INVESTIGATION OF ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF SOME SPICE PLANTS COLLECTED IN DAKLAK PROVINCE

KHẢO SÁT HOẠT TÍNH KHÁNG OXY HÓA VÀ KHÁNG KHUẨN CỦA MỘT SỐ CÂY GIA VỊ THU THẬP TẠI TỈNH ĐẮK LẮK

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ABSTRACT

Spice plants including Cinnamomum cassia Presl, Curcuma longa L, Zingiber officinale (Willd.) Roscoe, Piper nigrum L have been used as spice herbs as well as traditional medicinal plants for long ages. The aims of this study were to investigate the antioxidant and antimicrobial activities of some spice plants including cinamon bark, ginger root, curcuma root and piper leaves. The terms for investigation of antioxidant activity were DPPH radical scavenging activity and ferric reducing power. The antibacterial (E.coli and S.aureus) activity was determined by disc diffusion methods. The results indicated that all of investigated extracts display the antioxidant activity via DPPH radical scavenging and reducing power. Among the tested extracts, cinamon bark extract possessed the highest antioxidant activity which was higher effect than vitamin C and BHT. Moreover, these extracts also possessed the antibacterial activity against tested bacteria including E. coli and S. aureus and the highest effect also found in cinnamon extract. The lowest activities were found in pepper leaves extracts in both antioxidant and antibacterial activities. The results could be concluded that cinamon bark is a potential spice plant possessing antioxidant and antibacterial activities. It has a significant potential to use as traditional therapy and further study may lead to a novel antioxidant and antimicrobial compound.

Keywords: *DPPH radical scavenging, reducing power, polyphenol, cinnamon.*

TÓM TẮT

Các cây gia vị gồm quế, nghệ, gừng và tiêu đã và đang được sử dụng như một loại gia vị và cũng được sử dụng như một cây thuốc truyền thống từ lâu đời. Trong nghiên cứu này, chúng tôi tiến hành khảo sát khả năng kháng oxy hóa và kháng vi khuẩn của một số cao chiết từ vỏ quế, củ nghệ, củ gừng và lá tiêu cũng như cao chiết phân đoạn từ vỏ quế thông qua khả năng dập tắt gốc tự do 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH), khả năng khử sắt; khả năng kháng khuẩn E.coli *và* S.aureus *bằng phương pháp khuếch tán trên đĩa. Kết quả cho thấy, các cao chiết nghiên cứu đều thể hiện khả năng kháng oxy hóa thông qua khả năng dập tắt gốc tự do DPPH và khả năng khử sắt. Trong các cao chiết khảo sát, cao chiết từ vỏ quế cho có khả năng khá oxy hóa là cao nhất, cao hơn cả vitamin C và BHT. Ngoài ra, các cao chiết nghiên cứu cũng thể hiện khả năng kháng khuẩn đối với các vi khuẩn nghiên cứu. Trong đó, cao chiết từ quế thể hiện khả năng kháng khuẩn là mạnh nhất và thấp nhất là cao chiết từ lá tiêu. Từ những kết quả* *này có thể thấy quế là một loại gia vị có tiềm năng trong kháng oxy hóa và kháng vi sinh vật, nghiên cứu này nên tiếp tục tiến hành cô lập và xác định tên các hợp chất có khả năng kháng oxy hóa và kháng khuẩn.*

Từ khóa: *DPPH, reducing power, kháng nấm, polyphenol, quế.*

1. INTRODUCTION

Free radical and other, reactive oxygen species (ROS) are considered as a roots of many diseases including cardiovascular dysfunction, atherosclerosis, inflammation, carcinogenesis, drug toxicity, reperfusion injury and neurodegenerative diseases [1,2]. Moreover, free radical and ROS are the causes of food peroxidation; reduction of nutrition and sensory qualities [3] are also the major causes of lipid containing food products deterioration, commonly defined as rancidity. Significant changes can occur in product odor, taste, color, texture, nutritive value. Progressing oxidation results in complete spoilage of foods. Therefore, the addition of antioxidants to food products has become popular as a means of increasing shelf life to reduce wastage and nutritional losses by inhibiting and delaying oxidation [4]. Synthetic antioxidants such as 2, 3-tert-butyl-4-methoxy phenol (BHA) and 2, 6-di-tert-butyl-4-methyl phenol (BHT), etc. are widely used in food industry. However, there are serious concerns about the carcinogenic potential of these substances [5]. Therefore, the use of natural antioxidants has been considered to replace for synthetic antioxidants in food products. Dietary antioxidants are the supplements that may delay or reduce the effects of oxidative stress. Phenolic compounds widely present in the plant kingdom [6] and are considered as natural antioxidants that play an important role in our diet. They can absorb and neutralize free radicals by donating a hydrogen atom from their hydroxyl groups[7].

Besides that, infectious diseases are the primarily threats that account for death worldwide. In the last decades, the clinical efficacy of many synthetic antibiotics is being threatened by the emergence of a serious problem which can be defined as multi- drug resistant pathogens [8]. Multi-drug resistance in both human and plant pathogenic microorganisms has developed due to the indiscriminate usage of commercial antimicrobial drugs that have widely applied to treatment of infectious diseases. Therefore, scientists have tried to discover new antimicrobial substances from various sources including spice plants. It is known that, natural products and their derivatives currently hold more than 50% of all the drugs in clinical usage with one quarter originating from higher plants [8]. The mechanisms for antimicrobial activity of phenolic compounds might be due to interacting with membrane proteins rendering deformation in structure and functionality [9]; varying alkyls into phenol nucleus [10]; varying microbial cell permeability and obstructing membrane functions including electron transport, nutrient uptake, protein and nucleic acid synthesis, and enzyme activity [11].

Spice plants including *Cinnamomum cassia* Presl, *Curcuma longa* L, *Zingiber officinale* (Willd.) Roscoe and *Piper nigrum* L have been used as spice herbs as well as traditional medicinal plants for long ages. Nowadays, there are several reports indicating that these spice plants possess antioxidant and antimicrobial activities [12, 13, 2]. However, there are a few data published on antioxidant and antimicrobial activities of these plants in

Vietnam. The aims of this study is to investigate the antioxidant and antimicrobial activities of some spice plants including cinamon bark, ginger root, curcuma root and piper leaves.

2. MATERIALS AND METHODS

2.1 Chemicals

The following chemicals were obtained from Sigma-Aldrich (St Louis, MO, USA): 2,2-diphenyl-2-picrylhydrazyl hydrate, gallic acid, folin-ciocalteu, potassium ferricyanide, sodium nitrite, and aluminum chloride. Other chemicals and reagents were of analytical grade.

2.2 Spice plants and preparation

The trunk-bark of cinnamon, roots of ginger, curcumin and leaves of piper were collected at Buon Don District, Daklak Province in June, 2015. After collection, the samples were washed, cut and dried at ambient temperature in a room with active ventilation, packed in PE bags and stored at -30^{0}C or directly used.

The dried samples were grinded and extracted with methanol as a ratio of 1:10 (dried sample/methanol) for 24hrs and repeated 3 times. The combinations of each MeOH extract from sample were evaporated to produce dried crude extracts.

2.3 Evaluation of antioxidant and antimicrobial activities

Free radical scavenging activity of the extracts against stable DPPH radical (2,2-diphenyl-2-picrylhydrazyl hydrate) was determined by a spectrophotometer using the method described by Nguyen and Eun (2011) [14].

The reducing power of the plant extracts was determined using a mothod described by Nguyen and Eun (2011) [14].

Antimicrobial activities were determined by Disc diffusion test [15].

2.4 Data analysis

All experiments were performed in triplicate (n=3), and an ANOVA test (using STATGRAPHICS Centurion XV statistical software) was used to compare the mean values of each treatment. Significant differences between the means of parameters were determined by LSD test ($p<0.05$). The results were presented as mean ± standard deviation (STD) of three replicated determinations.

3. RESULTS AND DISCUSSION

3.1 Antioxidant activity of investigated extracts

3.1.1 DPPH radical scavenging activity

The assessment of the antioxidant activity showed that all of the tested extracts were able to scavenge the DPPH radical at all the concentration studied. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule.

The results of the DPPH inhibition assay using different extracts and a control (L-ascorbic acid) are also shown in Table 1. In general, the scavenging activity increased with increasing concentration of tested extracts and ascorbic acid up to 300µg/ml. The tested spice extracts possessed stronger DPPH radical scavenging activity than that of ascorbic acid. The IC_{50} of cinnamon trunk-bark, curcumin root and ginger root extracts were 82.21 µg/ml, 155.19 µg/ml and 180.20 µg/ml, respectively compared to IC_{50} value of 185.9 µg/ml of positive control (ascorbic acid), which is inversely related to antioxidant activity.

3.1.2 Reducing power of some spice plants

Antioxidant activity depends on the metallic catalyst used to generate the reactive species (Roedig-penman & Gordan, 1998). In this study, to measure reductive capability, we investigated the Fe^{3+}- Fe^{2+} transformation in the presence of spice plant extracts using the method of Oyaizu (1986). The reducing capacity of a compound may serve as an indicator of its potential antioxidant activity. The presence of reducing compounds causes reduction of the Fe^{3+}/ferricyanide complex to ferrous ion (Fe^{2+}).

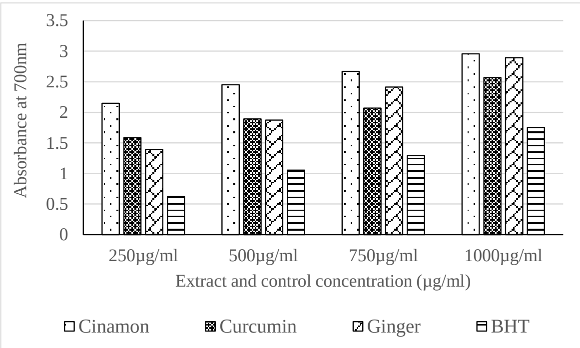

Figure 1. *Reducing power of investigated plant extracts*

Figure 1 represented the reducing power of investigated spice plants extracts as different concentration. Specifically, the reducing power of all extracts increased with increasing extract concentration, indicating that reducing agents were presented in these extracts. In general, all of the investigated extracts possessed stronger reducing power than positive control (BHT). Of these investigated extracts and positive control, the extract of cinnamon trunk-bark possessed the highest reducing power activity with the absorbent value of 2.96 at the concentration of 1000 µg/ml, which was 1.7 times higher than that of BHT of 1.75 at the same concentration. The lowest reducing power amongst the investigated extracts was found in curcuma extract with the absorbance value of 2.57 at the concentration of 1000 µg/ml.

Table 1. *DPPH radical scavenging activity of investigated plant extracts.*

Plant name (*)	Part of used	% DPPH radical scavenging activity				IC_{50} µg/ml
		50 µg/ml	100 µg/ml	200 µg/ml	300 µg/ml	
CCP	Trunk bark	32,83^{c}	66,33^{d}	81,46^{e}	90,44^{e}	82.21^{e}
CLL	Root	19,94^{b}	36,12^{b}	67,65^{c}	84,52^{d}	155.20^{c}
ZO	Root	17,11^{a}	36,75^{b}	64,41^{b}	79,59^{b}	165.20^{b}
PNL	Leaf	18,61^{b}	29,24^{a}	60,76^{a}	75,11^{a}	180.20^{a}
VC		33,206^{d}	49,309^{c}	76,197^{d}	82,898^{c}	114.40^{d}

(*) CCP (Cinnamomum cassia Presl), *CLL (Curcuma longa* L), *ZO (Zingiber officinale* (Willd.) Roscoe), *PNL (Piper nigrum* L), VC (Vitamin C)

Results are means ± SD of triplicate measurements. Different labels (a-e) in the same column indicate a significant difference at P<0.05.

Cinamon truk-bark, root of ginger and curcumar had been used as spice and colorant for long ages. Several researchers indicated that these extracts possessed antioxidant activity such as cinamon trunk-bark extract [16], root of giner [17] and root of curcuma [18].

3.2 Antibacterial activity of some spice plant extracts

The antimicrobial activity using the agar disc diffusion method of different spice plant extracts and control are represented in Table 2 the results indicated that almost all of the investigated spice plant extracts displayed the antimicrobial activity in regards to tested microorganisms (gram (-) *E. coli* và gram (+) *S. aureus*) at the concentration of 5 mg/disc. However, the antibacterial activity of these extracts was totally different from the investigated extracts. Amongst them, the highest effects to both investigated bacterial were found in the extract of cinnamon which had the inhibition zone diameter of 16.8 mm and 17.4 mm at a concentration of 5mg/disc against *S. aureus* and *E. coli*, respectively followed by root ginger and curcuma extracts and lowest effect was found in peper leaves extract. the results were accordance with the report of Huynh Kim Dieu 2011 [17] for the antibacterial of ginger and curcuma and Goni et al., 2009 [19] and Masih et al. 2012 [20] for cimamon extract and essencial oil. To confirm the antibacterial activity of the highest effect extracts, the experiment continued to carry out at a range of concentration of 1 mg/disc to 4 mg/disc. The results were represented in Table 3.

Table 2. *Antibacterial activity of investigated plant extracts.*

Plant name and control	**Conc.**	Inhibition zone diameter ***(mm)***	
		S. aureus	***E. coli***
Cinnamomum cassia Presl	5mg/disc	11.7^{bA}	11.4^{aB}
Curcuma longa L	5mg/ disc	11.2 ^{bA}	11.6^{aB}
Zingiber officinale (Willd.) Roscoe	5mg/ disc	16.8^{cA}	17.4^{cB}
Piper nigrum L	5mg/ disc	10.6^{aA}	12.1^{bB}
Streptomycin	0.5mg/disc	33.4^{eA}	33.5^{eB}
DMSO	10µl/disc	-	-

Results are means ± SD of triplicate measurements. Different labels (a-e) in the same column and (A – B) in the same raw indicate a significant difference at P<0.05; (-) no inhibition.

The inhibition zone diamete of cinnamon bark extract at different concentration was shown in Table 3. The results showed that increasing the treatment concentration from 1 to 4 mg/disc, the inhibition zone diameters against *S. aureus* and *E. Coli* increased in range of 10.4 mm to 14.1 mm and 9.7 mm to 14.3 mm, respectively. It indicated that there was the presence of broad spectrum antibiotic compounds in cinnamon bark extract.

3.3 Total poplyphenol contents in investigated plants

Phenolic compounds possessed the effects on antioxidant and related to inhibition of microorganisms, as well as played an important role in decreasing the risk of some diseases [4]. This led us to determine their total amount in selected spice plant extracts. Total phenolic contents varied widely in the selected medicinal plant extracts as shown in Table 4.

Table 3. *Antibacterial activity of cinnamon extract at different concentration.*

Plant name and control	**Conc.**	**Inhibition zone diameter (mm)**	
		S. aureus	***E. coli***
Cinnamomum cassia Presl	1mg/disc	10.4^{aA}	9.7^{aB}
	2mg/disc	11.8^{bA}	10.4^{bB}
	3mg/disc	12.2^{cA}	11.0^{cB}
	4mg/disc	14.1^{dA}	14.3^{dB}
Streptomycin	0.5mg/disc	34.4^{eA}	34.5^{eB}
DMSO	10µl/disc	-	-

Results are means ± SD of triplicate measurements. Different labels (a-e) in the same column and (A – B) in the same raw indicate a significant difference at P<0.05; (-) no inhibition.

Table 4. *Total poplyphenol contents in investigated plants.*

Plant name and control	**Total poplyphenol contents**(mg GAE/g)
Cinnamomum cassia Presl	140.03±0.026^{a}
Curcuma longa L	171.74±0.289^{c}
Zingiber officinale (Willd.) Roscoe	144.1±0.019^{b}
Piper nigrum L	199.59±0.037^{d}

Results are means ± SD of triplicate measurements. Different labels (a-d) in the same column indicate a significant difference at P<0.05

Results are means ± SD of triplicate measurements. Different labels (a-d) in the same column indicate a significant difference at P<0.05

Table 4 showed the different contents of total polyphenol the investigated spice plants. The results showed that the highest total of polyphenol content was found in the extract of piper leaves with the value of 199.59mg

GAE/g dried weight extract, followed by curcumin extracts (171.74mg GAE/g dried weight extract and ginger extract (144.1mg GAE/g dried weight extract). The lowest content was represented in cinnamon extract with the value of 140.03mg GAE/g dried weight extract. However, the antioxidant and antibacterial activities were showed in cinnamon extract and the lowest effects were found in piper extract (Data shown in Fig. 1, Table 1 and Table 2). It indicated that the antioxidant and antibacterial activities did not depend on the content of total polyphenol which is in accordance with previous study that found that the antimicrobial activity did not depend on the amount of total polyphenol, but depended on the structure of polyphenol compounds containing in plants [21]. However, it is contrast to the report of Nguyen and Eun, 2011 & 2013 [13-14] which indicated that antioxidant and antimicrobial activities correlated with total polyphenol content.

4. CONCLUSION

This work demonstrates antioxidant and antibacterial activities of spice plant extracts. The results have indicated that amongst four investigated spice extracts, cinnamon extract possessed the highest effect on both antioxidant and antibacterial activities. Further studies are required to determine the types of bioactive compounds in the effective extract, as well as the effective compounds are responsible for the antioxidant and antimicrobial activities of these spice plant extracts in the future.

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