Volume II

ICONC 2019

2019 ICONC TASP AND

"Integrated Approach in Advanced Animal Technology and Innovation in the Tropics"

"New Technology for Innovation and Synchrotron Applications"

The 2nd International Conference on Tropical Animal Science and Production (TASP 2019)

The 2nd International Conference on Native Chicken (ICONC 2019)

July 9-12, 2019
Surasammanakhan Hotel
Nakhon Ratchasima, Thailand

jointly organized by

ISBN: 978-974-533-745-9

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The 2nd International Conference on Native Chicken (ICONC 2019)

Assessment of extraction efficacy and antibacterial activity of ethanol extract of *Allium schoenoprasum* against *Escherichia coli* isolated from broiler chickens

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Abstract

Chive - *Allium schoenoprasum* and a traditional medicine in Vietnam. Maceration extraction of chive in ethanol solvent (96%, 72% and 48%, continuously) followed by solvent recovery distillation at 50^{o}C resulted in an average extract efficacy of 29.7%. It was clarified that the level of antibacterial activity depends on the concentration of extract. The effects were concentrationdependent and higher on standard *E. coli* strain in comparison to those on chicken-derived isolates. The diluted extract at a concentration of greater than 5 μg/μL was effectively against *E. coli* (antibacterial zone: 14±0.0 to 18.3±0.6 mm), but less than Gentamycin and better than Tetracycline/Amoxicillin. This extract also showed a MIC (MBC) value for *E. coli* of 63 (125) mg/mL. The 10% solution of chive extract exerted preventive and treatment effects on experimental infection with *E. coli* in 3F Viet chickens. The results can be considered as scientific basis for further studies on prevention and treatment potential of chive extracts.

Keywords: *Allium schoenoprasum,* ethanol extract, antibacterial activity

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I. Introduction

E. coli 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, it is not only cause economic losses but also effects to public health (Indu et al., 2006). 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 (Iram et al., 2016). Therefore, herbal extracts become a

potential alternative solution to antibiotic in livestock. Besides, antimicrobials of plant origin are effective treating infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (Jigna et al., 2005). Antimicrobial activity may involve complex mechanisms, such as inhibition of cell wall, cell membrane, nucleic acid, and protein synthesis, as well as the inhibition of nucleic acid metabolism. The substances identified in the extracts may act separately or in concert to exert these activities (Angiolella et al., 2018).

Chives (*Allium scordoprasum*) are common agricultural products in sandy soils in the central provinces of Vietnam. Chives were used as medicines for diarrhea treatment in animals (V.V. Chi, 1999) due to antibacterial chemical composition found in chives (Rattana and Phumkhachorn, 2008).

The aim of this study was to evaluate the antimicrobial activity of chives' bulbs on *E. coli* isolated from fecal of the chicken. The outcome of this study contributes to the application of these herbs in the prevention and treatment of diarrhea on chicken. Ethanol with medium polarity, used in the extract of the herb due to their soluble properties of many types of active ingredients, low impurities, high preservative drugs (Houghton and Raman, 1998).

II. Material and Methods

The solidified herbal extract

Fresh bulbs of chives (4-5 months old, in Hai Lang, Quang Tri) were washed, then crushed and dried at 50°C in 40 hours. Chives extract were collected by the cool extraction method according to the description of L.V. Kinh (2017). 100 grams of the materials were immersed in ethanol solvent (96%, 72% and 48% continuously within 5, 10 and 15 days for each concentration) with the ratio of materials to solvents is 1/3. The extract was mixed and solidified at 50^{o}C. Depending on the time of extraction, there were 3 types of the solidified chives extract (SCE) including chives 15 days (15D), 30 days (30D), and 45 days (45D).

Bacteria

Bacteria (Institute of Biotechnology, Hue University) was isolated from the fecal samples of 3F Viet chicken breed which has manifested diarrhea on Macconkey (*E. coli*) and SS Agar (*Salmonella* spp.).

Antibacterial activity assay

The antibacterial activity of SCE was examined according to the description of Bakhiet et al. (1995). *E. coli* was activated on the LB broth at 37°C overnight. Bacterial cell density is determined by OD value (λ = 600 nm), then bacterial cell density was adjusted at 10^6 (CFU/ml) on LB agar. SCE was dissolved with DMSO 10% (10 mg/µL). The paper discs were placed on LB agar plates, then 50, 75 and 100 µL of the dissolved herbal extract was dropped on it. The antibacterial activity

was calculated: diameter (mm) = diameter of the inhibitory zone – diameter of the paper discs. The results were considered to have antibacterial activity if the diameter > 8 mm (Fadia et al., 2012).

Antibacterial activity was tested by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using micro-dilution method with resazurin (Sarker et al., 2007). The bacteriophage reaches 10^6 CFU/ml into each well has 100µL the herbal extraction to be diluted from 1000 mg/ml to 1/2048 mg/ml on a 96 well plates containing Mueller–Hinton broth. The control wells contain bacterial, environmental and DMSO. The resazurin solution, as an indicator of microbial growth, was prepared by dissolving a 270 mg tablet in 40 µL of sterile distilled water. To each well 10 µL of resazurin indicator solution was added. The plates were prepared in triplicate, and placed in an incubator set at 37 °C for 24 h. MIC was defined as the lowest concentration of tested compound that prevented resazurin color change from blue to pink. MBC was determined by plating 10 µl of samples from wells, where no indicator color change was recorded, on nutrient agar. At the end of the incubation period the lowest concentration with no growth (no colony) was defined as minimum bactericidal concentration. Antibacterial activity was defined as bactericidal and bacteriostatic for MBC/MIC ratios <4 and ≥4, respectively (French, 2006).

Experimental chickens

3F Viet chickens at 1 - 37 days old were used in the experiments. Chickens were fed with mixed feed, no antibiotics, vaccinated against Newcastle disease and infectious bronchitis (IB) at 7 and 14 days of age; Gumboro at 10 and 21 days old.

The prevention and treatment effect of SCE 10% on chickens

Experimental chickens reared to 28 days of age were divided into 3 treatments (n=10, 3 replications) (See Table 1).

Table 1. Experimental design for the prevention and treatment of SCE 10% on broiler chickens

Age of chicken (days)	Intervention	Treatment 1 (T1)			Treatment 2 (T2)	Control
28	SCE 10% (1ml/10kg)		+		-	-
30	Oral infection with E. coli (1-3 x 106 CFU/ml)	+	+	+		
30-37	SCE 10% (1ml/10kg)		-		+	-

Note: + yes, - no

Infected/dead chickens were noted to be caused by E. coli when chickens have clinical symptoms, typical lesions and results of E. coli isolation from specimens.

Data were expressed by the mean ± SEM. For comparison among samples, data was analyzed by the the one-way analysis of variance (ANOVA). Chi-square test was used to evaluate the percentage difference. In all cases, p values < 0.05 were considered statistically significant. All statistical analyses were performed using SPSS v. 22 package.

III. Results and discussion

Performance of herbal extract

The product volume of three times extracted from chive's bulb (100g/time) is presented in Table 2.

Table 2. Recovery efficiency and sensory evaluation of extracts

Treatment	Volume (g) (Mean±SE)	Productivity (%)	Color
15D	19.3±1,1a	19.3	Reddish yellow
30D	33.5±1,2b	33.5	Orange
45D	36.33±1,0b	36.33	Reddish brown
Overall	29.71±1,1	29.71	Reddish -> yellow reddish brown

* Different letter (a, b) in each column indicate statically significant difference (P<0.05)

The productivity of SCE is relatively high due to the chive' bulbs contain a large amount of pectin, mucus, gum so after a long time of process of. However, these substances can reduce the mucosal irritation so there is no need to remove these compounds from the extract.

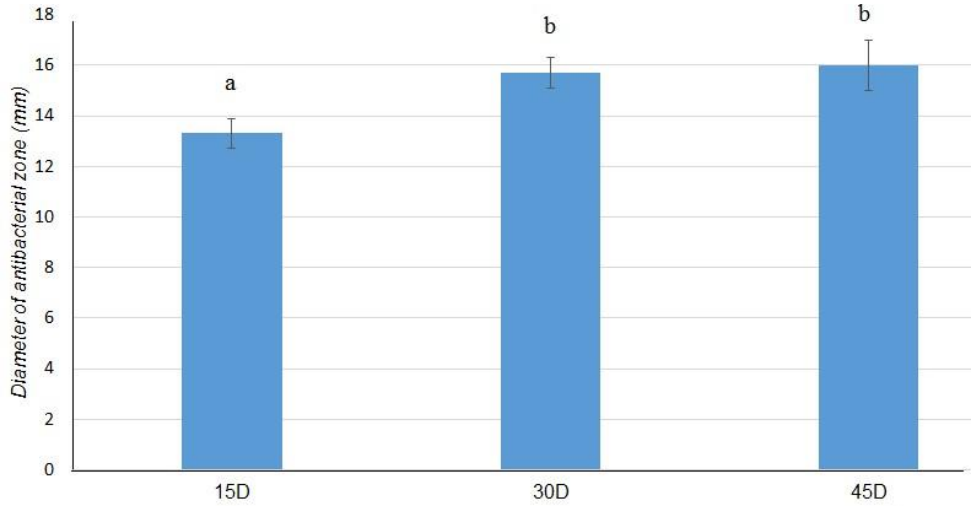
Results of Table 3 shows that the high volume of extraction was increasing over the time and reached the highest level from the 30th day of immersion onwards. The color of SCE was also darkening overtime: from reddish yellow to reddish brown).

Antibacterial activity of the herbal extract against *E. coli* compared with antibiotic

The results showed that the solidified herbal extract at 7.5 µg/µl in all treatment has antibacterial activity against *E. coli* (d>8mm, table 1). The SCE has effective antibacterial ability against *E. coli*.

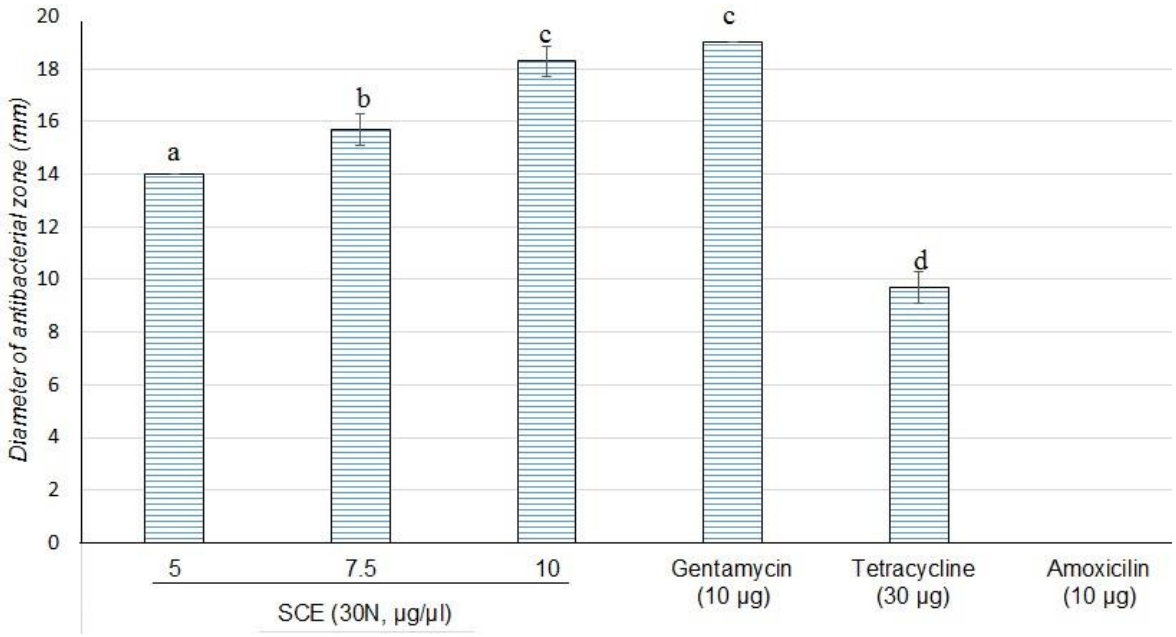
The antibacterial ability of SCE may be due to compounds such as diallyl monosulfide, dially disulfide, diallyl trisulfide and diallyl tetrasulfide were found in chive bulbs (Rattana and Phumkhachorn, 2008).

It was showed that 30D, 45D had higher antibacterial ability than 15D; therefore, 30D was used for subsequent experiments.



** Different letter (a, b, A, B) in each herbal extract indicate significant (P < 0.05) difference among groups.*

Figure 1. The antibacterial zone of the extract (mm)



** Different letter in each bacterium indicate significant (P < 0.05) difference among groups.*

Figure 2. *The antibacterial of herbal extract compared with atibiotic*

The herbal extract concentrations > 5 µg/µl from SCE has the antibacterial ability (d > 8mm). The bacteria were resistant to amoxicillin. In comparison with standard antibacterial indicators (CLSI, 2018), the antibacterial ability of gentamycin towards *E. coli* is standardized (ATCC 25922, 1926 mm). The 30D has an antimicrobial zone and an antibacterial active ingredients equivalent to gentamycin. Thus, the plant antibiotic content (phytocid) in 30D (10 µg/µl) may be equivalent to gentamycin and used as a replacement of this antibiotic 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.*

Treatment	30DC		
	MIC	MBC	MBC/MIC
E. coli	63	125	1.98

SCX had a bactericidal effect on *E. coli* (MBC/MIC <4).

Effect of SCE 10% on experimental *E. coli*-infected chickens

Results of 3 replicates of the experiment were presented in Table 4. After 24 hours of oral infection of E. coli, the chickens showed signs of moodiness, ruffled feathers, abandoned eating. The first chicken died after 72 hours. The results showed that the rate of diseased chickens of T1 and T2 were 23.33%; 20%, respectively, with a statistically significant difference (P <0.05) compared to a control treatment (56.7%).

Table 4. Results of monitoring chickens within 7 days of infection

Testing plot	Chickens /plot	T1		T2		Control	
		Chicken infected with *E. coli* disease	Chicken died with *E. coli* infection	Chickens infected with *E. coli* disease	Chicken died with *E. coli* infection	Chicken infected with *E. coli* disease	Chicken died with *E. coli* infection
1	10	3 (30%)	2 (20%)	3 (30%)	3 (30%)	6 (60%)	4 (40%)
2	10	2 (20%)	1 (10%)	1 (10%)	1 (10%)	5 (50%)	4 (30%)
3	10	2 (20%)	2 (20%)	2 (20%)	1 (10%)	6 (60%)	4 (30%)
Overall	30	7 (23.33%)[a]	5 (16.66%)[A]	6 (20%)[a]	5 (16.66%)[A]	17 (56.7%)b	12 (40%)[B]

* Different letter (a, b, A, B) in the row indicate significant (P < 0.05) difference among groups.

The results showed that an administration of SCE before and after infection with E. coli was effective for this bacterium. Preventive effects of SCE at a dose of 1 ml/10kg/day (T1) are equivalent to the therapeutic effect (T2). The rate of dead chickens in is 20%. The mortality rate of chickens in the control treatments (40%) is significantly higher (P <0.05) than those with SCE 10% to prevent (23.33%) and to treat (20%). The result of dead chicken examination and isolation of *E. coli* bacteria showed that the lesions are mainly sinuses, including yellow fluid in the cardiomyocytes and pericarditis. Thus, it can be observed that SCE 10% can reduce the ability of E. coli to infiltrate and cause disease.

The biological activity of chive extract has been demonstrated by some authors as antioxidant (Badami et al., 2003); resistance to some tumor-promoting agents (Benabadji et al., 2004); antimicrobial (Xu et al., 2004; Saravanakuma et al., 2013); protect and enhance liver function (Sathya Srilakshmi et al., 2010) and affect complement (Sarumathy et al., 2011). However, this was the first study to determine the ability to prevent and treat E. coli in experimental chickens. Currently "green" breeding, limiting antibiotics in the prevention and treatment of farm animal diseases are encouraged because of the downside of antibiotic use. This issue is of particular interest in developing countries, where antibiotics have been abused for the immediate profit of some manufacturers. The use of plant-based substances is one of the effective measures to limit the adverse effects of this situation. Use of plant-derived substances including chives for prevention and treatment of diseases is one of those measures. The results of this study can be considered as a basis for further studies to create products from chives for prevention and treatment of *E. coli* diseases in general and some other bacterial diseases in livestock.

IV. Conclusion

The yield of SCE was high (> 20%) and gradually increased with extraction time (45 days = 30 days > 15 days) with the darken color (from reddish yellow - reddish brown).

Chive's bulb had an antibacterial activity against *E. coli*. The antibacterial activity against *E. coli* of SCE was higher than those of amoxicillin and tetracycline. The MIC (MBC) of 30 SCE was 1663 (31-125) mg/ml. This indicates that SCX had bacteriostatic/bactericidal effects on *E. coli*.

SCE preparations 10% have the prophylactic and therapeutic effects on *E. coli* infection in chickens at a dose of 3 ml / animal.

This result is the basis for analytical studies assessing the antibacterial ability of SCE when used in combination with some other drugs and assessing the prevention and treatment effect of SCE by *E. coli*.

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