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STUDY ON THE BIOACTIVITY OF GINGER, GALANGAL EXTRACTS OF DIFFERENT REGIONS IN VIETNAM FOR PRESERVING SEAFOOD

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ABSTRACT

Ginger and galangal have long been known as common spices in Vietnam and referred in many reports as antibacterial, anti-inflammatory, well-digestive in traditional medicine. This study assessed the components with biological activities such as antibacterial and antioxidant from ginger, galangal extracts in a number of different regions in the country (Hai Duong, Bac Ninh, Nghe An, Tay Nguyen and Quang Nam).

The results showed that the polyphenols components (based on gallic acid) in ginger, galangal extracts in ethanol/water higher than in water: the highest ginger extract in ethanol/water (50 % v/v) of 19.93 mg/g dry matter was Nghe An ginger, the lowest of 17.2 mg/g dry matter was Tay Nguyen ginger. The highest galangal extracts in ethanol/water (60 % v/v) of 11.58 mg/g dry matter was Quang Nam galangalandthelowest of 10.5 mg/g dry matter was Tay Nguyen galangal. The highest extraction in water of 9.27 mg/g dry matter was NgheAnginger, the lowest of 6.6 mg/g dry matter was Tay Nguyen ginger. These extracts werewellanti-bacterialto certain human pathogenic microorganisms,whichare contaminated in food and seafood.The highest antibacterial diameter to *V. parahamolyticus* ATCC 17802 of the extract in ethanol/ water was 35 mm (Nghe An ginger) and the lowest was 31mm (Tay Nguyen galangal), while for the extracts in water the highest was 33 mm (Nghe An ginger) and the lowest was 25 mm (Tay Nguyen galangal). Antioxidant ability of these extracts was determined by ability of eliminating DDPH free radical, for extracts in ethanol/water, the highest was Bac Ninh galangal with 72.9 %, the lowest was Hai Duong ginger with 62.17 %.

Exploiting the components from ginger, galangal with intention to preserve seafood materials, help prolong and ensure safety is very meaningful job, which helps replace the misuse of toxic chemicals as urea, formol, etc., which are currently used widespread, which can severely impact the health of consumers.

Keywords: galangal, ginger, phenolic, polyphenols, bioactive, antioxidant, antibacterial, preservation, seafood.

1. INTRODUCTION

The antibacterial and antioxidant roles of ginger *(Zingiberofficinale)*, galangal *(Alpiniagalanga)*are recently reported in many studies on specialized magazines in the world. The main role of the components having biological activity is referred to phenolic compounds, the selected recovery method is extraction in solvents such as ethanol/water or water,... in which, the total polyphenols components recovered when extracting in the presence of ethanol are higher, the content isfrom 21.1 to 43.2 mg/g dry matter [1, 2] for ginger, while for galangal the highest content is 31.49 mg/g dry matter when extracting inethanol/water and much lower 8.25 mg/g dry matter when extracting in water [3,4].

By using paper disk diffusion method, the antibacterial and antioxidant properties of ginger and galangal extractsshowed that the ginger extract in ethanol/water inhibited *E. coli*showing9mm diameter while*S.*typhiwasinhibited with 10 mm diameter; theginger extract in water was 8 mm with the *S.* typhi [5]. Furthermore, those extracts inethanol / water with the concentration of 2.5 and 2.7 % have antioxidant effectequivalent to 92.9 % of ascorbic acid [2]. Similarly, galangal extracts in ethanol/water showed the best inhibitory effect against *S. aureus*, theextracts in water have significant effect against *K. pneumonia*, *E. coli* [6]. The ethanol extractsof galangal showed the highest DPPH free radial scavenging ability when compared to the water extract and the essential oil. TheIC_{50}values ofthe galangal ethanol extract (10.66 mg/ml), water extract (55.48 mg/ml) and essential oil (455.43 mg/ml), were higher than those of α-tocopherol (1.45 mg/ml) andBHA (0.41 mg/ml). The ethanol extract of galangal was also advantageous as an antioxidant in food due to its mild odor compared with the essential oil [4]. The results of a previous study by the authors exploiting antibacterial and antioxidant ability of extracts of ginger, galangal (G-NA, G-HD and R-BN) to preserve squid and cuttlefish also showed very positive results [7]. This capability helps the selection of extracts as a natural antibacterial and antioxidant applied in foods in order to replace components of chemical nature.

2. MATERIALS AND METHODS

2.1. Materials

There were four types of ginger with small rhizomes planted in different regions as Hai Duong, Nghe An, Tay Nguyen and Quang Nam. Threetypesofgalangal purchased from regions of Bac Ninh, Tay Nguyen and Quang Nam.

Human pathogenic microbial strains of *V. parahaemolyticus*ATCC 17802 (from microbial strains collection of Department of Food Technology), was used for determining the antibacterial activities of the extracts of ginger and galangal.

2.2. Research methods

2.2.1. Methods for determining the microbiological criteria

- Quantitative analysis of*V. parahaemolyticus*infoodsaccording to TCVN 8988-2012

- Determination of antibacterial ability of ginger, galangal extracts by using paper disk diffusion method (dripping indicator solution of 10^6 CFU/ml onto the paper disk with a diameter of 6 mm) and the method of determining the minimum bactericidal concentration (MBC -

Minimum Bactericidal Concentration) with bacteria indicator *V. parahaemolyticus*ATCC 17802 of 10^4 CFU/ml [5].

2.2.2. Methods for determining the physicochemical and biochemical criteria

- Determination of total phenolic and polyphenols content using improved experimental method of Folin-Ciocalteu: reagents FC, Na_2CO_3, gallicacidas a standard, colorimetric measurementatwavelength of 760 nm [4].

- Determination of antioxidant abilities byusing method of removingDPPH free radicals (2,2-diphenyl-1-picrylhydrazyl) [1, 4]

2.2.3.Preparation of ginger, galangal extracts in ethanol and water solvent

Fresh ginger and galangal were sliced and dried at 45 ^oC for 18 – 20 hours to reach moisture of 7 - 8.5 %, then, milled into powder and stored at -20 ^oC. Conducting extraction by using the ethanol/water solvents, for ginger selected the ethanol/water solvent of 50 % (v/v) and for galangal the solvent of 60 % (v/v), extracted at the temperature of 50 °C; and extraction with water at the temperature of 60 ^oC, (From the previous research result, the suitable extraction mode for each kind of ginger, galangal has been chosen.) [8]. The extraction rate of ginger (galangal) powder/solvent is 1/30 (extracting 2 times). The recovered extracts were concentrated in vacuum for detaching ethanol (extracted with ethanol/water) and to norm the volume to the same volume at the ratio 1/40 (weight of powder/normed volume) and stored at the temperature of -12 ^oC [2, 4]. And then antibacterial and anti-oxidant ability of these extracts were assessed.

Symbols of extracts are as follows

- Ginger/galangal from Hai Duong, Bac Ninh, Nghe An, Quang Nam, Tay Nguyen extracted withethanol/water: G-HD, G-NA, G-QN, G-TN/ R-BN, R-QN, R-TN

- Ginger/galangal from Hai Duong, Bac Ninh, Nghe An, Quang Nam, Tay Nguyen extracted with water: G-HD(N), G-NA(N), G-QN(N), G-TN(N)/R-BN(N), R-NA(N), R-TN(N).

3. RESULTS AND DISCUSSION

3.1. Studying some material properties of Ginger and Galangal in some regions of Vietnam

Table 1. Study results on humidity and color of ginger and galangal material.

Materials		Material properties		
		Moisture of fresh rhizomes (%)	Moisture of powder (%)	Color (L-a-b)
Ginger	G-HD	88.6	8.25	30.70-4.65-23.57
	G-NA	86.4	7.8	36.01-4.20-20.56
	G-QN	87.45	8.41	39.43-2.24-22.58
	G-TN	85.73	7.94	29.33-4.70-19.42
Galangal	R-BN	81.12	6.95	33.05-5.99-22.84
	R-QN	81.31	7.17	36.82-4.64-18.56
	R-TN	78.02	7.74	28.16-5.37-14.57

Fresh ginger and galangal were purchasedfrom different regions of the country. Ginger and galangal are at same age (buds). Due to different geographical characteristics of land and soil,

climate, the characteristics of shape, color, moisture content of fresh rhizomes as well as powdersaftertreating are quite different, the results were shown in the Table 1, Figure 1.

Materials were purchased and selected at the same time in the buds stage (not old, not young) but the ginger, galangal materials in regions of the country are slightly different in the initial moisture content of fresh rhizomes (78 - 88.6 %) and powder after drying (6.95 - 8.41 %), particularly in rhizome size and color, ginger powders are brighter than galangal powders and easily distinguish powders from different regions.

Figure 1. Images of ginger and galangal powders from some regions of Vietnam.

3.2. Evaluation of some biological properties of extracts of ginger, galangal collected from some regions of Vietnam

3.2.1. Determination of the total polyphenols content of ginger, galangal materials

According to the research reports, the phenolic compounds (mainly polyphenols) that having bioactivities of antibacterial and antioxidant activities. Polyphenols contents are high in ginger and galangal [1 - 4].

To determine the total polyphenols content of the obtained ginger, galangal extracts using the colourimetric method with Folin-Ciocalteu reagents. Received results of polyphenols content of ginger and galangal extracts from some regions of Vietnam were expressed in Figure 2 and 3.

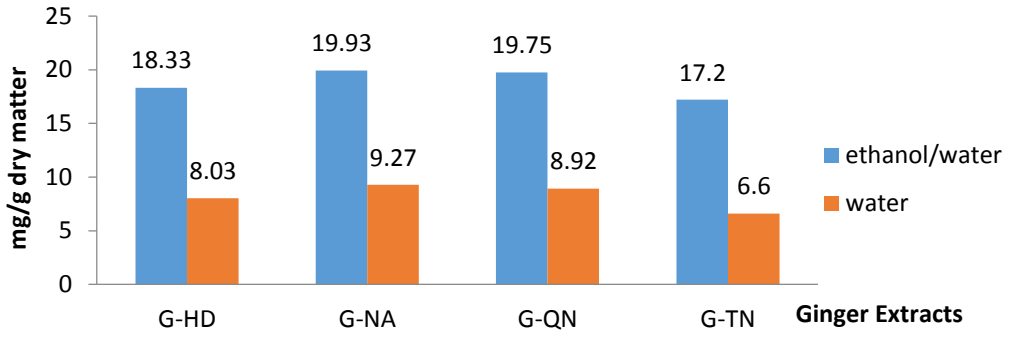

Figure 2. Polyphenols content of extracts of ginger powders in solvents ethanol/water and water in mg/g dry matter (the average result of repeated 3 times).

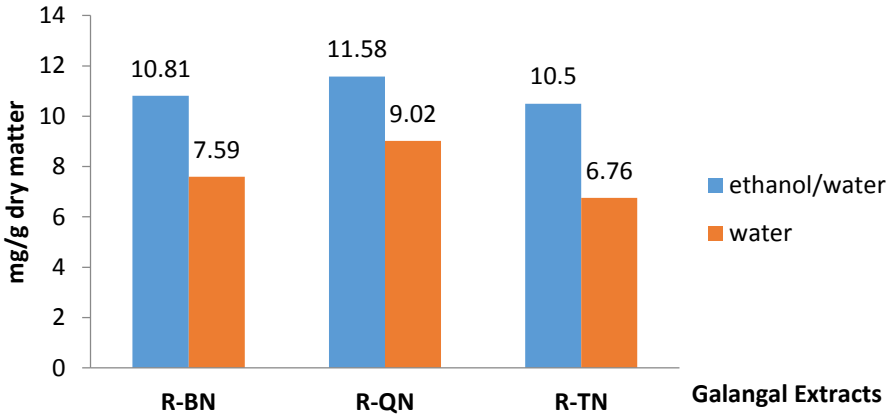

Figure 3.Polyphenols content of extracts of galangal powders in solvents ethanol/water and water in mg/g dry matter(the average result of repeated 3 times).

All samples of ginger, galangalextracts in solvents ethanol/water had polyphenols content higher than the samples extracted in water. This is consistent with other researches because the polyphenol content is better extracted in solvent with suitable polarization than in ethanol/water [4, 5]. The polyphenols content of G-NA sample was the highest compared to other ginger samples, was 19.93 mg/g dry matter (with the solvent ethanol/water); 9.27 mg/g dry matter (with water). And R-QN sample was the highest compared toothergalangalsamples, was 11.58 mg/g dry matter (with the solvent ethanol/water); 9.02 mg/gdry matter (with water). The polyphenols contentofG-TN andR-TNsampleswere the lowestcomparedtootherginger and galangal samples. Theseresults were quite consistent with other studies showing that if galangal extracted in water had the concentration of 0.5; 0.75 and 1 % (w/v) the water temperature showed the best performance at 60 ^{o}C, obtained polyphenols content was8.25 mg/g extract. When extracted in 50 % ethanol (v/v) andtheratesample/solvent = 1/10, thermostable at 50 ^{o}C (with stirring) obtained content is 31.49 mg GAE/g extract [4].

3.2.2. Determination of the antioxidant ability of ginger, galangalextracts

Antioxidant activity by means of the ability ofremoving DPPH free radicals (1,1-diphenyl-2-picrylhydrazyl) of ginger, galangal extracts in ethanol/water and water at different concentrations are shown in Figure 4.

Results showed that galangal extracts have higher antioxidant ability than ginger extracts. R-BN extracts in ethanol/water 60 % (v/v) reached the highest of 72.09 %; the lowest was R-TN with 61.55 %. With raw ginger, the high estpercentage of removing DPPH was G-NA extracted in ethanol/water 50% (v/v) with 68.16 %, the lowest was G-TN with 51.01 %. Meanwhile, this percentage of extracts in water was much lower in the same concentration (25 mg/water) with R-BN(N) of 51.25 % and G-NA(N) of just 44.08 %. With the decreasing concentration, the antioxidant ability decreases, however, in concentration 12.5 mg/ml it did not decreaseas significantly as in concentration of 25 mg/ml (based on method of a ½ serial dilution. The obtained results were quite close to the results of antioxidant ability of ginger/galangal in the world [1 - 4]. This result shows the potential application of the extract components in different concentrations in limiting lipid oxidation and discoloration of food, especially seafood in the preservation process is very feasibility.

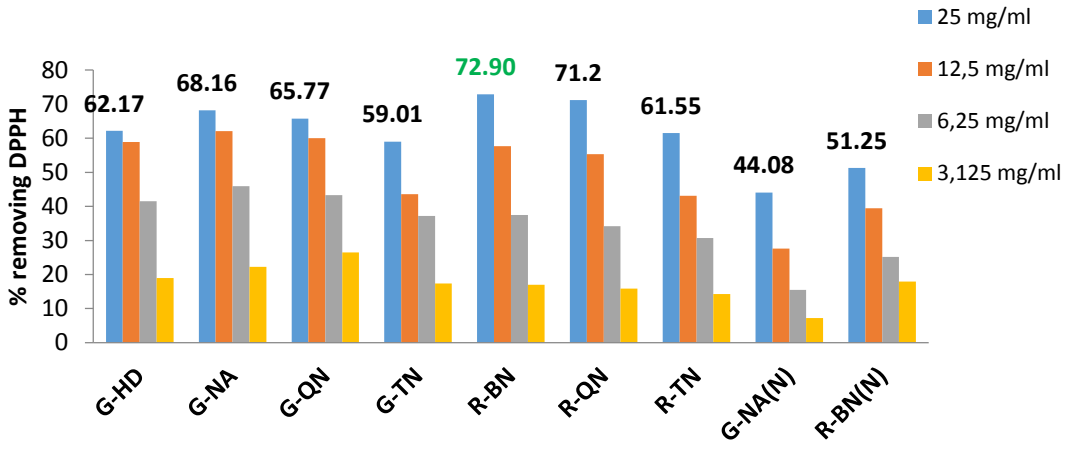

*Figure 4.*Ability of removing DPPH free radicals of ginger, galangal extracts in ethanol/water and water in different concentrations (the average result of repeated 3 times).

3.2.3. Determination of the antibacterialabilityof ginger, galangalextracts

Using paper disk diffusion method to test antibacterial ability of ginger, galangal extracts against *Vibrio parahaemolyticus* ATCC17802 strain (a common group of human pathogenic microorganisms in fresh aquatic, seafood products) to apply latter in preserving seafood. Results about inhibition zone wereshownin Figures 5, 6.

The results show that the diameter of antibacterial ring of ginger, galangal extracts against *V. parahaemolyticus* ATCC 17802 indicator strain (the contaminants in seafood) was quite large. The extracts in ethanol/water from the diameter of inhibition zone (D-d) = 31 – 35 mm showed larger antibacterial diameter of inhibition zone than in water from 25 - 33 mm. The highest was G-NA extract (35/34 mm) and the lowest was R-TN (31/15 mm).

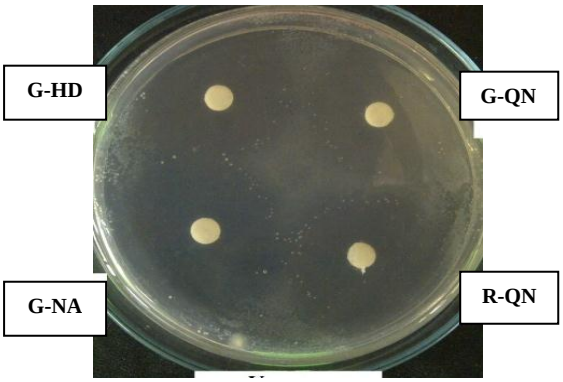

*Figure 5.*Diameter of inhibition zonewith*V. parahaemolyticus*of ginger, galangal extracts in water.

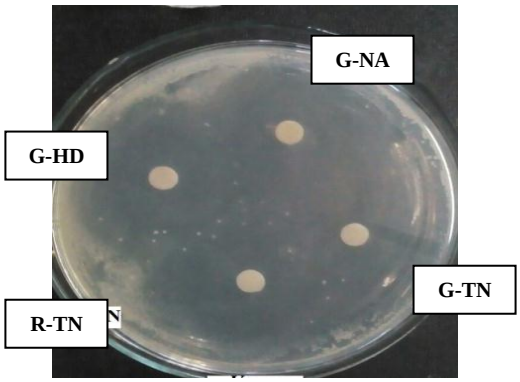

*Figure 6.*Diameterof inhibition zone with *V. parahaemolyticus*of ginger, galangal extracts in ethanol/water.

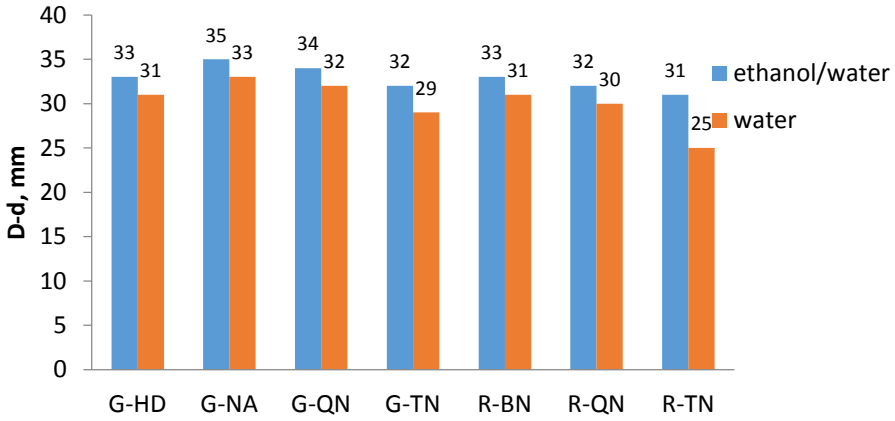

Figure 7. Diameter of inhibition zone of ginger, galangal extracts in ethanol/water and water with *V. parahaemolyticus*ATCC 17802.

To choose the concentration of ginger, galangal extract for antibacterial effectiveness when preserving, the MBC-Minimum Bactericidal Concentration against *V. parahaemolyticus* ATCC 17802 indicator strain was assessed, results shown in Table 2.

Table2. Minimum Bactericidal Concentration(MBC) of ginger, galangal extracts in ethanol/water inhibit *V. parahaemolyticus*ATTC 17802.

Ginger/Galangal	Extracts	**25** (mg powder/ml)	**12.5** (mg powder/ml)	**6.25** (mg powder/ml)	**3.125** (mg powder/ml)
G-HD	Ethanol/Water	-	-	++	++
	Water	-/+	+	++	++
G-NA	Ethanol/Water	-	-	+	++
	Water	-/+	+	++	++
G-QN	Ethanol/Water	-	-	+	++

	Water	-/+	+	++	++
G-TN	Ethanol/Water	-	-/+	+	++
	Water	-/+	+	++	++
R-BN	Ethanol/Water	-	-	+	++
	Water	-/+	+	++	++
R-QN	Ethanol/Water	-	-	+	++
	Water	-	-/+	++	++
R-TN	Ethanol/Water	-	-/+	+	++
	Water	-/+	+	++	++

Note: Indicator strain: (-): do not grow; (-/+): very little grows;(+): less grows;(++): grows

With *V. parahaemolyticus*ATCC 17802 strains, all extracts of ginger, galangal in ethanol/water at a concentration of 25 mg/ml and with G-NA, R-BN, R-QN at a concentration of 12.5 mg/ml inhibited completely indicator strain at the initial density of 10^4 CFU/ml, these extracts have MBC of 12.5 - 25 mg/ml. When diluted to concentration of 6.25 mg/ml, bacteria appeared but in small quantities. Extracts of ginger, galangal in water were less capable of destroying, at a concentration of 25 mg/ml most strains grew but were very less, except for R-QN extract, which could destroy completely, therefore, MBC of extracts in water were > 25 mg/ml (with R-QN of 25 mg/ml).

4. CONCLUSION

Ginger and galangal in some regions in Vietnam are different in size, color, moisture... because of soil and climate conditions. Polyphenols content of ginger, galangal extracts in ethanol/water was higher than in water, the highest was G-NA (NgheAnginger) extracted in ethanolreaching 19.93 mg/g dry matter, the lowest was R-TN (Tay Nguyen galangal) with 9.69 mg/g dry matter. The highest extract in water wasG-NA (NgheAnginger) with 10.5 mg/g dry matter and the lowest was G-TN (Tay Nguyen ginger) with 6.6 mg/g dry matter.

Antioxidant ability of the extracts in ethanol/water was higher in water, the highest was at concentration of 25 mg/ml, with the extracts in ethanol/water, R-BN (BacNinhgalangal) was able to remove DPPH free radicals to 72.09 %, then R-QN (QuangNamgalangal) with 71.2% , G-NA (Nghe An ginger) with 68.16 %,....With extracts in water, the highest was R- BN (Bac Ninh galangal) with 51.25 %, G-NA (Nghe An ginger) with 44.08 %.

Antibacterial ability against *Vibrio parahaemolyticus*indicatorstrains was quite good, the diameter of inhibition zone of the extracts in ethanol/water reached 31 – 35 mm, and of the extracts the water reached 33 - 25 mm. Determining the minimum bactericidal concentration MBC with this indicator strain (density of 10^4 CFU/ml) of these extracts showed that MBC of ginger, galangal extractsin ethanol/water was from 12.5 – 25 mg/ml while of extracts in water >25 mg/ml (only of R-QN was 25 mg/ml), hereby makes the selection of the appropriate concentration extracts to preserve seafood effectively.

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TÓM TẮT

NGHIÊN CỨU HOẠT TÍNH SINH HỌC CỦA DỊCH CHIẾT TỪ GỪNG, RIỀNG Ở MỘT SỐ VÙNG MIỀN VIỆT NAM NHẰM ỨNG DỤNG BẢO QUẢN THỦY HẢI SẢN

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Gừng và riềng từ lâu đã được biết đến là các loại gia vị khá phổ biến ở Việt Nam và được nhiều báo cáo nhắc đến với các vai trò kháng khuẩn, chống viêm, tiêu hóa tốt trong y học cổ truyền. Nghiên cứu này đã đánh giá thành phần có hoạt tính sinh học như khả năng kháng khuẩn và chống oxy hóa từ dịch chiết gừng, riềng ở một số vùng miền khác nhau trong cả nước (Hải Dương, Bắc Ninh, Nghệ An, Tây Nguyên và Quảng Nam).

Kết quả cho thấy: thành phần polyphenols (tính theo axit gallic) các dịch chiết gừng, riềng trong etanol/nước cao hơn trong nước: dịch chiết gừng trong etanol/nước (50 %v/v) cao nhất là gừng Nghệ An 19,93 mg/g chất khô, thấp nhất là gừng Tây Nguyên 17,2 mg/g chất khô. Dịch chiết riềng trong etanol/nước (60 %, v/v) cao nhất là riềng Quảng Nam đạt 11,58 mg/g chất khô và thấp nhất là riềng TâyNguyên 10,5 mg/g chất khô. Chiết trong nước/nước thì gừng Nghệ An cao nhất 9,27 mg/g chất khô, thấp nhất là gừng Tây Nguyên là 6,6 mg/g chất khô. Các dịch chiết này đều có khả năng kháng khuẩn tốt với một số loại vi sinh vật gây bệnh, sinh độc tố hay ô nhiễm trong thực phẩm và thủy sản, đường kính vòng kháng khuẩn với chủng chỉ thị *Vibrioparahamolyticus* ATCC 17802 của dịch chiết trong etanol/nước cao nhất là 35 mm (gừng Nghệ An) và thấp nhất là 31 mm (riềng Tây Nguyên), còn với dịch chiết trong nước cao nhất là 34 mm (gừng Nghệ An) và thấp nhất là 25 mm (riềng Tây Nguyên). Khả năng chống oxy hóa của các dịch chiết này cũng được xác định bằng khả năng loại gốc tự do DDPH, dịch chiết trong etanol/nước với riềng Bắc Ninh cao nhất là loại 72,9 %, thấp nhất là gừng Hải Dương 62,17 %.

Khai thác các thành phần từ gừng, riềng nói trên với mong muốn ứng dụng để bảo quản nguyên liệu thủy sản giúp kéo dài thời gian và đảm bảo an toàn là việc làm rất có ý nghĩa, giúp thay thế việc lạm dụng các hóa chất độc hại như urê, foocmol,... hiện đang được sử dụng tràn lan gây ảnh hưởng nghiêm trọng đến sức khỏe người tiêu dùng.

Từ khóa: gừng, riềng, polyphenols, chống oxy hóa, kháng khuẩn.