International Journal of Pharmaceutical Science and Research
ISSN: 2455-4685; Impact Factor: RJIF 5.28
Received: 08-02-2019; Accepted: 13-03-2019
www.pharmacyjournal.net
Volume 4; Issue 2; April 2019; Page No. 32-36

Antioxidant and antimicrobial activities of ethanolic extracts of onions (*Allium cepa*) and shallots (*Allium ascalonicum*) cultivated in Vietnam

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Abstract
The objective of this study was to evaluate the antimicrobial and antioxidant capacities of four *Allium* species grown in Vietnam including purple onion, white onion, purple shallot and white shallot. Under ethanolic extraction, the highest total phenolic and total flavonoid content was detected in purple onion (22.66 ± 1.35 mg FAE/g dried sample and 11.00 ± 3.74 mg RE/g dried sample, respectively), which correlated to the strong antioxidant activity (84.70% for 50 mg/ml extract concentration) measured by DPPH-radical scavenging assay; white onion ranked second, neutralized 67.82% DPPH-radical; while purple shallot and white shallot ranked third for three mentioned criteria. The antimicrobial activities were tested against two gram-positive pathogenic bacteria: *S. aureus* and *B. cereus* (VTCCB-1005); and two gram-negative pathogenic bacteria: *P. aeruginosa* (ATCC-9027) and *S. typhi* - by agar well diffusion method and the dilution method for minimum inhibitory concentration (MIC). Although not being a prominent antioxidant in four test subjects, white shallot was appreciated for expressing the best antimicrobial activity, followed by purple onion and white onion. *S. typhi* resisted against all extracts while purple shallot showed no antimicrobial activity against reference strains. The distinction in growth suppression between gram negative and gram positive bacteria were not significant.

Keywords: onion, shallot, antioxidant, antimicrobial, phenolic compound

1. Introduction

Allium species is one of the oldest cultivated vegetables, belongs to the genus *Alliaceae* family. The most familiar and notable members are onion, garlic, leek, and shallot [1]. With more than 700 wild and domestic varieties identified all over the world, *Allium* species are mostly distributed in temperance climate, subtropical regions with limited water supply such as Central Asia and Southeast Asia [2], including Vietnam. For thousand years they have been attended widely in human diet as spices and vegetables; in home remedies against minor diseases such as cough, flu, indigestion, etc.; or as therapeutic herbs in alternative medicinal treatment [3]. Lately scientific evidences have confirmed that *Allium* species possess a wide range of antimicrobial, antithrombotic, antitumor and anticancer, hypo-lipidemic, anti-arthritic, anti-inflammatory and hypoglycemic properties [3].

Studies have shown that consuming vegetables and fruits could lower the risk of chronic diseases due to the intake of phenolic compounds. High phenolic content is often associated with high antioxidant activity. These compounds exhibit potent antioxidant power through action of chelating metal ion, neutralizing free radicals and inhibiting lipid peroxidation [4], result in as a lowered risk of coronary heart diseases and various types of cancer. Flavonoids are phenolic compounds and responsible for various functions in plant including formation of color pigment, protection from UV radiation, defensing against microorganisms causing plants diseases, and for fertility. Being flavonoid compounds, anthocyanins contributes as red/purple pigments while quercetin and its derivatives contribute primarily to the brown skin or yellow color of flesh of onion [5]. Moreover, study by Singh *et al*. [6] reported that the total phenolic compounds and total antioxidant activity of the outer layers of *Allium* bulbs are higher than the flesh and reduce towards the inner edible part of the bulbs, mainly due to the distribution of color pigments outside.

Organo-sulfur and saponins are best known to be effective antimicrobial compounds from plant. Organo-sulfur compounds are precursors of chemicals defining the pungent, characteristic odor, taste and flavor of onion and shallot. Over 50 sulfur-containing compounds will be produced in the reactions happen when onion or shallot is chopped down and cell walls are disrupted [3]. Saponins are a diverse group of plant-derived bioactive compounds. Saponins taste bitter and play a role in defensive mechanism of plant. Different saponins have been identified in different *Allium* species. Saponins family possesses potent antimicrobial and anti-inflammatory activities [7]. Besides, antioxidants such as flavonoids also contribute to antimicrobial activity, which is validated in the case of onion [8].

The current project shifts attention to the Vietnamese varieties of onion and shallot. Although the exceptional antioxidant and antimicrobial activity of onion and shallot have been known, there is not enough study conducted on

different varieties of shallot as well as the Vietnamese species. Therefore, the objective of this study was to evaluate the antimicrobial and antioxidant capacities of four *Allium* species grown in Vietnam including purple onion, white onion, purple shallot and white shallot.

2. Materials and Methods

2.1 Materials

Four types of Vietnamese onion and shallot species: purple onion (*Allium cepa*), white onion (*Allium cepa*), purple shallot (*Allium ascalonicum*) and white shallot (*Allium ascalonicum*) were purchased from local supermarket. The two kinds of onions were cultivated in Đà Lạt province, purple shallot was from Sóc Trăng province, and white shallot which has red-brownish outer skin was retrieved from Hải Dương province of Vietnam.

Initial treatment of onions and shallots followed method of Lu *et al*. [9]. Raw materials were selected to be free from blemish or defects. The bulbs were wrapped in plastic bag and stored at 4 ºC within 2 weeks until working. Next, the outer skin of the bulbs was removed manually, only edible flesh was kept. They were sliced into 2-4 mm thickness, put in a freezing chamber of -20 ºC for three days, and dried in a vacuum freeze-drier. Dried samples were ground in a blender to obtain fine powder. The powder was put in air-tight plastic bag and stored at 4 ºC for further analysis.

All chemicals and reagents used were of analytical-grade and purchased from local agents (Merck and Sigma) in Vietnam.

2.2 Extraction of crude extracts

Extraction of bioactive compounds of onion and shallot was modified from the method of Hung *et al*. [10]. Two grams of each freeze-fried sample was dissolved in 20 ml diluted ethanol (75:25 v/v with distilled water) and extracted in an orbital shaker for 20 min at room temperature (ca. 24 ºC). Next, the suspension was centrifuged and supernatant was recovered. Solid remained was extracted for two more times, with another 20 ml solvent each time. All supernatant eventually was combined. Light exposure was avoided during the extraction process.

Solvent was evaporated completely from the collected solution using the rotary evaporator (45 ºC, 150 rpm) at reduced pressure. Volume of the concentrated extracts was adjusted to 12 ml with sterilized distilled water. The solution was stored in the dark at -20 ºC until analysis. Extraction was performed in duplicate.

2.3 Determination of total phenolic compounds (TPC)

The amount of phenolic compounds in extract solution was measured by colorimetric method, followed procedures of Hung *et al*. [10]. A volume of 0.5 ml methanolic-diluted extract solution was added to 0.5 ml Folin-Ciocalteu reagent; 1 ml Na_2CO_3 13%; 8 ml distilled water and thoroughly mixed. The solution was incubated for 45 min in the dark at room temperature. Then, it was centrifuged at 4000g for 5 min and supernatant was recovered for measuring the absorbance at 725 nm.

Control blank was prepared with methanol. Calibration curve was built with Ferulic acid of varying concentration: 0, 20, 40, 60, 80, 100 µg/ml. Total phenolic compounds were expressed as milligram of Ferulic acid equivalent per gram of dry matter of sample (mg FAE/g dried sample).

2.4 Determination of total flavonoid compounds

The concentration of flavonoid compounds in extract solution was also measured by colorimetric method followed procedures of Hung *et al*. [10]. A volume of 0.5 ml methanolic-diluted extract was mixed with 1.5 ml 95% ethanol; 0.1 ml $AlCl_3$ 10%; 0.1 ml CH_3COOK 1M; 2.8 ml distilled water and thoroughly mixed. The solution was incubated for 30 min in the dark at room temperature. The absorbance of the solution was measured by UV-spectrophotometer at 415 nm.

Control blank was prepared with methanol. Calibration curve was built with Rutin of varying concentration: 0, 20, 40, 60, 80, 100 µg/ml. Total flavonoid compounds were expressed as milligram of Rutin equivalent per gram of dry matter of sample (mg RE/g dried sample).

2.5 DPPH-radical scavenging assay

Antioxidant activity was determined by DPPH-radical scavenging assay, followed procedure described by Huang *et al*. [11]. Stock extract solution was first diluted to half in methanol and 15 µM DPPH solution was also prepared by dissolving DPPH in absolute methanol. A volume of 0.1 ml of each diluted extract was mixed with 3.9 ml DPPH solution. The mixture is incubated for 30 min in the dark at room temperature. Absorbance is measured with UV-spectrophotometer at 515 nm. The control blank is prepared with methanolic solvent and measured absorbance immediately without incubation. The inhibition effect was calculated by the following equation:

$$Inhibition\ (\%) = \frac{absorbance_{control} - absorbance_{sample}}{absorbance_{control}} x\ 100$$

To understand the capacity of onion / shallot sample to scavenge DPPH radical, standard vitamin C was used for comparison. Vitamin C of 0, 30, 60, 120 µg/ml was dissolved in methanol and similar step of DPPH assay was performed.

2.6 Determination of antimicrobial activities

2.6.1 Microbial strains

Antimicrobial activity of the extract solution was tested against four pathogenic bacterial strains. These were two gram-positive bacteria: *Staphylococcus aureus* (provided by Marine Biotechnology Laboratory of International University) and *Bacillus cereus* (VTCCB-1005, provided by Vietnam National University Institute of Microbiology and Biotechnology); and two gram-negative bacteria: *Pseudomonas aeruginosa* (ATCC-9027) and *Salmonella typhi* (both provided by Institute of Drug Quality Control – Ho Chi Minh City). These are frequent food-borne bacterial pathogens.

The inhibitory effect was measured by agar well diffusion method and minimum inhibitory concentration (MIC). Procedures in both testing method followed by recommendation of National Committee for Clinical Laboratory Standards (NCCLS, 1997a and 1997b).

2.6.2 Agar well diffusion test

The effect of various plant extracts on several bacterial strains was measured by agar well diffusion test, followed procedures recommended by National Committee for Clinical Laboratory Standards [12].

The TSA petri plates were prepared using 90 mm petri discs containing 22 ml TSA medium giving a depth of 3 mm. Next, the 9 mm-diameter wells were bored in the agar plates and culture suspension of respective bacteria (10^6 CFU/ml) was swabbed over the plate. A volume of 100 µl of each extract solution was poured into each well. For the negative control, sterilized distilled water was poured into a separated well. Totally five wells were made to test four sample solution and one was for the negative control. The plates were incubated at 37 ^oC for 24 h. The clearance zone around each well signifies bacterial inhibition. Antimicrobial activity of different test solution was assessed by measuring the diameter of inhibition zone in millimeter (mm), including 9 mm of the well.

2.6.3 Minimum inhibitory concentration test

In this study the broth dilution method was employed to study the MIC of onion and shallot extract. The test followed procedures recommended by National Committee for Clinical Laboratory Standards [12].

Concentration of extract compounds in the obtained solution was determined by drying 1ml solution in the oven for 24 h and recorded the weight of solid remained. For each onion or shallot sample, stock solution was adjusted to the same concentration of 50 mg/ml with sterilized distilled water. A series of diluted extract in sterilized distilled water were also prepared by ratio of 100%, 50%, 25% and 12.5%. Next, 4 ml TSB medium was added with 500 µl diluted extract solution and 500 µl bacterial cultures (10^6 CFU/ml) was inoculated to make up to totally 5 ml. The suspension is agitated continuously during incubation period at 37 ^oC for 24 h. There are three controls in the experiment: the negative-control tubes with growth medium (TSB) and extract solution without adding microorganism; the positive-control tubes with growth medium and bacterial cultures; and solvent-control tubes with alcoholic solvent, growth medium and bacterial cultures. After incubation, 100 µl of each tube is spread on TSA plate and incubated for another 24 h prior to MIC determination. Minimum inhibitory concentration (MIC) is determined by the lowest concentration of the extract that is able to inhibit the apparent growth of test microorganism (or <3 colonies are observed).

2.7 Statistical analysis

All experiments were done at least in duplicate and the results were presented as the average value. Analysis of variance (ANOVA) was used to analyze data by SPSS and Excel software.

3. Results and Discussion

3.1 Total phenolic and total flavonoid contents of onion and shallot extracts

The average total phenolic content (TPC) and total flavonoid content (TFC) of crude extracts of purple onion, white onion, purple shallot and white shallot are summarized in Table 1.

Table 1: TPC and TFC of crude extracts of purple onion, white onion, purple shallot and white shallot

Sample	TPC	TFC
Purple shallot	11.61 ± 1.27a	4.96 ± 0.34d
Purple onion	22.66 ± 1.35b	11.00 ± 3.74e
White shallot	10.01 ± 0.31a	3.04 ± 0.34f
White onion	18.66 ± 0.76c	9.95 ± 0.76g

Data followed by the same letter within a column were not significantly different ($p<0.05$).

Purple onion had the highest content of total phenolic and total flavonoid compounds (22.66 ± 1.35 mg FAE/g dried sample and 11.00 ± 3.74 mg RE/g dried sample, respectively), followed by white onion (18.66 ± 0.76 mg FAE/g dried sample and 9.95 ± 0.76 mg RE/g dried sample, respectively). While white shallot and purple shallot had the least TPC and TFC content. Study of Lu *et al.* [9] stated similar results in comparing the red and white onion. Total phenolic content of purple shallot and white shallot showed no significant difference at $p<0.05$. TFC was arranges in descending order as purple onion > white onion > purple shallot > white shallot. TFC of all four *Allium* species in the test were statistically different from each other.

According to Gorinstein *et al.* [13], onion is a rich source of polyphenols, flavonoids, flavonol, and tannin among species of onion. The main flavonoid compounds of both onion and shallot are anthocyanin, kaempferol, and quercetin and its conjugates. Anthocyanin, although contributes to the red/purple pigments of *Allium* bulbs, concentrates in the outer skin and is only a minor constituents in the edible portion. Kaempferol presents in much smaller amount than quercetin. The quercetin level of purple onion is 14-fold greater than that of garlic and two-fold greater than that of white onion [13] while its outer skin contains 48-fold the flavonoid level comparing to the edible flesh.

Regardless of color, it was found that onion had higher TPC and TFC value than shallot of Vietnamese varieties. Contradicting to study of Lu *et al.* [9], the TPC, TFC and antioxidant value of shallot was significantly higher than four varieties of onion in their test. Further analysis of chemical composition may be necessary to verify the case. In fact, the biosynthesis of phytochemicals are affected, not only by genetic variation, but also by different cultivating conditions, weather condition, plant location, harvesting season, etc. which may explain for the finding. The flavonoids contents will directly affect the exhibition of TPC and antioxidant activity of plants [14].

3.3 Antioxidant activity of onion and shallot extracts

Antioxidant capacity exhibits through different mechanisms, such as transfer a hydrogen or an electron to radical, deactivate singlet oxygen, oxidize enzymes, chelate the transition metals, or detoxify enzyme of reactive oxygen species [15]. The radical-scavenging capacity of DPPH is a simple, sensitive and popular technique to assess the antioxidant activity of various materials, including plants. DPPH assay measures total antioxidant capacity by both the ability to donate an electron or a hydrogen atom [9]. DPPH• is a stable organic nitrogen radical and is a receiver [16]. In the DPPH assay, antioxidants are able to reduce DPPH radical to the yellow colored diphenylpicryhydrazine.

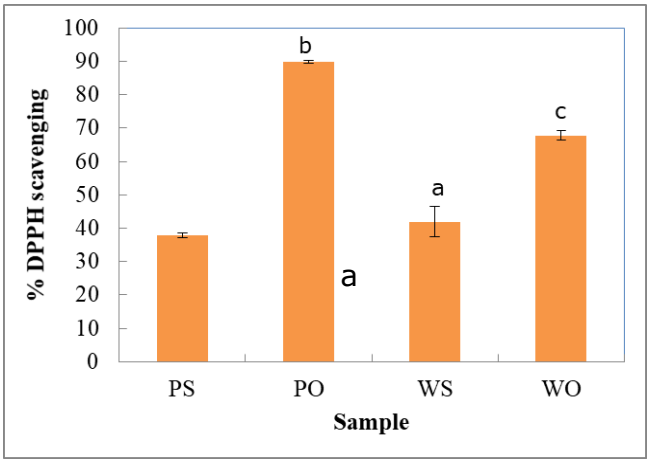

Fig 1: Inhibition percentage of DPPH-radical scavenging by different Vietnamese onion and shallot varieties. PS, purple shallot; PO, purple onion; WS, white shallot; WO, white onion.

Upon half dilution (50 mg/ml) all *Allium* sample displayed an antioxidant activity, as showed in Figure 1. Purple onion exhibited great antioxidant capacity, which is 89.74 ± 0.43% inhibition. For purple onion, the purple color of DPPH-radical turned completely into the yellow color of neutralized form DPPH-H. Inhibition percentage of the extract was ranked as following: purple onion > white onion > white shallot and purple shallot; with purple onion and white onion had neutralized more than 50% of the free radical. Purple shallot and white shallot had statistically similar antioxidant capacity; and were generally less powerful than both kinds of onion. Onions possess a high content of flavonoid compounds (quercetin and its conjugates) and sulfur compounds (e.g thiosulphinates), both of which contribute to a high level of antioxidant activity [5]. Therefore, onions, particular the purple ones may exert important protective affects against oxidative stress related diseases. In general, all *Allium* species in the study were recommended for their great value as antioxidant materials. Considering shallots, another finding of Leelarungrayub *et al*. [17] indicated that largely dependence on the phenolic compounds and diallyl disulfide in the bulbs, shallot extracts can function as a potential source of antioxidant.

In order to understand the antioxidant capacity of onion / shallot extract, standard Vitamin C was prepared. At concentration 50 µg/ml, Vitamin C was able to inhibit 11.23% DPPH radical. Onion and shallot extract at 50 mg/ml was 1000-times more concentrated than Vitamin C. The amount Vitamin C required to produce the same inhibition percentage as onion and shallot extracts were as following: purple shallot (89.74 ± 0.43% equivalent to 399.6 µg/ml of vitamin C), white onion (67.82 ± 1.52% equivalent to 302 µg/ml of vitamin C), white shallot (41.97 ± 4.42% equivalent to 186.7 µg/ml of vitamin C), and purple shallot (37.93 ± 0.75% equivalent to 164.7 µg/ml of vitamin C).

3.3 Antimicrobial activities of onion and shallot extracts

The average inhibition diameter and MIC of purple onion, white onion, purple shallot and white shallot are summarized in Tables 2 and 3. Among four microorganisms, *S. aureus* was the most sensitive bacteria to alcoholic extract of four *Allium* samples, except for purple shallot which was not effective. In study of Ye *et al*. [18] tested on nine different bacteria and molds versus essential oil of onion (*Allium cepa*), the high sensitivity of *S. aureus* to *Allium* sample was confirmed. Its sensitivity was ranked according to the MIC as following: white shallot > purple onion > white onion. In the agar well diffusion test of *S. aureus*, purple onion and white shallot produced statistically similar in diameter of clearance zone around the wells, while white onion gave smaller inhibition zone. For *P. aeruginosa*, white shallot exerted statistically stronger activity than purple onion in agar well diffusion test. Moreover, MIC determination indicated that, for white shallot only 25 mg/ml was required to inhibit is microbial growth in the broth; however purple onion needed double the concentration (50 mg/ml). For *B. cereus*, the inhibition zone of white shallot was larger than that of purple onion. Since MIC was recorded when less than 3 colonies had appeared in the agar dish, therefore MIC of purple onion was 50 mg/ml, which was the stock concentration (un-diluted). In short, purple onion and white shallot had the same MIC of 50 mg/ml for *B. cereus*. *S. typhi* resisted against all of the extract solution, in both agar well diffusion and MIC test. In general, no obvious difference was observed in the sensitivity of gram positive and gram negative bacteria against extract of onion and shallot, except for *S. typhi*. Similar results were reported in study of Nantaporn *et al*. [19] and Amin *et al*. [20].

Table 2: Diameter of inhibition zone inhibited by onion and shallot extracts against different bacteria

Pathogens	Inhibition diameter (mm) including 9 mm of well			
	White shallot	Purple onion	White onion	Purple shallot
S. aureus	16.50 ± 0.58aA	15.50 ± 0.58bA	13.75 ± 0.96dB	No inhibition
P. aeruginosa	15.50± 0.58aC	12.25 ± 0.50cD	No inhibition	No inhibition
B. cereus	16.75± 0.50aE	13 ± 0.82cF	No inhibition	No inhibition
S. typhi	No inhibition	No inhibition	No inhibition	No inhibition

Data followed by the same letter within a column were not significantly different ($p<0.05$).

In fact, the MIC of the onion and shallot extracts required to inhibit visible growth of microorganisms was considered to be relatively high. It can be explained due to the method of determining extract concentration was different from other studies. Instead of freeze-drying the extract solution completely into powder (as seen in other studies), soluble concentration was determined by taking 1ml extract solution and applying to the heating oven until all liquid solvent was evaporated. After measuring the weight of solid remained, the stock solution was diluted to appropriate concentration to measure the MIC of bacterial pathogen.

Table 3: MIC (mg/ml) of onion and shallot extracts against different bacteria

MIC (mg/ml)	*S. aureus*	*P. aeruginosa*	*B. cereus*	*S. typhi*
Purple shallot	No inhibition	No inhibition	No inhibition	No inhibition
Purple onion	25	50	50	No inhibition
White shallot	12.5	25	50	No inhibition
White onion	50	No inhibition	No inhibition	No inhibition

4. Conclusion

Unsafe and possible side effects are the major problem in the use of synthetic antioxidant and antimicrobial substances nowadays. Scientific community is currently interested in plant materials that have been long attended in traditional medicinal treatment, such as herbs and spices. In this study, extracts of local Vietnamese varieties of onion and shallot were appreciated for their antimicrobial and antioxidant capacity. Purple onion displayed a prominent antioxidant activity while white shallot showed to be an effective antimicrobial against referenced bacterial strains. The results have revealed that onion and shallot could be considered as a natural, safer substitution for traditional food preservatives. Optimization in extraction should also be tried to obtain better yield and higher concentration. Furthermore, study to find out which active substances are responsible for these actions will also be very useful.

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