Antioxidant and Antibacterial activity of Lotus tea in Thua Thien Hue province from Vietnam

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Abstract
In this study, we determined the antioxidant and antibacterial activity in different types of lotus tea (Nelumbo nucifera Gaernt.*) in Thua Thien Hue province, Vietnam. The results showed that in four types of lotus tea, the catalase enzyme activity was 0.92-1.35 U/mg protein, the peroxidase enzyme activity was 0.97-2.67 U/mg protein, ascorbic acid content was 0.088-0.135%, the IC50 value was 4.98-6.03 mg/mL and all four kinds of lotus tea inhibited bacteria to a variable extent with low sensitivity.*

The inhibition zone's mean diameter increased from 0.23 cm to 0.77 cm. The flower lotus tea gave the highest results on most of the research criteria and the lowest was lotus rhizome tea. From obtained results, tea produced from lotus plants grown in Thua Thien Hue province, Vietnam has a high potential for antioxidant and antibacterial properties.

Keywords: Antibacterial activity, Antioxidant activity, DPPH assay, Lotus tea, Thua Thien Hue, Vietnam.

Introduction

Nelumbo nucifera is native to tropical Asian countries and is now commonly grown in many parts of the planet[1]. Almost all parts of the lotus plant have some biological activities such as anti-bacterial, antioxidant, free radical scavenging, immune-modulatory activities and anti-inflammatory[24]. Today, the lotus plant is widely used in food, cosmetics, medicine and more[17,31]. Especially, some different parts of this plant could also be used to make herbal tea. The tea lotus has many benefits for the health of humans[34]. Besides, lotus tea has been proven to be a healthy food product that is in high demand today in leading consumer markets such as the European community, the United States and Japan[14].

In Korea, lotus leaf tea is widely used to help reduce the level of lipids in the human body and antioxidation[17]. Lotus pollen tea has high antioxidant activity as well as a pleasant scent and flavor[14]. Kim et al[19] reported that lotus seed tea has a long-term effect on the prevention of skin moisture loss, decreases the growth of abnormal keratinocytes and aids in the inhibition of protein oxidation.

Napradit et al[27] evaluated the efficacy and safety of oral administration of lotus stamen tea in the treatment of hyperlipidemia. After two weeks of intervention, this tea could reduce LDL-cholesterol levels and total cholesterol levels in the blood. Tea is also made from lotus flowers. It has a 32.19% antioxidant activity[20]. Kaewka et al[18] reported that lotus petal tea has high total phenolic content and antioxidant activities

Many types of tea are produced from lotus plants grown in Thua Thien Hue province of Vietnam including lotus flower tea, lotus leaf tea, lotus root tea and lotus embryo tea. Those teas are very popular and frequently consumed[34]. Our previous research indicated that lotus tea has high and varied bioactive compounds depending on the part of the lotus plant used for processing[34]. Our study aimed to determine the antioxidant and antibacterial activity of lotus tea produced from embryos, rhizomes, leaves and flowers of lotus plants. Thereby, it provides more evidence to elucidate the important role of lotus tea in human health and the scientific basis for developing the current lotus tea industry.

Material and Methods

Materials: The study was conducted on four types of dried lotus tea samples which were collected from tea factories in Thua Thien Hue province of Vietnam. The tea samples include lotus embryo tea, lotus flower tea, lotus rhizome tea and lotus leaf tea. All of these teas were produced from lotus plants *(Nelumbo nucifera Gaernt.)* grown in Thua Thien Hue province.

Methods

Determination of catalase enzyme activity: Determination of catalase enzyme activity was done by the titrimetric method using $KMnO_4$[10]. About 2 g of the lotus tea sample was ground in a porcelain mortar with 0.3 g of $CaCO_3$, add 20 mL distilled water, carefully ground to a homogenous solution, transfer to 50 mL volumetric flask and make upto the mark level. A magnetic stirrer was used to constantly stir the extraction mixture. After 30 minutes, Whatmann no. 4 filter paper was utilized to filter the extraction mixture. The tea solution was cooled to room temperature.

Place in two triangular flasks, each containing approximately 1mL of the tea sample extract, mix with 4 mL of distilled water and 10 mL of H_2O_2 0.1%. Add 3 mL of 10% H_2SO_4 to the test flask. Set all two to the warm 30°C for 30 minutes. Remove and add 3 mL of 10% H_2SO_4 to the test flask. Titrate both flasks with 0.1N $KMnO_4$ until a pink color persists for about 30 seconds. Duplicate analyses were performed on all samples. An average result was obtained by repeating each experiment three times.

The following formula was used to calculate the catalase activity in the tea sample:

$$C = \frac{(A - B) \times 1.7 \times f}{W}.$$

where A is the volume of $KMnO_4$ 0.1N for the blank sample, B is the volume of $KMnO_4$ 0.1N for the titration of the test sample, C is mg of H_2O_2 decomposed, f is correction coefficient of $KMnO_4$ 0.1N solution (f=1) and W is mass (g) of tea sample for analysis.

Determination of Peroxidase enzyme activity: Approximately 2 ± 0.001g of ground tea samples were extracted with 100 mL of hot distilled water (at 95°C). A magnetic stirrer was used to stir the extraction mixture continuously. Use Whatmann no. 4 filter paper to filter the extraction mixture after 10 minutes. The residues were washed three times with distilled water (10 mL each). We then cooled the tea solution to room temperature[32].

Peroxidase enzyme activity in lotus tea extracts was determined (30 seconds on 1 measurement) by measuring the absorbance of guaiacol produced by the reaction between 2 mL of lotus tea extract, 0.05 mL guaiacol solution, 0.03 mL of 0.042% hydrogen peroxide solution and 0.92 mL phosphate buffer (pH 7.0, at 25°C) at 470 nm. During the first three minutes of the reaction, the rate of guaiacol peroxidation was linear. As explained above, an activity unit is defined as the amount of enzyme required to cause an increase of 0.01% in absorbances per minute[36].

Determination of Vitamin C content: Vitamin C content was determined by titration method by I_2[5]. About 5 g of lotus tea was added into the porcelain mortar with 10 mL HCl 2%, crushing small, extracting water in the glass cup. Add 10 mL HCl 2% into crushed porcelain mortars and extract as above. Repeat 3 times and end the extraction process. Use 10mL HCl 2% coated mortars and transfer the extraction to a volumetric flask (V = 50 mL). Use distilled water to the level of the bottle. Leave the vessel in the darkness for 10 minutes and filter the extract. Take 10 mL filter into the conical flask, add 10 drops of 0.5% starch and shake lightly. Use the titration I_2 solution until the color of blue appears.

The following formula was used to calculate the vitamin C content:

$$X = \frac{V \times V1 \times 0.00088 \times 100}{V2 \times w}$$

where X is the content of vitamin C in the materials (%), V is the volume of diluted sample solution (mL), V_1 is the volume of 0.01N I_2 solution (mL), V_2 is the volume of analyzed solution (mL), w is the weight of the sample (g) and 0.00088 is the weight (g) of vitamin C which was equivalent to 1 mL of 0.01N I_2.

Determination of antioxidant activity: The antioxidant activities of lotus tea liquid extracts were measured by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method as described by Chan et al[0] with minor modifications[0].

Lotus teas extract preparation: Weigh 0.2 g of all kinds of lotus tea samples and grind them into small pieces, then add to 10 mL tubes. Raise the temperature of the extraction tubes to 70°C for 1 minute. Add 5 mL of the thermally stabilized 70% methanol solution at 70°C for 30 minutes.

The tube was then shaken well on a shaker and extracted within 10 minutes at 70°C. After extraction, cool naturally and centrifuge (Hermle Z400) at 3500 rpm for 10 minutes. Decant the tea extract and conduct further extraction 2 times[16].

DPPH assay: The solution of lotus tea extract and ascorbic acid (1 mg/mL) was diluted with the same ratios of 1:100, 1:200, 1:400, 1:800 and 1:1000. 0.2 mM of DPPH solution was prepared in 70% methanol solution. Add 1 mL test sample at each dilution in the test tube and 1 mL of DPPH of 0.2 mM, shake well and incubate in the dark for 30 minutes. After that, the absorbance of the extraction mixture was measured at 517 nm. The control ascorbic acid was performed similarly. The percentage of free radical scavenging DPPH was determined by the formula:

$$SC\% = \frac{OD_c - OD_m}{OD_c} \text{x}100$$

where Odc is the optical density of blank sample after subtracting blank (without DPPH), Odm is the optical density of the experimental sample after subtracting blank (without DPPH). Building regression equation shows percentages of DPPH inhibitions at different concentrations. From there, determine the IC50 value based on the regression equation to find the sample content that inhibits 50% of DPPH free radicals.

Determination of Antibacterial activity

Tested Bacteria: Three strains of bacteria were tested including *Samollena, Escherichia coli (E. coli) and Bacillus subtilis (B. subtilis)*. Those bacteria were obtained from the Institute of Biotechnology, Hue University, Hue city, Vietnam.

Prepare antibacterial solution: Tea lotus was brewed in boiling water (90°C) with different concentrations (2, 20, 40, 80 mg/mL). After four minutes, the tea extract was filtered through Whatmann filter paper no. 4 and allowed to cool to room temperature. The antibiotic ampicillin at a dose of 10 µg was used as a positive control.

Distilled water used for sample preparation was used as a negative control.

Determination of Antibacterial activity: The antibacterial activity of four kinds of lotus tea extract was determined by Bauer et al.[0] Each experiment was performed three times.

Table 1
The content of antioxidative enzymes in lotus tea

Type of lotus teas	Catalase enzyme (U/mg protein)	Peroxidase enzyme (U/mg protein)	Vitamin C (%)
Lotus rhizome tea	0.92[b]	0.97[b]	0.126[a]
Lotus embryo tea	1.33[a]	1.09[b]	0.088[b]
Lotus flower tea	1.35[a]	2.67[a]	0.135[a]
Lotus leaf tea	1.20[ab]	2.00[a]	0.106[b]

Note: Different letters on the same column indicate a statistically significant difference of the sample mean with $p < 0.05$ (Duncan's test).

To be considered sensitive, the bacteria need to be in a clear zone of inhibition of more than 12 mm[4]. The tested bacteria strains'sensitivity to lotus tea extracts was compared with antibiotic ampicillin and distilled water.

Data Analysis: All experiments were performed three times and mean values were calculated. Collected data are processed by Duncan's test analysis method ($p<0.05$) by the SPSS program (ver. 20.0).

Results and Discussion

The content of antioxidants in lotus tea: In the body of plants and animals, there are many types of antioxidants such as catalase enzyme, peroxidase enzyme, superoxide dismutase, ascorbate peroxidase, glutathione reductase and ascorbic acid to scavenge free radicals generated by redox reactions[33]. In the present work, antioxidants in four types of lotus tea were determined including catalase enzyme, peroxidase enzyme and vitamin C. The result is presented in table 1.

Catalase enzyme activity: Catalase enzyme plays an important role in the breakdown of hydrogen peroxide and in maintaining cellular redox homeostasis[9]. In addition, it can inhibit the resolution of neurons, apoptosis, inflammatory processes, aging and a variety of malignancies as well as it can assist intracellular medication transport and can be utilized in quantitative cholesterol measurement[3].

Catalase deficiency or dysfunction is thought to be involved in the etiology of many age-related degenerative disorders including hypertension, diabetes, vitiligo, anemia, Parkinson's disease, Alzheimer's disease, bipolar disorder, schizophrenia and cancer[12,13].

The catalase enzyme activity of the studied lotus teas varied from 0.92 to 1.35 U/mg protein. Teas made from lotus embryos and lotus flowers reached the highest value (1.33-1.35 U/mg protein). Lotus leaf tea reached the value of 1.20 U/mg protein. The lowest result is dried lotus rhizome tea with 0.92 U/mg protein (Table 1).

The results of the present study are higher than values detected in green tea (1,20 µM H_2O_2 reduced min-1 mg-1 protein)[28] or the catalase activity of the extracts of *N. Nouchali* dried flowers (Aqueous, Methanol, Ethanol) respectively reached the following values: 0.925, 0.117, 0.64 U/mg[26].

Peroxidase enzyme activity: Peroxidase catalyzes the breakdown of hydrogen peroxide into water releasing molecular oxygen. In the body, peroxidases form a network that may have beneficial effects. Peroxidases are enzymes that participate in the detoxification of oxidizing drugs and xenobiotics, innate immunity, hormone biosynthesis and the pathogenesis of inflammatory diseases[21,29,37].

The peroxidase enzyme activity in the tested teas varied from 0.97 to 2.67 U/mg protein. The lowest values were lotus rhizome tea and lotus embryo tea (0.97-1.09 U/mg protein), next to lotus leaf tea (2.0 U/mg protein). The highest value belongs to lotus flower tea reaching 2.67 U/mg protein (Table 1).

Shah et al[29] showed that *Camellia sinensis* leaves in Assam, India have peroxidase enzyme activity of 6.19 U/mg protein. Tea buds still showed peroxidase activity which reached values ranging from 0.8 to 1.4 U/mg protein[33]. Peroxidase activity of the extracts of *N. nouchali* (NN) dried flowers (Aqueous, Methanol, Ethanol) respectively reached the following values: 0.349; 0.0149 and 0.023 U/mg[26]. This result is similar to the peroxidase enzyme activity of four kinds of lotus tea in our study.

Vitamin C (Ascorbic acid) content: Vitamin C has various actions in the body that contribute to the general health of the human body. Vitamin C is used for enzyme stimulation, collagen production, nitrosamine formation, hormone activation, histamine detoxification, leukocyte phagocytic activity and hydroxylation of proline. It is mainly used as antioxidant. These roles depend on a person's vitamin C level and its health implications. In the body of humans, ascorbic acid can reduce the risk of hypertension and cancer, increases immunity, hydroxyproline excretion in urine, regenerates tissues and helps in metabolism of drugs[38].

All four kinds of lotus tea contain vitamin C, although only in trace amounts ranging from 0.088% to 0.135%. Lotus rhizome tea has the lowest vitamin C value, next to teas made from lotus leaves and lotus embryos. The lotus flower tea still gives the highest vitamin C content with 0.135% (Table 1).

Cennet[7] reported that herbal teas produced from lemon balm (*Melissa officinalis* L) and sage (*Salvia officinalis* L.) have ascorbic acid content ranging from 0.24 to 615.8 µg/mL. In unbrewed Japanese green tea leaves, this vitamin content has been found to exceed 250 mg/100 g[30]. Green tea (*Camellia sinensis*) of China exhibits a vitamin C content of 1-2% weight of extract solid and 0.3 mg/100g[1]. In fresh lotus leaves, the ascorbic acid content reached 33.29 mg/100g and after drying, this value decreased ranging from 15.31 to 22.47 mg/100g[22]. Thus, in the studied lotus teas, the vitamin content was present at very low concentrations.

Antioxidant activities: The DPPH test is a common and widely used method for determining a compound's potential to act as a free radical scavenger or hydrogen donor as well as to assess the antioxidant activity of foods[23]. In this work, we used assays of DPPH scavenging activity to evaluate the antioxidant capacity of different lotus teas. The results of these assessments are expressed via the percentage of free radical scavenging activity DPPH (SC%) and IC50 values presented in table 2 and 3.

The values of antioxidant potential of the lotus teas are presented as SC% values (Table 2). The DPPH radical scavenging activity ranging in lotus teas increases gradually with the ratios 1:1000, 1:800, 1:400, 1:200 and 1:100. Tea produced from flowers lotus has the highest SC% values, ranging from 29.76% to 71.86%, next to lotus leaf tea (25.47%–69.33%) and lotus embryo tea (24.79%-67.53%). Lotus rhizome tea has the lowest result, ranging from 24.04% to 66.99%. This result is similar to the antioxidant activity of these parts of the lotus plant when not dried, specifically as follows: Flowers (59.3%) > leaves (47.8%) > seeds (42.15%) > rhizomes (21.8%)[17]. Compared with other kinds of tea, the antioxidant capacity of white tea (10.70-73.46%), green tea (10.84-79.60%), oolong tea (6.46-75.27%), black tea (2.98- 69.26%) and Pu-erh Tea (4.50-72.94%) is higher than four kinds of lotus teas in our study[11].

The linear regression equations obtained from the extract of four types of lotus tea with a high correlation coefficient from 0.956-0.981 are shown in table 3. Therefore, these regression equations can be used to determine the IC50 value of lotus tea extracts. The IC50 value is the concentration of the sample that can scavenge 50% of DPPH free radicals in the DPPH free radical scavenging method. The IC50 value is inversely proportional to the antioxidant activity.

As observed in table 3, the IC50 value was in the order: lotus rhizome tea > lotus embryo tea > lotus leaf tea > lotus flower tea. According to the IC50 value, lotus flower tea was the most effective in terms of antioxidant capacity (IC50 = 4.98 mg/mL) followed by lotus leaf tea (IC50 = 5.40 mg/mL) and lotus embryo tea (IC50 = 5.40mg/mL). The lowest antioxidant capacity was found in lotus rhizome tea (IC50 = 6.03 mg/mL). There was a significant difference between the lotus tea tested. Antioxidant capacity in these lotus teas was significantly lower than the ascorbic acid standard (IC50=3.03 µg/mL).

The IC50 values of aqueous extracts of some teas have been reported including white tea (0.285 mg/mL), oolong tea (0.468 mg/mL), green tea (0.329 mg/mL), Pu-erh tea (1.432 mg/mL) and black tea (1.944 mg/mL), demonstrating the ability to scavenge 50% of free radicals of lotus teas as lower than that of the above kinds of tea[11].

Table 2
DPPH free radical scavenging activity of lotus tea extracts

Dilution rate / Type of lotus teas	1:1000	1:800	1:400	1:200	1: 100
Lotus embryo tea	24.79^{b}	32.97^{a}	38.57^{a}	49.81^{ab}	67.53^{b}
Lotus rhizome tea	24.04^{b}	27.65^{b}	32.27^{b}	51.40^{b}	66.99^{b}
Lotus flower tea	29.76^{ab}	32.41^{a}	40.60^{a}	54.26^{a}	71.86^{ab}
Lotus leaf tea	25.47^{a}	32.45^{a}	37.54^{a}	53.56^{a}	69.33^{a}

Note: Different letters on the same column indicate a statistically significant difference of the sample mean with $p < 0.05$ (Duncan's test).

Table 3
IC_{50} values of lotus tea liquid extract and ascorbic acid control

Type of lotus tea	Unit	R^2	IC50
Lotus embryo tea	mg/mL	0.956	5.71^{a}
Lotus rhizome tea		0.966	6.03^{ab}
Lotus leaf tea		0.981	5.40^{bc}
Lotus flower tea		0.956	4.98^{c}
Ascorbic acid	µg/mL	0.963	3.03^{d}

Note: Different letters on the same column indicate a statistically significant difference of the sample mean with $p < 0.05$ (Duncan's test).

Table 4
Diameter of Inhibition Zones Induced by Extract of lotus tea and ampicilin antibiotic

Bacterial	Concentrations					
	Tea	2	20	40	80	Ampicillin
		mg/mL				µg
Salmonella	Lotus embryo tea	-	0.23^{d}	0.37^{c}	0.53^{b}	0.90^{a}
	Lotus leaf tea	-	0.23^{c}	0.37^{c}	0.53^{b}	0.95^{a}
	Lotus rhizome tea	-	-	0.33^{a}	0.37^{a}	1.15^{a}
	Lotus flower tea	-	0.32^{d}	0.43^{c}	0.55^{b}	1.10^{a}
E. coli	Lotus embryo tea	-	0.47^{d}	0.60^{c}	0.63^{b}	1.43^{a}
	Lotus leaf tea	-	0.37^{d}	0.50^{c}	0.57^{b}	1.40^{a}
	Lotus rhizome tea	-	0.33^{d}	0.43^{c}	0.53^{b}	1.38^{a}
	Lotus flower tea	-	0.43^{d}	0.53^{c}	0.77^{b}	1.38^{a}
B. subtilis	Lotus embryo tea	-	-	0.43^{c}	0.57^{b}	1.40^{a}
	Lotus leaf tea	-	0.33^{d}	0.50^{c}	0.67^{b}	1.36^{a}
	Lotus rhizome tea	-	0.23^{d}	0.47^{c}	0.57^{b}	1.40^{a}
	Lotus flower tea	0.27^{e}	0.37^{d}	0.57^{c}	0.70^{b}	1.36^{a}

Note: Different letters on the same row indicate a statistically significant difference of the sample mean with $p < 0.05$ (Duncan's test); "-": no zone of inhibition

In China, flower teas with IC50 values of 11.38 to 218.45 µg/mL. Rosa rugosa (IC50=11.38 µg/mL) and troll flower (IC50=12.69 µg/mL) both had strong antioxidant activity. Lower IC50 values were found for lily (92.47 µg/mL), praecox (94.34 µg/mL) and magnolia flowers (218,45 µg/mL)[15].

Antibacterial activities: The antibacterial capacity of lotus tea extracts was evaluated based on the diameter of the inhibition zone. As shown in table 4, all four types of lotus tea studied had an antibacterial effect on three strains of different bacteria viz. *Salmonella, E.coli and B. subtilis*. In 2 mg/mL concentration, almost four kinds of lotus tea did not appear in inhibition zone. The mean diameter of the inhibition zone increases gradually with the amount of tea from 20 to 80 mg/mL, ranging from 0.23 cm to 0.77 cm. Among the different lotus teas tested, lotus flower tea gave the best results with values reaching 0.55 cm (with *Salmonella bacteria)*, 0.77 (with *E.coli bacteria)* and 0.70 cm (with *B. subtilis*) respectively.

The lotus rhizome tea still gave the lowest anti-bacterial activity. Gallic acid, polyphenols, flavins and catechins compounds present in tea contribute to its antibacterial properties[25]. Our previous reports indicated that lotus flower tea had the highest polyphenol content and rhizome lotus tea had the lowest result[34]. That contributed to explaining our antibacterial results in this study.

Salmonella, E.coli and B. subtilis were sensitive to ampicillin antibiotics. The mean diameter of the inhibition zone with ampicillin was 0.90 - 1.43 mm, higher than that of lotus tea extracts (Table 4).

Tea is considered as an herb that has the effect of preventing tooth decay[9]. Bacterial inhibitory effects of black and green tea have been studied and published[4,9,25]. Our present results indicate that lotus teas can inhibit *Salmonella, E. coli* and *B. subtilis*. This finding determined the anti-bacterial effects of lotus tea, a common daily beverage in Vietnam.

Conclusion

In our study, the antioxidant and antibacterial capacity of lotus tea made from four parts of the lotus plant including flowers, leaves, embryos and rhizomes were evaluated and compared. Four types of lotus tea studied were all high in antioxidants: catalase enzyme activity (0.92-1.35 U/mg protein), peroxidase enzyme activity (0.97-2.67 U/mg protein), ascorbic acid content (0.088-0.135%) and IC50 value was 4.98-6.03 mg/mL. All four kinds of lotus tea have anti-*Salmonella, E. coli and B. subtilis* effects. Especially, the flower lotus tea gave the highest results on most of the research criteria. These results have increased the value of the lotus plant in tea production.

In addition, these findings may also facilitate the development of lotus tea as a new and inexpensive source of natural antibacterials and antioxidants, especially for the functional food industry which is developing very fast today.

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