toan Thu [The Encyclopedia of Medicinal Plants]*. A DK Publishing House, UK. 1999. Translated to Vietnamese by Kim Dan Nguyen. Ho Chi Minh publishing house, Vietnam. 2006. pp: 418.
3. Bui Thi Tho and Nguyen Thi Thanh Ha. *Text-book of Veterinary Medicine Plants. Teaching documents in faculty of Veterinary Medicine, Vietnam National University of Agriculture, Hanoi, Vietnam. Agriculture publishing House, Vietnam, 2009.*
4. Bui Thi Tho, PhD thesis. *A study on the effect of common synthesized drugs and phytoncide with E. coli isolated from diarrhea piglets, Hanoi University of Agriculture Publishing House, 1996.*
5. CN Fokunang, V Ndikum, OY Tabi, RB Jiofack, B Ngameni, NM Guedje, EA Tembe-Fokunang, P Tomkins, S Barkwan, F Kechia, E Asongalem, J Ngoupayou, NJ Torimiro, KH Gonsu, V Sielinou, BT Ngadjui, F Angwafor, III A Nkongmeneck, OM Abena, J Ngogang, T Asonganyi, V Colizzi, J Lohoue, and Kamsu-Kom. *Traditional Medicine: Past, Present and Future Research and Development Prospects and Integration in the National Health System of Cameroon. Afr J Tradit Complement Altern Med*. 8: 284-295, 2011.
6. Do, T.L. Hat tieu. In: Do TL (eds.) *Nhung cay thuoc va vi thuoc thuong dung tai Vietnam [Book in Vietnamese]*. Vietnam Medical publishing house, Hanoi, Vietnam, pp. 370-372, 2005. (a).
7. Do, T.L. Toi. In: Do TL (eds.) *Nhung cay thuoc va vi thuoc thuong dung tai Vietnam [Book in Vietnamese]*. Vietnam Medical publishing house, Hanoi, Vietnam, pp. 181-182, 2005. (b).
8. Ha Viet Le, Ryuji Kawahara, Diep Thi Khong, Hoa Thi Tran, Thang Nam Nguyen, Khai Ngoc Pham, Michio Jinnai, Yuko Kumeda, Tatsuya Nakayama, Shuhei Ueda and Yoshimasa Yamamoto. *Widespread dissemination of extended-spectrum  $\beta$ -lactamase-producing, multidrug-resistant Escherichia coli in livestock and fishery products in Vietnam. International Journal of Food Contamination*. 2:17, 2015.
9. Maryam Zarringhalam, Jalal Zarringhalam, Mehdi Shadnough, Firouzeh Safaeyan, and Elaheh Tekieh. *Inhibitory effect of black and red pepper and thyme extracts and essential oils on Enterohemorrhagic Escherichia coli and DNase Activity of Staphylococcus aureus. Iran J Pharm Res*. 12: 363–369, 2013.
10. Nguyen Thanh Hai and Bui Thi Tho. *A study on the in vitro antibacterial effect of garlic extract (Allium sativum L. ) with pathogen E. coli and E. coli resistant to ampicillin, kanamycin. Journal of Science and Development*. 16: 804-808, 2013.
11. Nguyen Thanh Hai, Nguyen Van Thanh, Bui Thi Tho, Miyamoto Atsushi. *A study about althelminthic effect of Punica gramatum L bark on veterinary endoparasites. Asian journal of Pharmaceutical and Clinical Research*. 7: 148-152, 2014.
12. Nguyen Van Thanh and Nguyen Thanh Hai. *In vitro antibacterial activity of Clerodendrum fragans Vent. extract on E. coli and Salmonella spp. isolated from feces of diarrhea piglets and experimental clinical treatment. Journal of Science and Development*. 12: 683-689, 2014.
13. Nguyen Van Thanh, Nguyen Thanh Hai, Bui Thi Tho, Miyamoto Atsushi. *The effect of garlic (Allium sativum L.) bulbs and ginger (Zingiber officinale) rhizomes extracts on Escherichia coli and Salmonella spp isolated from duck diarrhea syndrome. International Journal of Medical Research and Pharmaceutical Sciences*. 2: 8-12, 2015.
14. Nivo H. Rakotoarivelo, Fortunat Rakotoarivony, Aro Vonjy Ramarosandratana, Vololoniaina H. Jeannoda, Alyse R. Kuhlman, Armand Randrianasolo, and Rainer W. Bussmann. *Medicinal plants used to treat the most frequent diseases encountered in Ambalabe rural community, Eastern Madagascar. J Ethnobiol Ethnomed*. 11: 68. 2015. (doi: 10.1186/s13002-015-0050-2).
15. Ponmurugan Karuppiah and Shyamkumar Rajaram. *Antibacterial effect of Allium sativum cloves and Zingiber officinale rhizomes against multiple-drug resistant clinical pathogens. Asian Pac J Trop Biomed*. 2: 597–601, 2012.
16. V. Viswanathan, A. G. Phadataré, and Alka Mukne. *Antimycobacterial and Antibacterial Activity of Allium sativum Bulbs. Indian J Pharm Sci*. 76: 256–261, 2014.

17. Xiaonan Lu, Barbara A. Rasco, Jamie M. F. Jabal, D. Eric Aston, Mengshi Lin, and Michael E. Konkel. *Investigating antibacterial effects of garlic (*Allium sativum*) concentrate and garlic-derived organosulfur compounds on *Campylobacter jejuni* by using fourier transform infrared spectroscopy, raman spectroscopy, and electron microscopy. *Appl Environ Microbiol.* 77: 5257–5269, 2011.*