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Original Article

Initial Evaluation of Antioxidant and Antibacterial Activities of Several Medicinal Plant Extracts Collected in Vietnam

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Abstract: The diversity of medicinal plants in Vietnam is a rich source to develop novel health products. In this study, the antioxidant and antimicrobial activities of ethanolic extracts of *Pluchea indica*, *Drynaria fortunei*, *Stephania glabra*, lemongrass (*Cymbopogon citratus*) and lime (*Citrus aurantifolia*) mixture, and ginger (*Zingiber officinale*) and kumquat (*Citrus japonica*) mixture, were evaluated. The extract of ginger and kumquat mixture exhibited the highest free radical scavenging activity (85%), followed by *P. indica* and *S. glabra* (60% and 53%, respectively) at the concentration of 0.1 mg/ml. Moreover, the agar well diffusion assay revealed that the extract of *S. glabra* and the extract of ginger and kumquat mixture were effective in inhibition against *Bacillus cereus*, *Staphylococcus aureus*, and *Vibrio parahaemolyticus*. Especially, the extract of *S. glabra* at the concentration of 100 mg/ml showed the highest inhibition zone diameter (13±1.73 mm) against *B. cereus*, which was significantly larger than that of Ceftriaxone (5 μg/ml) and other extracts.

Keywords: Medicinal plant, antioxidant, free radical scavenging, antibacterial activity.

1. Introduction

Plants contain a wide range of natural bioactive compounds that are good for human health due to their antioxidant and antibacterial activities [1, 2]. Various plants have been used as traditional medicines in different countries for bacterial infection treatments. Although antibiotics are the most effective in treatment of bacterial infections, other solutions should be developed because of the increase in antibiotic resistance. For example, *Staphylococcus aureus* is resistant to many common antibiotics, including penicillin, methicillin and oxacillin [3]. In addition, foodborne pathogens, *Bacillus cereus* and *Vibrio parahaemolyticus* are resistant to several antibiotics [4, 5]. Therefore, plant materials can be a supplement or an alternative therapy for conventional antibiotics.

Medicinal plants in Vietnam are diverse and can be a potential source to develop health products [1]. In fact, the Vietnamese people have a long tradition of using a variety of medicinal plants as decoction as well as tea and medicinal liquor. For instance, plants such as

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Pluchea indica, *Drynaria fortunei*, *Stephania glabra*, are components of many traditional remedies and health care products [2]. Some commonly used spices such as ginger (*Zingiber officinale*), lime (*Citrus aurantifolia*), kumquat (*Citrus japonica*) and lemongrass (*Cymbopogon citratus*) have been used to treat coughs, sore throats, colds and stomachaches [2]. Several studies have evaluated biological activity of these spices but there are few study on their mixtures although they have often been used in combination.

In the present study, the antioxidant and antimicrobial potential of ethanolic extracts of *P. indica*, *D. fortunei*, *S. glabra*, the mixture of lemongrass and lime, and the mixture of ginger and kumquat, were investigated.

2. Methodology

2.1. Plant Materials

All plant materials were collected in Vietnam (Table 1). Plant materials of *P. indica*, *D. fortunei*, *S. glabra* were traditionally dried under shade at temperature of about 35-37 ^{o}C for 2 weeks before being grinded to powder. To prepare the mixture of lemongrass and lime, thin slices of fresh lemongrass stalks were put into the peels of lime (at the ratio of 1:1 in fresh weight), which were then dried at 55 ^{o}C for 5 days and were subsequently grinded to achieve a powder mixture. The mixture of ginger tuber and kumquat was prepared similarly.

Table 1. Origin of plant materials

Sample	**Collected part**	**Location**
P. indica	Leaves and young stem	Hanoi
D. fortunei	Rhizome	Bac Kan
S. glabra	Tuber	Bac Kan
Ginger	Tuber	Bac Kan
Lemongrass	Stalk	Bac Kan
Lime	Fruit	Bac Kan
Kumquat	Fruit	Bac Kan

2.2. Preparation of Plant Extracts

Dried powder of plants (20 g) was extracted with 200 ml ethanol for 24 hours in a flask, on a shaker at room temperature, followed by filtration through filter paper. The extraction step was repeated 3 times. The combined extracts were concentrated by vacuum rotary evaporator (RE300 Bibby Stuart) to obtain dry extracts. Dry extracts were dissolved in dimethyl sulfoxide (DMSO) 100% to obtain a proper test concentration.

2.3. Bacterial Strains

Three strains of bacteria, namely *Bacillus cereus*, *Staphylococcus aureus* and *Vibrio parahaemolyticus* preserved at -20 ^{o}C were provided by the Department of Microbiology, VNU University of Science. Bacterial strains were grown in Luria-Bertani (LB) broth medium, in a shaking incubator (180 rpm) at 37 ^{o}C for 24 hours before the antimicrobial test.

2.4. Agar Well Diffusion Assay

The agar diffusion assay was carried out as described by Athanassiadis et al., [6] with minor modifications. Suspension of bacteria (50 µl, OD_{600} = 0.1-0.3), was spread on petri dishes containing 15 ml LB agar medium and incubated at 37 ^{o}C for 24 hours. Then, seven wells (6 mm in diameter) were punched on each agar plate. Among the wells, five wells were filled with 20 µl extracts with a final concentration of 100, 50, 25, 17.5, and 8.75 mg/ml. For the negative and positive control, two wells were filled with 20 µl DMSO 100% and 20 µl antibiotic Ceftriaxone (5 µg/ml), respectively. After incubation for 24 hours, the inhibition zone diameter was recorded. The experiment was carried out in triplicate.

2.5. DPPH Radical Scavenging Assay

The 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical scavenging assay was performed in triplicate as described by Okawa et al., [7] with some modifications. The plant

extracts were diluted into tested concentrations (0.02 and 0.1 mg/ml). Samples (10 µl) were incubated with 190 µL (0.1 mM) DPPH dissolved in methanol at 37 ^{o}C for 20 min. The absorbance was read at 517 nm. Ascorbic acid dissolved in distilled water at 0.01 and 0.0025 µM was used as the positive control. The percentage of free radicals scavenging was calculated as followed: %Scavenging = 100 - [(ODs/ODc) x 100%], where ODs is an average optical density of the sample, ODc is an average optical density of the control.

2.6. Statistical Analysis

Data analysis was carried out using R version 4.1. Comparison between treatments was performed using one-way analysis of variance (ANOVA), followed by a Tukey test.

3. Results and Discussion

3.1. Free Radical Scavenging Activity

The free radical scavenging activity of ethanolic extracts of investigated plants are shown in Figure 1. Among the extracts, the mixture of ginger and kumquat possessed the highest activity (32% and 85% at the concentration of 0.02 and 0.1 mg/ml, respectively). The mixture of lemongrass and lime had the lowest activity (0% and 8% at the concentration of 0.02 and 0.1 mg/ml, respectively). At concentration of 0.02 mg/ml, three among five extracts were mixture of ginger and kumquat, *D. fortunei* and *S. glabra* had higher radical scavenging activity than 0.0025 µM ascorbic acid. At concentration of 0.1 mg/ml, extract of mixture of ginger and kumquat and extract of *P. indica* had higher radical scavenging activity than 0.01 µM ascorbic acid.

Although the percentage of free radical scavenging of *P. indica* extract (8%) was lower than that of *D. fortunei* and *S. glabra* at the concentration of 0.02 mg/ml, it was highest (60%) among 3 extracts at the concentration of 0.1 mg/ml. When the concentration increased 5 folds (from 0.02 to 0.1 mg/l), *P. indica* shoot extract increased its DPPH radical scavenging activity by 7.5 times, while extracts of *D. fortunei* rhizome and *S. glabra* tuber increased by 2.07 and 2.21 times, respectively. Possessing the highest free radical scavenging activity, the mixture of ginger and kumquat extract only induced approximately 2.66 times of scavenging enhancement. A previous study showed that the methanolic extract of *P. indica* root had antioxidant activity in radical hydroxyl (OH) and hydrogen peroxide (H_2O_2) scavenging assays [8]. In this study, the extract of *P. indica*'s aerial part also exhibited a radical scavenging activity. Therefore, *P. indica* is a potent source of antioxidants.

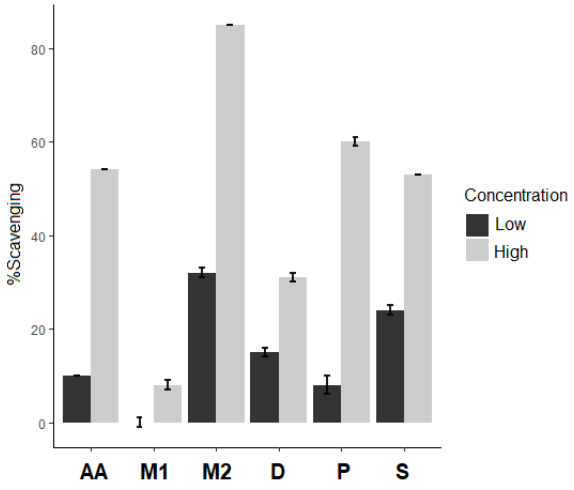

Figure 1. DPPH radical scavenging activity. AA: Ascorbic acid (control), M1: mixture of lemongrass and lime, M2: mixture of ginger and kumquat, D: *D. fortunei*, P: *P. indica*, S: *S. glabra*. Low concentration: AA at 0.0025 µM and extracts at 0.02 mg/ml. High concentration: AA at 0.01 µM and extracts at 0.1 mg/ml. Bars represent means ± SD.

Besides the ethanolic extract (this study), aqueous extract of *D. fortunei* also showed antioxidant effect in previous studies [9]. Regarding *S. glabra*, the results from this study agreed with other studies about the antioxidant activity of *S. glabra* and other plants belonging to *Stephania* genus [10].

The extract of lemongrass and lime possessed a weak antioxidant activity. Balakrishnan et al., (2014) showed that the percentage of free radical scavenging of lemongrass extract ranged from 10-40% in DPPH assay [11]. In the mentioned study, chloroform, methanol and water were used as solvents [11], while ethanol was used to prepare plant extracts in this study. Therefore, the antioxidant activity of lemongrass in the present study can be in a lower range. Additionally, according to Phi et al., (2015), the secondary metabolite profile of lime varies considerably depending on growth locations, that can lead to differences in antioxidant activity [12]. The authors reported significant differences in the amount of chemical compositions such as α-terpinene, between limes from two provinces in Vietnam, Long An and Da Lat [12].

The strong antioxidant activity of the ginger and kumquat mixture extract can be explained by the high antioxidant activity of ginger and kumquat alone. The extract of kumquat peel at the concentration of 1000 µg/100 µl possessed a high scavenging percentage (64.98%) in a DPPH assay [13]. Stoilova et al., (2007) showed that the extract of ginger in Vietnam scavenged DPPH radical up to 90.1% at the concentration of 0.02 mg/ml [14]. It is higher than the percentage of free radicals scavenging of ginger and kumquat mixture at concentration 0.02 mg/ml in this study (32%). However, there are differences related to the preparation of plant extracts and DPPH assay between the study by Stoilova et al., (2007) and the present study. Hence, further study is needed to compare the antioxidant effect between ginger and kumquat mixture and ginger alone while they have been traditionally used together.

3.2. Antibacterial Activity

The tested plant extracts differ in their antibacterial activity (Table 2). The extract of *S. glabra* and ginger and kumquat mixture can inhibit the growth of both Gram-positive bacteria (*S. aureus*, *B. cereus*) and Gram-negative bacteria (*V. parahaemolyticus*), at all the concentrations. The extract of lemongrass and lime mixture showed a weak antibacterial activity. Its inhibition zone can be observed only at high concentration (50 and 100 mg/ml), and the inhibition zone diameter was small (1.67-4.33 mm at concentration 100 mg/ml). The extract of *D. fortunei* can inhibit the growth of *B. cereus* and *V. parahaemolyticus*, but it did not inhibit *S. aureus*. The extract of *P. indica* only showed inhibition against *B. cereus*.

The higher means of inhibition zone diameter can be seen at higher concentrations of extract. However, the differences in the diameter among extract concentrations are not always significant. For example, the inhibition zone diameter at different concentrations of *P. indica* extract against *B. cereus* and that of ginger and kumquat mixture extract against *S. aureus*, are not statistically significant.

Regarding the bacterial strain *B. cereus*, all plant extracts showed antibacterial activities at different levels. At the concentration of 100 mg/ml, the extract of *S. glabra* showed the highest inhibition zone diameter (13±1.73 mm), which was significantly larger than that of control (9.33±0.58 mm) and other extracts. The lemongrass and lime mixture extract did not produce an inhibition ring at the concentration of 25 mg/ml or lower.

The growth of *S. aureus* can be suppressed by *S. glabra* or mixture of ginger and kumquat extract, although the inhibition rings produced by these extracts were significantly smaller than that of control. The *P. indica* and mixture of lemongrass and lime extract only produced a tiny inhibition zone against *S. aureus* at the concentration of 100 mg/ml.

For *V. parahaemolyticus*, the *S. glabra* extract at the concentration from 17.5 to 100 mg/ml was similar to the control (5 µg/ml Ceftriaxone) in antibacterial activity. There were no significant differences in inhibition zone diameter between the control and 100 mg/ml of *D. fortunei* or mixture of ginger and kumquat extract, though the mean values of these extracts were smaller than that of control.

Table 2. Antibacterial activity of the plant extracts against *B. cereus*, *S. aureus* and *V. parahaemolyticus* (Means of Inhibition zone diameters ± SD in mm)

B. cereus					
Concentration of extracts	Lemongrass + lime	Ginger + kumquat	*D. fortunei*	*P. indica*	*S. glabra*
100 mg/ml	$^{A}2.67\pm2.31^{a}$	$^{B}6.67\pm0.58^{a}$	$^{AB}7.00\pm1.00^{a}$	$^{A}3.33\pm3.06^{a}$	$^{A}13.00\pm1.73^{b}$
50 mg/ml	$^{A}0.67\pm0.58^{c}$	$^{B}6.33\pm0.58^{b}$	$^{BC}5.67\pm1.15^{b}$	$^{A}2.33\pm2.08^{c}$	$^{AB}11.33\pm1.15^{a}$
25 mg/ml	0	$^{B}5.53\pm0.58^{b}$	$^{BC}4.33\pm0.58^{bc}$	$^{A}2.00\pm1.73^{c}$	$^{BC}10.00\pm1.00^{a}$
17.5 mg/ml	0	$^{BC}4.33\pm0.58^{a}$	$^{C}2.67\pm1.53^{a}$	$^{A}2.00\pm1.73^{a}$	$^{BC}9.00\pm0^{b}$
8.75 mg/ml	0	$^{C}2.33\pm1.53^{ab}$	$^{C}1.67\pm1.53^{ab}$	$^{A}1.33\pm1.53^{b}$	$^{C}8.00\pm0^{a}$
Control (Ceftriaxone)	$^{B}8.00\pm1.00^{a}$	$^{A}10.67\pm1.53^{a}$	$^{A}10.00\pm1.73^{a}$	$^{B}11.33\pm1.53^{a}$	$^{BC}9.33\pm0.58^{a}$
S. aureus					
Concentration of extracts	Lemongrass + lime	Ginger + kumquat	*D. fortunei*	*P. indica*	*S. glabra*
100 mg/ml	$^{A}4.33\pm0.58^{b}$	$^{B}3.67\pm0.58^{bc}$	0	$^{A}1\pm0^{c}$	$^{B}16.33\pm2.31^{a}$
50 mg/ml	0	$^{B}2.00\pm1.00^{a}$	0	0	$^{BC}14.33\pm0.58^{b}$
25 mg/ml	0	$^{BC}1.33\pm0.58^{a}$	0	0	$^{CD}12.33\pm1.15$
17.5 mg/ml	0	$^{BC}1.33\pm0.58^{a}$	0	0	$^{D}9.33\pm1.53^{b}$
8.75 mg/ml	0	$^{BC}0.67\pm0.58^{a}$	0	0	$^{D}7.67\pm1.53^{b}$
Control (Ceftriaxone)	$^{B}24.33\pm1.53^{a}$	$^{A}24.67\pm0.58^{a}$	23.33 ± 0.58^{a}	$^{B}24.00\pm0^{a}$	$^{A}23.67\pm0.58^{a}$
V. parahaemolyticus					
Concentration of extracts	Lemongrass + lime	Ginger + kumquat	*D. fortunei*	*P. indica*	*S. glabra*
100 mg/ml	$^{A}1.67\pm2.89^{c}$	$^{AB}8.33\pm3.21^{ab}$	$^{AB}6.67\pm0.58^{bc}$	0	$^{A}13.67\pm1.53^{a}$
50 mg/ml	$^{A}1.00\pm1.73^{c}$	$^{AB}6.67\pm2.08^{ab}$	$^{B}5.67\pm1.15^{bc}$	0	$^{AB}11.67\pm2.52^{a}$
25 mg/ml	0	$^{AB}5.67\pm2.08^{a}$	$^{BC}4.00\pm1.00^{a}$	0	$^{AB}10.67\pm1.53^{b}$
17.5 mg/ml	0	$^{B}4.33\pm2.31^{a}$	$^{CD}1.67\pm1.53^{a}$	0	$^{AB}9.33\pm1.53^{b}$
8.75 mg/ml	0	$^{B}3.00\pm3.00^{a}$	$^{D}0.67\pm1.15^{a}$	0	$^{B}8.33\pm1.53^{b}$
Control (Ceftriaxone)	$^{B}9.00\pm2.65^{a}$	$^{A}13.00\pm3.61^{a}$	$^{A}9.33\pm1.53^{a}$	9.33 ± 3.51^{a}	$^{AB}10.67\pm1.53^{a}$

Uppercase and lowercase letters indicate significant differences in a same column and row, respectively.

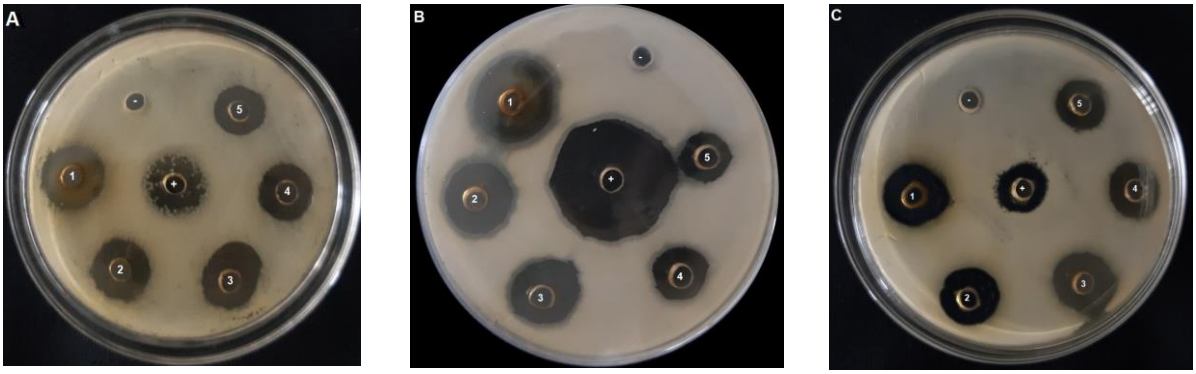

Figure 2. Antibacterial activity of *S. glabra* against *B. cereus* (A), *S. aureus* (B) and *V. parahaemolyticus* (C). 1-5: *S. glabra* extract at the concentration of 100, 50, 25, 17.5, and 8.75 mg/ml, respectively; -: DMSO 100%; +: Ceftriaxone 5 µg/ml.

As shown in this study, ethanolic extract of mixture of lemongrass and lime was not an effective antibacterial agent against *S. aureus*, *B. cereus* and *V. parahaemolyticus*. However, aqueous and methanolic extract of lemongrass can inhibit the growth of *S. aureus* in a previous study [11]. The essential oil of lime from Vietnam also suppressed *S. aureus* and *B. cereus* in an agar disk diffusion assay [12]. It illuminates why lemongrass and lime (also lemon) are rarely pickled in wine.

The extract of ginger and kumquat mixture produced inhibition rings against all tested bacteria although the inhibition zone diameters were lower than that of the control. Moreover, Gao et al., [15] showed that ethanolic extract of ginger can inhibit the growth of *S. aureus* and several Gram-negative bacteria, but its antibacterial activity was not strong. Al-Saman et al., [13] reported that ethanolic extract of kumquat peel can inhibit *S. aureus*, however, kumquat in that study belonging to margarita variety, is different from the plant materials in the present study.

Previous studies showed the antibacterial activity of methanolic or other solvents extract of *D. fortune* against bacteria [16]. Nevertheless, the antibacterial effect of ethanolic extract of *D. fortune* is rarely evaluated. This study showed that 100 mg/ml *D. fortune* extract was similar to the antibiotic ceftriaxone (5 μg/ml) in inhibition against *B. cereus* and *V. parahaemolyticus*.

Methanolic root extract of tissue cultured *P. indica* possessed antibacterial activity against some bacteria, including *S. aureus* and *V. parahaemolyticus* [17]. However, the results in this study showed that ethanolic shoot extract of *P. indica* almost did not inhibit the growth of *S. aureus* and *V. parahaemolyticus*.

The extract of *S. glabra* in this study showed a strong antibacterial activity against *S. aureus*, *B. cereus* and *V. parahaemolyticus* (Figure 2). Semwal et al., [18] reported the antibacterial and antifungal activities of *S. glabra* extract, but *B. cereus* and *V. parahaemolyticus* were not included in that study.

4. Conclusion

In this study, the antioxidant and antimicrobial activities of five ethanolic extracts of medicinal plants in Vietnam were evaluated. Among them, the extract of ginger and kumquat mixture, *S. glabra* and *P. indica* possessed a high free radical scavenging activity. The extract of *S. glabra* was an effective antibacterial agent against *S. aureus*, *B. cereus* and *V. parahaemolyticus*. Further studies are required to determine minimum inhibitory concentration of the extracts against bacteria as well as the compounds responsible for the antimicrobial activity in these plant extracts.

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