



ANTIMICROBIAL ACTIVITY OF CHIVE AND GINGER EXTRACT ON *ESCHERICHIA COLI* AND *SALMONELLA* SPP. ISOLATED FROM DIARRHOEIC BROILER CHICKENS

Phan Vu Hai*, Nguyen Van Chao, Nguyen Dinh Thuy Khuong, Tran Ngoc Liem,
Ho Thi Dung, Thuong Thi Thanh Le, Le Xuan Anh, Pham Hoang Son Hung

University of Agriculture and Forestry, Hue University, 102 Phung Hung St., Hue, Vietnam

Abstract. The chive and ginger bulbs were extracted with ethanol 96%, 72%, and 48% within 5, 10, and 15 days for each concentration (15, 30, and 45 days in total). The solidified extract was then used for antibacterial activity against *E. coli* and *Salmonella* spp. isolated from fecal of chickens with diarrhoea. The results show that both ginger and chive soaked and leached for greater than 30 days give better antibacterial ability. The extracts of ginger and chive bulbs, diluted at concentrations of 5 $\mu\text{g}/\mu\text{L}$, 7.5 $\mu\text{g}/\mu\text{L}$, and 10 $\mu\text{g}/\mu\text{L}$ are resistant to both bacteria. However, chive extract is more resistant to *Salmonella* spp. The antibacterial activities of both herbs are superior to those of amoxicillin and tetracycline (for *E. coli*) and gentamicin and amoxicillin (for *Salmonella* spp.). The minimum inhibitory concentration of chives extract (30 days) is 16–63 (31–125) mg/mL, and that of ginger extract (30 days) is 16–80 (2–4) mg/mL; overall, both extracts have bacteriostatic/bactericidal effects on *E. coli* and *Salmonella* spp.

Keywords: chicken, chives, ginger, *E. coli*, *Salmonella* spp.

1 Introduction

E. coli and *Salmonella* spp., belonging to the family Enterobacteriaceae, are considered as the major cause of diarrhea in humans and animals. These pathogens are widely distributed in the environment, and they not only cause economic losses but also affect public health [8]. Antibiotics are widely used in the prevention and treatment of bacterial diseases. However, antibiotic-resistant bacteria have been observed with increasing frequency over the past several decades. In addition, the use of antibiotics also has undesirable effects such as hypersensitivity, immunodeficiency, and antibiotic residues in animal products [9]. Therefore, herbal extracts have become a potential alternative solution to antibiotics in livestock.

Ginger and chives are common agricultural products in mountainous areas and sandy soils in the central provinces of Vietnam. Ginger and chives were used as medicines for diarrhea treatment in animals [4] because they have antibacterial chemical compounds [11, 13].

This study evaluates the antimicrobial activity of chives and ginger bulbs against *E. coli* and *Salmonella* spp. isolated from fecal of the chicken with diarrhoea. The outcome of this study

Corresponding: hai.phanvu@huaf.edu.vn

Submitted: Jun 20, 2019; Revised: July 20, 2019; Accepted: October 16, 2019.

contributes to the application of these herbs in the prevention and treatment of diarrhea on chickens.

2 Material and methods

Solidified herbal extract

Fresh bulbs of ginger (12–14 months old from A Luoi, Thua Thien Hue) and fresh chives (4–5 months old from Hai Lang, Quang Tri) were used in the study. The PCR (Polymerase Chain Reaction) product of ITS1-4 gene fragment from DNA from ginger and chive bulb samples was sent to Macrogen Korea (dna.macrogen.com) for sequencing with the Sanger method [3]. The sequencing results were processed using BioEdit software and Blast tool on the NCBI database (www.ncbi.nlm.nih.gov) to determine the species name. The results show that the ginger and compressed samples are in the same branch and closely related to *Zingiber officinale* and *Allium scordoprasum*.

The bulbs were washed, crushed, and dried at 50 °C for 40 h. Ginger and chive extract was collected using the cool extraction method described by Kinh [10] (Figure 1). One hundred grams of the materials was immersed in ethanol (96%, 72%, and 48% continuously within 5, 10, and 15 days for each concentration) with the ratio of materials to solvents is 1/3 (gr/ml). The extract was mixed and solidified at 50 °C. Depending on the extraction time, three types of the solidified chive extract (SCE) are obtained, namely chives 15 days (15DC), 30 days (30DC), and 45 days (45DC). Similarly, three types of solidified ginger extract (SGE) are 15DG, 30DG, and 45DG.

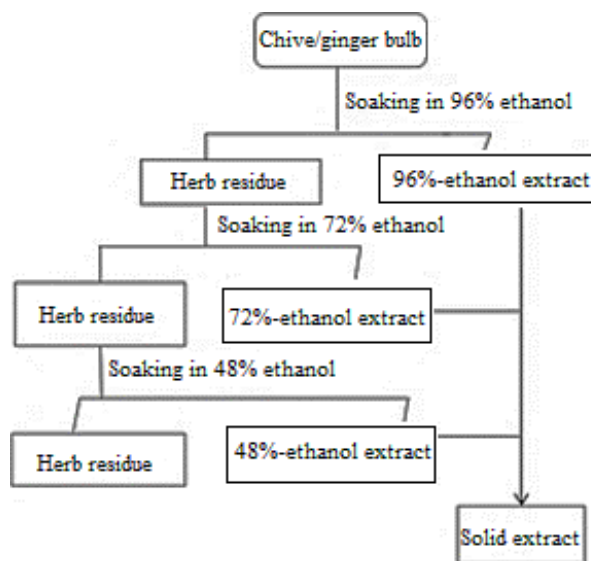


Figure 1. Schematic diagram of ethanol extraction process of chive and ginger bulb

Bacteria

Bacteria (Institute of Biotechnology, Hue University) were isolated using the routine culture method: *E. coli* (TCVN 6846: 2007; ISO 7251: 2005), *Salmonella* (TCVN 4829: 2005; ISO 6579: 2002) from samples of 3F Vietnamese chickens with diarrhea on selective Macconkey (*E. coli*) and SS Agar media (*Salmonella* spp.).

The PCR products of rDNA 16S gene fragments from DNA extracted from bacterial colonies were sent to MacroGen Korea company (dna.macrogen.com) for sequencing using the Sanger method [3]. The sequencing results were processed using BioEdit software and Blast tool on the NCBI database (www.ncbi.nlm.nih.gov) to determine the species name. The results show that colonies are closely related to *Escherichia coli* and *Salmonella* spp.

Bacterial virulence was determined using the infectious model in white mice, as described by Picard et al. [12]. The results show that 100% of experimental mice died within 6 to 18 h with typical lesions of *E. coli* and *Salmonella* spp.

Antibacterial activity assay

The antibacterial activity of the solidified herbal extract was examined according to Bakhiet et al. [1]. *E. coli* and *Salmonella* spp. were activated on the LB broth at 37 °C overnight. Bacterial cell density is determined as OD value ($\lambda = 600$ nm). Then, the bacterial cell density was adjusted at 10^6 (CFU/mL) on LB agar. The solidified herbal extract was dissolved in dimethyl sulfoxide 10% (10 mg/mL). Paper discs were placed on LB agar plates. Then, 50, 75, and 100 μ L of the dissolved herbal extract were dropped on the discs. The antibacterial activity was calculated as the difference between the diameter of the inhibitory zone and the diameter of the paper discs (D , mm). The sample is considered to have antibacterial activity if the difference is greater than 8 mm [6].

The determination of antibiotic susceptibility by the method of diffusion on Kirby-Bauer Mueller agar [2] is based on the sterile ring diameter of antibiotic-resistant paper plates according to CLSI standards [5] (Table 1).

Table 1. Antibiotics used in the research

Antibiotic	Producer	Standard diameter (mm)
Amoxicillin (10 μ g)		12–22
Gentamycin (10 μ g)	Nam Khoa Biotek Co.	19–26
Tetracycline (10 μ g)		18–24

If $D \leq$ standard diameter, the bacteria are resistant to antibiotics, and if $D >$ standard diameter, the bacteria are susceptible to antibiotics.

Antibacterial activity was tested by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using the micro-dilution method with resazurin [14]. The bacteria with a cell density of 10^6 CFU/mL were put into each well that has 100 μ L herbal extract diluted from 1000 mg/mL to 1/2048 mg/mL on 96 well plates containing Mueller-Hinton broth. The control wells contain bacteria in the Mueller-Hinton broth and dimethyl sulfoxide. The resazurin solution as an indicator of microbial growth was prepared by dissolving a 270 mg tablet in 40 mL of sterile distilled water. 10 μ L of the resazurin indicator solution was added to each well. The plates were prepared in triplicates and placed in an incubator set at 37 °C for 24 h. MIC was defined as the lowest concentration of the tested compound that prevents resazurin color to change from blue to pink. MBC was determined by plating 10 μ L of samples from wells, where no indicator color change was recorded on nutrient agar. The lowest concentration with no growth (no colony) is the minimum bactericidal concentration. Antibacterial activity is bactericidal or bacteriostatic if $MBC/MIC < 4$ or $MBC/MIC \geq 4$, respectively [7].

Data were expressed as the mean \pm SEM (Standard Error of the Mean). For comparison, the data were analyzed using a one-way analysis of variance. In all cases with $p < 0.05$, the difference is considered statistically significant. The statistical analysis was performed using SPSS v. 22 package.

3 Results and discussion

Antibacterial activity of the herbal extract against *E. coli* and *Salmonella* spp. compared with antibiotics

The results show that the solidified herbal extract at 7.5 μ g/ μ L in all treatment has antibacterial activity against *E. coli* and *Salmonella* spp. ($d > 8$ mm, Table 2). SCE has an effective antibacterial ability against both *E. coli* and *Salmonella* spp. Meanwhile, SGE is more effective against *E. coli* than against *Salmonella* spp.

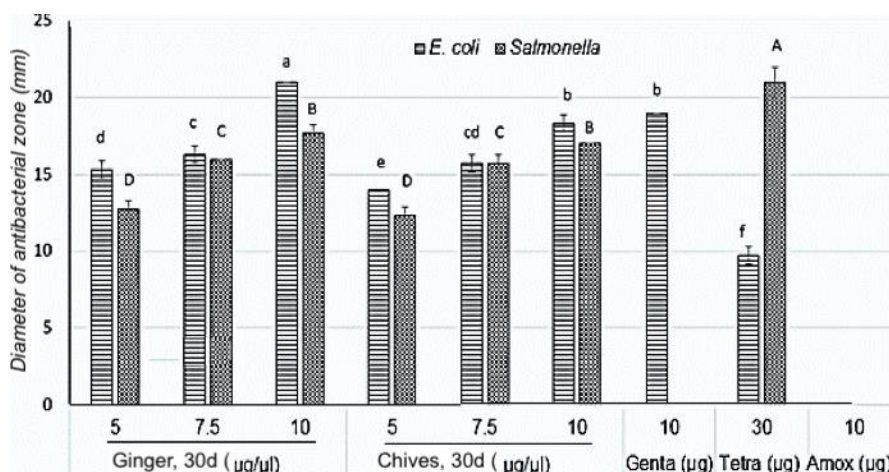
The antibacterial ability of SGE may be due to compounds such as sesquiterpenoid, zingiberene, bisabolene, farnesene, and monoterpenoid [11]; meanwhile, diallyl monosulfide, diallyl disulfide, diallyl trisulfide, and diallyl tetrasulfide are found in chive bulbs [13].

It is obvious that 30DC, 45DC, 30DG, and 45DG have higher antibacterial ability than 15DG and 15DC. Therefore, 30DC and 30DG were used for subsequent experiments.

Table 2. Antibacterial zone of the extract (mm)

Treatment	<i>E. coli</i>	<i>Salmonella spp.</i>
15DC	13.3 ± 0.6 ^a	14.3 ± 0.6 ^a
30DC	15.7 ± 0.6 ^b	15.7 ± 0.6 ^b
45DC	16.0 ± 1.0 ^b	16.7 ± 0.6 ^b
15DG	15.3 ± 0.6 ^A	14.3 ± 0.6 ^A
30DG	16.3 ± 0.6 ^A	16.0 ± 0.0 ^B
45DG	18.3 ± 0.6 ^B	16.3 ± 0.6 ^B

* Different letters (a, b, A, B) in each herbal extract indicates a statistically significant difference among groups ($p < 0.05$)



* Different letters a, b, c, d, e, f, A, B, C, D for each bacterium indicate a statistically significant difference among groups ($p < 0.05$)

Figure 2. The antibacterial of herbal extract compared with antibiotics

Results of Figure 2 show that the herbal extract from SCE and SGE, with concentrations greater than 5 µg/µL, has an antibacterial ability ($d > 8$ mm). The bacteria are resistant to amoxicillin (*E. coli* and *Salmonella spp.*) and gentamicin (*Salmonella spp.*). In comparison with standard antibacterial indicators (CLSI, 2018), the antibacterial ability of gentamycin against *E. coli* is standardized (ATCC 25922, 19–26 mm). The 30DC has an antimicrobial zone and antibacterial active ingredients equivalent to gentamycin. Thus, the plant antibiotic content (phytocid) in 30DC (10 µg/µL) may be equivalent to gentamycin and used as a replacement in the treatment of *E. coli* in chicken.

MIC and MBC of the herbal extract

Table 3. MIC (mg/mL) and MBC (mg/mL) of herbal extract on *E. coli* and *Salmonella* spp.

Treatment	30DC			30DG		
	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC
<i>E. coli</i>	63	125	1.98	16	80	5.00
<i>Salmonella</i> spp.	16	32	2.00	2	4	2.00

It is showed on Table 3 that the antibacterial activity of SGE (MIC = 16-80 mg/mL, MBC = 2-4 mg/mL) is 2–5 times higher than that of SCE (MIC = 16-63 mg/mL, MBC = 32-125 mg/mL). Both SGE and SCE have a bactericidal effect on *E. coli* and *Salmonella* spp. (MBC/MIC < 4). Meanwhile, the SGE has bacteriostatic effects (MBC/MIC > 4) on *Salmonella* spp.

4 Conclusion

Both ginger and chive bulbs have antibacterial activity against *E. coli* and *Salmonella* spp. However, SCE is more effective against *Salmonella* spp. than against *E. coli*. The antibacterial activity of SGE and SCE against *E. coli* is higher than that of amoxicillin and tetracycline. The antibacterial activity of SGE and SCE against *Salmonella* spp. is more effective than that of gentamicin and amoxicillin. The MIC (MBC) of 30 SCE and 30 SGE is 16–63 (31–125) mg/mL and 16–80 (2–4) mg/mL, respectively. This indicates that both SCE and SGE have bacteriostatic/bactericidal effects on *E. coli* and *Salmonella* spp.

References

1. Bakhiet A.O., Adam S.E. (1995), Therapeutic utility, constituents and toxicity of some medicinal plants: a review, *Veterinary and Human Toxicology*, 37, 255–258.
2. Bauer A.W., Kirby W.M., Sherris J.C. (1966), Antibiotic susceptibility testing by a standardized single disk method, *American Journal of Clinical Pathology*, 45, 493–496.
3. Blazej R. G., Kumaresan P., Mathies R. A. (2006), Microfabricated bioprocessor for integrated nanoliter-scale Sanger DNA sequencing, *Proceedings of the National Academy of Sciences*, 103(19), 7240–7245.
4. Chi Vo Van (1999), Dictionary of Medicinal Plants in Vietnam. Medicine Publisher, Hanoi, Vietnam.
5. Clinical and Laboratory Standards Institute (CLSI) (2018), CLSI Document M07-A9, 28th Edition.
6. Fadia M., AL-Haiali Z., AL-Rassam T., Yassen M. (2012), The inhibition effect of some plant

- extracts on some gram-negative and gram-positive bacteria, *Academic Scientific Journals*, 23, 22–38.
7. French G. L. (2006), Bactericidal agents in the treatment of MRSA infections—the self-stability of processed frozen beef sausage, *Life Science Journal*, 9, 2012, 448–461.
 8. Indu M. N., Hatha A. A. M., Abirosh C., Harsha U., Vivekanandan G. (2006), Antimicrobial activity of some South Indian spices against serotypes of *Escherichia coli*, *Salmonella*, *Brazilian Journal of Microbiology*, 37, 153–158.
 9. Iram S., Akbar K. J., Aman N., Nadhman A., Zulfiqar Z. and Arfat Y. M. (2016), Enhancing the anti-enterococci activity of different antibiotics by combining with metal oxide nanoparticles, *Journal of Microbiology*, 12, 9(3), e31302.
 10. La Van Kinh (2017), The use of finished products of medicinal herbs in chicken and pig production, *Agricultural publishing house*, Hanoi, Vietnam.
 11. O'Hara M., Kiefer D., Farrell K., Kemper K. (1998), A review of 12 commonly used medicinal herbs, *Archives of family medicine*, 7, 523–536.
 12. Picard, B., Duriez, P., Gouriou, S., Matic, I., Denamur, E., Taddei, F. (2001), Mutator natural *Escherichia coli* isolates have an unusual virulence phenotype, *Infection and Immunity*, 69, 9–14.
 13. Rattana P. and Phumkhachorn P. (2008), Diallyl sulfide content and antimicrobial activity against food-borne pathogenic bacteria of chives (*Allium schoenoprasum*), *Bioscience, Biotechnology and Biochemistry*, 72, 2987–2991.
 14. Sarker S.D., Nahar L., Kumarasamy Y. (2007), Microliter plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals, *Methods*, 42, 321–324.