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Research Article

ANTIOXIDANT CAPACITY AND FLAVOINOIDS, TRITERPENOIDS, POLYPHENOL, POLYSACCHARIDE CONTENT FROM TUBERS OF TWO *AMORPHOPHALLUS* SPECIES (ARACEAE)

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ABSTRACT. *Amorphophallus opertus* Hett. and *A. lanceolatus* (Serebryanyi) Hett. & C. Claudel, two members of the Araceae family, are recognized as endemic species of Vietnam. Due to lacking samples of these species, the bioactivity and phytochemicals of these species have not been elucidated yet. This study was firstly investigated the antioxidant capacity and flavoinoid, triterpenoid, polyphenol, polysaccharide contents of *A. opertus* and *A. lanceolatus* tubers using spectrophotometric assay (UV-VIS) and UVWin6 Software v6.0.0, which is important step for further utilization and application of these species. The results showed that *A. opertus* tuber contained several bioactive compounds, such as flavonoids (0.313 ± 0.009 mg QE/g dry mass), triterpenoids (0.020 ± 0.001 mg OAE/g dry mass), polyphenols (0.026 ± 0.001 mg GAE/g dry mass), with the polysaccharide content about 1.653 ± 0.157 mg GE/g dry mass, and the strong antioxidant capacity (DPPH scavenging activity about 0.027 ± 0.001 mg Trolox/g dry mass and ABTS scavenging activity about 0.017 ± 0.001 mg Trolox/g dry mass). On the other hand, *A. lanceolatus* tuber comprised about flavonoids (0.032 ± 0.004 mg QE/g dry mass), triterpenoids (0.0109 ± 0.0003 mg OAE/g dry mass), polyphenols (0.029 ± 0.003 mg GAE/g dry mass), with the lower polysaccharide content (0.0131 ± 0.0003 mg GE/g dry mass) amd antioxidant capacity (DPPH scavenging activity about 0.017 ± 0.001 mg Trolox/g dry mass and ABTS scavenging activity about 0.012 ± 0.001 mg Trolox/g dry mass)..

Keywords: *Amorphophallus, flavonoids, triterpenoids, polysaccharide, polyphenol, DPPH, ABTS*

INTRODUCTION

Amorphophallus Blume ex Decaisne. is a large genus of Araceae family which comprise of 200 species growing over the world, Among them, 30 species are found in Vietnam [1, 2, 3]. Tubers from some *Amorphophallus* species can be used as the material to produce medicinal and food powder. For instance, Singh & Wadhwa suggested that tuber of *A. paeoniifolius* contained high starch and carbohydrate content, essential minerals. Furthermore, methanolic extract of *A. paeoniifolius* tuber had analgesic, gastroprotective, antihelmitic, antibacterial activities [4]. Tran et al. recorded 6 species of *Amorphophallus* genus in northern region of Vietnam (*A. konjac, A. corrugatus, A.*

krausei, A. yunnanensis, A. yuloensis and *A. paeoniifolius*). Of note, the starch from these species poccesses a high percentage of fiber and glutamannan, a low energy carbohydrate, and effectively absorb water; therefore, it can be used in production of dietary foods [5].

A. opertus and *A. lanceolatus* are two rare species of the *Amorphophallus* genus which are mainly distributed in southern regions of Vietnam and recorded as endemic species of Vietnam [1, 2, 3]. Recently, Van et al. also identified 12 compounds in ethanolic extract of *A. lanceolatus* tuber, including of glycol–diglycidyl ether, lycopersin, heptacosane, n–heneicosylcyclopentane, homalomenol F, maltitol, pyrinuron, octadecane, 2 (1 *H*) naphthalenone, 3,5,6,7,8,8a–hexahydro–4,8a–dimethyl–6–(1–methylethenyl), 2–methylfluoranthene, quinic acid, 1H–imidazole, and the extract exhibited antibacterial activity against *B. cereus*, *E. coli*, *P. aeruginosa*, *S. enteritidis*, *S. typhimurium*, and *S. aureus* [6]. Accordingly, the concentration of bioactive compounds and antioxidant activity of *A. opertus* and *A. lanceolatus* have not been studied yet, especially in the concentration of antioxidant compounds such as flavonoids, triterpenoids, polyphenols. These bioactive compounds have an important role in prevention from cancer, neurodegenerative, cardiovascular diseases, and diabetes [7].

In this study, the flavonoids, triterpenoids, polyphenol, polysaccharide contents and antioxidant activity of *A. opertus* and *A. lanceolatus* tubers were determined. Furthermore, results from this study provide more information for further application of these species in functional food and medicine.

MATERIALS AND METHODS

Specimens Collection

Specimens of *A. opertus* and *A. lanceolatus* were collected from Binh Chau-Phuoc Buu Nature Reverse, Bung Rieng ward, Xuyen Moc district, Ba Ria-Vung Tau province, location about $10^{0}32^{'}28^{''}$N; $107^{0}31^{'}39^{''}$E and $10^{0}30^{'}56^{''}$N; $107^{0}29^{'}42^{''}$E, December 9, 2018, 47m in elevation (Fig. 1).

The fresh tubers were washed to remove the surface pollutants, and subsequently sliced, drided at 55-65 ^{o}C until the moisture below 10%. The dried tubers were pulverized using an electric grinder into fine powder and used for further studies. To determine the moisture content of powder, 0.5 g *A. opertus* or *A. lanceolatus* powder was analyzed by Sartorius Moisture Analyzer MA150 Balance (Germany).

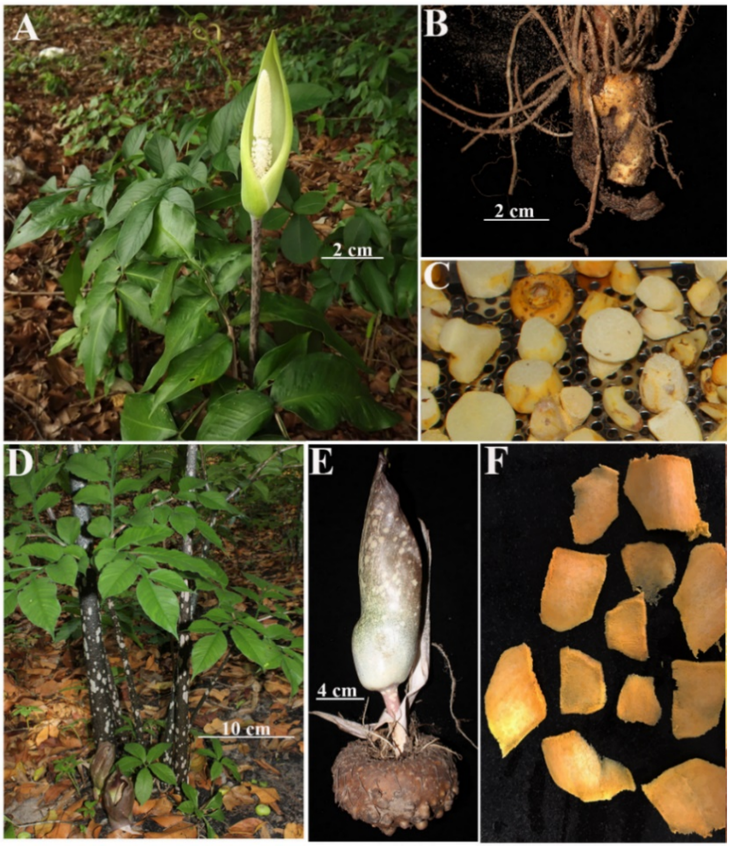

Fig. 1. *A-C: A. lanceolatus (A. habitat, B. tuber, C. Flesh of tuber). D-F: A. opertus (D. habitat, B. tuber and flower, C. Flesh of tuber).*

Preparation of Ethanolic Extracts

1 g of *A. opertus* or *A. lanceolatus* powder was soaked with 100 mL of 98% ethanol for 24 hours. The extract was filtrated using Whatman number 1 filter paper, and the extraction procedure was repeated 2 times more to obtain the filtrate. Resulting filtrates were combined and eventually concentrated in reduced pressure (130 mmBar) at 60ºC to eliminate the residual ethanol [8]. Ethanol was added into the obtained residue up to 100 ml, and the adjusted extracts were used for further experiments.

Determination of Total Flavonoid Content

Total flavonoid contents (TFC) of *A. opertus* and *A. lanceolatus* tuber extracts were evaluated by aluminium chloride reagent using UV-vis spectrophotometer (UVS 2800, Labome, USA). In concise, 0.3 ml of extract was mixed with 0.3 ml of 2% $AlCl_3$ solution, the mixture was stand in room temperature for 5 minutes. 2 ml of 1M NaOH was subsequently added to the mixture, and water was added up to 10 mL. The absorbance of the mixture was measured at 510 nm using an UV-vis spectrophotometer (UVS 2800, Labome, USA). A standard curve of quercetin (5-100 µg/ml) was plotted with the equation $y = 0.0007x + 0.0078$, $R^2 = 0.9991$ in which y was the absorbance at 510 nm and x was sample concentration µg/ml. The sample concentration was calculated from the standard curve equation and result was expressed as mg QE/g dry mass. Total flavonoid contents of tubers were calculated using equation (I). The experiments were performed in triplicate [9].

Determination of Total Triterpenoid Content

Total triterpenoid contents (TTC) of *A. opertus* and *A. lanceolatus* extracts were determined according to Wei et al. [10] with some modifications. Briefly, a 0.2 ml of sample was added with 0.2 ml of 5% (W/V) vanillin-acetic solution. The mixture was mixed with 1.2 ml of perchloric acid ($HClO_4$). The mixture was subsequently incubated at 70°C for 15 min and cooled for 2 min. The mixture was diluted to 5 ml with ethyl acetate. The absorbance was measured at 550 nm using UV-vis spectrophotometer. A standard curve of oleanoic acid (2-10 µg/ml) was plotted with the equation y = 0.0167x – 0.0009, R^2 = 0.9995, in which y was the absorbance at 550 nm and x was sample concentration µg/ml. The sample concentration was calculated from the standard curve equation and result was expressed as mg OAE/g dry mass. Total triterpenoid contents of tubers were calculated using equation (I). The experiments were performed in triplicate.

Determination of Total Polyphenol Content

Total polyphenol contents (TPC) of *A. opertus* and *A. lanceolatus* tuber extracts were evaluated based on UV-vis spectrophotometry assay with Folin- Ciocalteu reagent. Briefly, 1 ml of the extract was mixed with 5 ml of Folin-Ciocalteu, and the mixture was kept at room temperature for 5 min. Thereafter, 4 ml of sodium carbonate Na_2CO_3 7,5% was added to the mixture, the mixture was diluted to 10 ml with water. The adsorbance of the mixture was measured at 765 nm. A standard curve of gallic acid (0-50 µg/ml) was plotted with the equation y = 0.0119x + 0.0047, R^2 =0.9997, in which y was the absorbance at 765 nm and x was sample concentration µg/ml. The sample concentration was calculated from the standard curve equation and result was expressed as mg GAE/g dry mass. Total polyphenols contents of tubers were calculated using equation (I). The experiments were performed in triplicate [9].

Determination of Total Polysaccharide Content

0.5 g of tube powder was soaked with 100 ml of distilled water and boiled for 30 min. The mixture was filtered by filter paper, this procedure was performed three times to obtain the filtrate. Total polysaccharide contents of *A. opertus* and *A. lanceolatus* tuber extracts were determined via UV-vis spectrophotometry assay. In this assay, polysaccharide of extract produces yellow-orange complex with phenol and concentrated sulfuric acid. The color intensity of this reaction determined at 488 nm is relavant with the polysaccharide content [11].

5 ml of 5% phenol solution were mixed with 1 ml of extract and the mixture was incubated in room temperature for 5 min. 4 ml of concentrated sulfuric acid were added and absorbance of the mixture was determined at 488 nm using UV-vis spectrophotometry assay. A standard curve of D-glucose (10-100 µg/ml) was plotted with the equation y = 0.0065x + 0.0262, R^2 = 0.9976, in which y was the absorbance at 488 nm and x was sample concentration µg/ml. The sample concentration was calculated from the standard curve equation and result was expressed as mg GE/g dry mass. Total polysaccharide contents of tubers were calculated using equation (I). The experiments were performed in triplicate [12].

Determination of DPPH Radical Scavenging Activity

Antioxidant activities of ethanolic extracts of *A. opertus* and *A. lanceolatus* tubers were evaluated via the DPPH radical scavenging activity (1,1-diphenyl-2- picrylhydrazyl). The remaining DPPH radicals of solution are determined by colometric assay at 517 nm. Briefly, 0.1 ml of ethanolic extract were taken and mixed with 4 ml of 0.1 mM DPPH solution. The mixture was diluted to 5 ml by 99.5% ethanol and shaken vigorously. The tube was incubated at dark room temperature for 30 min, and the absorbance of mixture was evaluated at 517 nm using UV-vis spectrophotometer (UVS 2800, Labome, USA) and UVWin6 Software v6.0.0, and Trolox was used as the reference sample. A standard curve of Trolox (0-10 µg/ml) was plotted with the equation y = -0.0029x + 0.1609, R^2 = 0.9965, in which y was the absorbance at 517 nm and x was sample concentration µg/ml. The sample concentration was calculated from the standard curve equation and result was expressed as mg Trolox/g dry mass. DPPH radical scavenging activities of tubers were calculated using equation (I). The experiments were performed in triplicate [13].

Determination of ABTS Radical Scavenging Activity

Determination of 2,2-azinobis 3-ethylbenzothiazoline-6-sulfonate (ABTS) radical scavenging effect of the ethanolic extracts of *A. opertus* and *A. lanceolatus* tubers was conducted as indicated in Maeng et al. procedures with some modifications. Firstly, 7 mM ABTS solution and 2.45 mM K_2SO_4 were mixed in a 1:1 ratio and incubated at dark room temperature over 18 hours (solution A). 0.1 ml of extract was mixed with 3 ml of solution A, the mixture was diluted to 5 ml by ethanol and kept in dark for 15 min. The absorbance of mixture was recorded at 734 nm using UV-vis spectrophotometer (UVS 2800, Labome, USA) and UVWin6 Software v6.0.0. A standard curve of Trolox was established (0-10 µg/ml) with the equation y = -0.0278x + 0.421, R^2 = 0.9990, in which y is the absorbance at 734 nm and x is sample concentration µg/ml. The sample concentration was calculated from the standard curve equation and result was expressed as mg Trolox/g dry mass. ABTS radical scavenging activities of tubers were calculated using equation (I). The experiments were performed in triplicate [13].

Data Analysis and Statistical Analysis

Concentrations of some compounds, such as flavonoids, polyphenols, triterpenoids, polysaccharides, DPPH and ABTS radical scavenging activities, of the extracts were calculated using the following equation: M (*mg/g dry matter*) = $\frac{Ax}{A}.\frac{1}{a}.\frac{Vdm}{100-W}$. k (I), in which: M was the concentration per gram tuber powder (mg/g dry mass), Ax was the concentration of solution which was calculated from calibration curve (µg/ml), a was the amount of tuber in extract (g), A was conversion factor (ppm), V_{dm} was the volume after dilution, k was dilution factor, W was the moisture content in sample (%). Statistical analysis was conducted using Statgraphics Centurion XVI software (Statpoint Technologies Inc., Warrenton, Virginia, USA). The data were presented as mean ± standard error of the mean. Differences between means of different groups were analyzed using ANOVA variance analysis followed with multiple range tests, and the criterion of statistical significance was set as $p < 0.05$.

RESULTS AND DISCUSSION

Moisture Content of Tuber Powder

After determination of moisture content of powder, avenge of moisture content of *A. opertus* was 8,63%, whereas that of *A. lanceolatus* is 7,45%. Those numbers were applied to the equation (I) to calculate the polyphenols, triterpenoids, flavoinoids, polysaccharide contents of tuber powders.

Total Flavonoid Content

Total flavonoid contents (TFC) of *A. opertus* and *A. lanceolatus* tuber powders were presented in Table 1. As shown in Table 1, *A. opertus* tuber powder contained 0.313 ± 0.009 mg QE/g dry mass with SD = 0.0035, and repeatability of this experiment (RSD% = 1.10%) was below the accepatable limit (5%) whereas those of *A. lanceolatus* was 0.032 ± 0.004 mg QE/g dry mass with SD = 0,0015, and RSD% was 4.8238% (<5%). Quercetin not only is a flavonoid abundantly found in fruits and vegetables and commonly used in food and beverage industry, but is classified as antioxidant compounds [14]. These results revealed that TFC of *A. opertus* powder was 9.78 times as high as that of *A. lanceolatus*. As compared to other species, such as *Brassica oleracea* (0.23mg), *Vaccinium spp.* (0.15mg), *Ipomoea batatas* (0.1mg), *Brassica oleracea* (0.03mg), *Camellia sinensis* (0.02mg), TFC of *A. opertus* powder was higher and could be applied in food industry, but both TFC of *A. opertus* and *A. lanceolatus* were lower other species, including of *Capparis spinosa* (2.34mg), *Levisticum officinale* (1.73mg), *Rumex acetosa* (1.7mg), *Raphannus sativus* (0.7mg) [15, 16].

Table 1. *Total flavonoid contents of A. opertus and A. lanceolatus tubers*

No.	Sample Code*	TFC (mg quercetin/g dry mass)	Mean of TFC (mg quercetin/g dry mass) **SD, RSD%
1	AO1	0.317	$\overline{M}$= 0.313 ± 0.009
2	AO2	0.311	SD = 0.0035
3	AO3	0.311	RSD% = 1.10%
4	AL1	0.033	$\overline{M}$= 0.032 ± 0.004
5	AL2	0.032	SD = 0.0015
6	AL3	0.030	RSD% = 4.8238 %

Note: *AO: *A. opertus* tuber, AL: *A. lanceolatus* tuber
**SD: standard deviation, RSD %: Relative standard deviation

Total Triterpenoid Content

We determined that total triterpenoid content (TTC) of *A. opertus* powder was 0.020 ± 0.001 mg OAE/g dry mass with SD = 0.0006 while that of *A. lanceolatus* was 0.0109 ± 0.0003 mg OAE/g dry mass with SD = 0.0001 (Table 2). Repeatabilities of of these experiments were acceptable and lower than 5% (RSD% of *A. opertus* and *A. lanceolatus* powders were 2.8394% and 1.0626%, respectively). The data showed that TTC of *A.*

opertus was 1.82 times as compared to that of *A. lanceolatus*. Oleanolic acid, a well-known member of triterpenoids, is a non toxic subtance which possesses strong antibacterial, anti-fugal, and antioxidant activities [17, 18], anti-infllammatory [19, 20, 21, 22], hepatoprotective and anti-viral effects [23]. Furthermore, anti-HIV activity of oleanolic acid has been proved human cells [24]. Recently, Zhang et al. also suggested that oleanolic acid could prevent high glucose impaired vasoliadation [25]. This study has determined triterpenoids of *A. opertus* and *A. lanceolatus* tuber powders for the first time, which suggests the further application of these species in functional foods and medicine.

Table 2. *Total triterpenoid contents of A. opertus and A. lanceolatus tubers*

No.	Sample Code*	TTC (mg OAE/g dry mass)	Mean of TTC (mg OAE/g dry mass) **SD, RSD%
1	AO1	0.021	$\overline{M}$= 0.020 ± 0.001
2	AO2	0.020	SD = 0.0006
3	AO3	0.020	RSD% = 2.8394%
4	AL1	0.0110	$\overline{M}$= 0.0109 ± 0.0003
5	AL2	0.0108	SD = 0.0001
6	AL3	0.0108	RSD% = 1.0626%

Note: *AO: *A. opertus* tuber, AL: *A. lanceolatus* tuber
**SD: standard deviation, RSD %: Relative standard deviation

Total Polyphenol Content

As shown in Table 3, total polyphenol content of *A. opertus* tuber powder was 0.026 ± 0.001 mg GAE/ g dry mass whereas that of *A. lanceolatus* was 0,029 ± 0,003 mg GAE/g dry mass. Repeatabilities of of these experiments were acceptable and lower than 5% (RSD% of *A. opertus* and *A. lanceolatus* powders were 2.1925% and 3.4483%, respectively). The data indicated that TPC of two species was identical. Gallic acid, a member of polyphenols, is a strong anoxidant which possesses anti-cancer, antimicrobial [26, 27], and anti-diabetic effects [28].

Table 3. *Total polyphenol contents of A. opertus and A. lanceolatus tubers*

No.	Sample Code*	TPC (mg GAE/g dry mass)	Mean of TPC (mg GAE/g dry mass) **SD, RSD%
1	AO1	0.027	$\overline{M}$= 0.026 ± 0.001
2	AO2	0.026	SD = 0.0006
3	AO3	0.026	RSD% = 2.1925%
4	AL1	0.028	$\overline{M}$= 0.029 ± 0,003
5	AL2	0.030	SD = 0.001
6	AL3	0.029	RSD% = 3.4483%

Note: *AO: *A. opertus* tuber, AL: *A. lanceolatus* tuber
**SD: standard deviation, RSD %: Relative standard deviation

Total polysaccharide content

In Table 4, we determined that total polysaccharide content of *A. opertus* powder was 1.6527 ± 0.1568 mg GE/g dry mass with SD = 0.0631 while that of *A. lanceolatus* was 0.0131 ± 0.0003 mg GE/g dry mass with SD = 0.001 (Table 2). Repeatability of this experiment was acceptable and lower than 5% (RSD% of *A. opertus* and *A. lanceolatus* powders were 3.8184% and 0.8837%, respectively). The data showed that total polysaccharide content of *A. opertus* was 126.2 times as hight as that of *A. lanceolatus.*

Table 4. *Total polysaccharide contents of A. opertus and A. lanceolatus tubers*

No.	Sample Code*	Total polysaccharide content (mg GE/g dry mass)	Mean of total polysaccharide content (mg GE/g dry mass) **SD, RSD%
1	AO1	1.583	M̅= 1.6527 ± 0.1568
2	AO2	1.669	SD = 0.0631
3	AO3	1.706	RSD% = 3.8184%
4	AL1	0.0132	M̅= 0.0131 ± 0.0003
5	AL2	0.0130	SD = 0.0001
6	AL3	0.0130	RSD% = 0.8837%

Note: *AO: *A. opertus* tuber, AL: *A. lanceolatus* tuber
**SD: standard deviation, RSD %: Relative standard deviation

DPPH Radical Scavenging Activity

As shown in Table 5, DPPH scavenging activity of *A. opertus* tuber powder was 0. 027 ± 0.001 mg Trolox/g dry mass with SD = 0,0006, and repeatability of this experiment (RSD% = 2.1123%) was below the accepatable limit (5%). On the other hand, DPPH scavenging activity of *A. opertus* tuber powder was 0.017 ± 0.001 mg Trolox/g dry mass with SD = 0.0006, and RSD% was 3.4641% (<5%). Furthermore, DPPH scavenging activity of *A. opertus* was 1.59 times as as compared to that of *A. lanceolatus.*

Table 5. *DPPH radical scavenging activities of A. opertus and A. lanceolatus tubers.*

No.	Sample Code*	DPPH scavenging activity (mg Trolox/g dry mass)	Mean of DPPH scavenging activity (mg Trolox/g dry mass) **SD, RSD%
1	AO1	0.028	M̅= 0.027 ± 0.001
2	AO2	0.027	SD = 0.0006
3	AO3	0.027	RSD% = 2.1123%
4	AL1	0.017	M̅= 0.017 ± 0.001
5	AL2	0.017	SD = 0.0006
6	AL3	0.016	RSD% = 3.4641%

Note: *AO: *A. opertus* tuber, AL: *A. lanceolatus* tuber
**SD: standard deviation, RSD %: Relative standard deviation

ABTS Radical Scavenging Activity

In Table 6, we determined that ABTS radical scavenging activity of *A. opertus* tuber powder was 0.017 ± 0.001 mg Trolox/g dry mass with SD = 0.0006. On the other hand, scavenging activity of *A. opertus* tuber powder was 0.012 ± 0.001 mg Trolox/g dry mass with SD = 0.0006. Repeatability of this experiment was acceptable and lower than 5% (RSD% of *A. opertus* and *A. lanceolatus* powders were 3.4641% and 4.9487%, respectively). The data showed that ABTS radical scavenging activity of *A. opertus* was 1.42 times higher as compared to that of *A. lanceolatus*. As shown in Table 5 and Table 6, both *A. opertus* and *A. lanceolatus* tuber powder exhibited antioxidant activity through DPPH and ABTS radical scavenging assays. The difference antioxidant and bioactive compound contents of two species, such as polyphenols and flavonoids, may be an explanation for their antioxidant capacity [29].

Table 6. *DPPH radical scavenging activities of A. opertus and A. lanceolatus tubers*

No.	Sample Code*	ABTS scavenging activity (mg Trolox/g dry mass)	Mean of ABTS scavenging activity (mg Trolox/g dry mass) **SD, RSD%
1	AO1	0.017	$\overline{M}$= 0.017 ± 0.001
2	AO2	0.017	SD = 0.0006
3	AO3	0.016	RSD% = 3.4641%
4	AL1	0.011	$\overline{M}$= 0.012 ± 0.001
5	AL2	0.012	SD = 0.0006
6	AL3	0.012	RSD% = 4.9487%

Note: *AO: *A. opertus* tuber, AL: *A. lanceolatus* tuber
**SD: standard deviation, RSD %: Relative standard deviation

CONCLUSION

This study determined concentration of some bioactive compounds in *A. opertus* tuber, including flavonoids (0.313 ± 0.009 mg QE/g dry mass), triterpenoids (0.020 ± 0.001 mg OAE/g dry mass), polyphenols (0.026 ± 0.001 mg GAE/g dry mass), as well as the polysaccharide content (1.653 ± 0.157 mg GE/g dry mass), DPPH radical (0.027 ± 0.001 mg Trolox/g dry mass) and ABTS radical scavenging activities (0.017 ± 0.001 mg Trolox/g dry mass). On the other hand, *A. lanceolatus* tuber contained flavonoids (0.032 ± 0.004 mg QE/g dry mass), triterpenoids (0.0109 ± 0.0003 mg OAE/g dry mass), polyphenols (0.029 ± 0.003 mg GAE/g dry mass), as well as polysaccharide (0.0131 ± 0.0003 mg GE/g dry mass), DPPH radical (0.017 ± 0.001 mg Trolox/g dry mass) and ABTS radical scavenging activities (0.012 ± 0.001 mg Trolox/g dry mass). The results showed that concentration of some compounds in ethanolic extract of *A. opertus* was higher than those of *A. lanceolatus* and other species, especially in polysaccharide, DPPH and ABTS radical scavenging activities. Due to lack of the samples, the main constituents of classes of bioactive compounds and their bioactivities have not identified yet. The further application of these species in functional food and medicine requires more experiments to determine other bioactivities and toxicity of these extracts.

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