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Antioxidant and antimicrobial potential of *Aspidistra elatior* extracts

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ABSTRACT

Aspidistra elatior (Asparagaceae) has been used for relief backache and no data support their antioxidant and antimicrobial properties. The aim of this study was determined phytochemical constituents, antioxidant and antimicrobial activities of aqueous (AE) and ethanolic (EE) extract of A. elatior. Total phenolic, flavonoid and tannin contents were investigated for phytochemical contents. 2, 2' -azino-bis (3-ethylbenthiazoline-6-sulphonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, hydrogen peroxide (H_2O_2), nitric oxide (NO), hydroxyl radical (OH•) scavenging and ferric reducing antioxidant power (FRAP) methods were selected to study antioxidant properties. The antimicrobial effect of AE and EE were tested against Staphylococcus aureus. The results showed that both fractions contained phenolic, flavonoid and tannin. The AE fraction scavenged NO, H_2O_2, ABTS and OH• more than EE. While, the EE had ability to scavenge DPPH• and reduce Fe^{3+} to Fe^{2+} better than the AE. Both the extracts were not affected to Staphylococcus aureus growth. This study indicated that A. elatior extract had potential to scavenge free radicals but had no antimicrobial potentials.

Keywords: *Aspidistra elatior, phytochemical constituents, antioxidants, antimicrobial*

INTRODUCTION

The natural plants and their products are an importance for prevention some diseases caused from free radicals. Antioxidant compounds including phenolic, flavonoid, tannin and anthocyanin are most important in the plants because their ability to scavenge free radical-mediated degradation of cells and tissues [1,2]. Many reports have been showed that fruits, vegetables and herbs had antioxidant properties. However, many plants still lack to study their antioxidant and antimicrobial which are basic screening method of plants' utilities. *Aspidistra elatior* is the popular herbal tea due to its pharmacological activity. It has been used in the treatment of back pain, waist pain, aphthous ulcer, hypercholesterolemia and fatigue [3-5]. Moreover, aspidistrin, its bioactive compound, also has antifungal effect against *Saccharomyces cerevisiae*, *Mucor mucedo* and *Candida albicans* [6]. But the study of antioxidant activity and antimicrobial effect were lacked. So, the aim of this study was focused to determined phytochemical constituents including total phenolic, total flavonoid and total tannin contents, antioxidant activity and antimicrobial effect of this plant against *Staphylococcus Aureus Aspidistra elatior* is a local plant in Asparagaceae family that found in China, Japan and Northern Thailand. In Japanese also known as "Haran", an ornamental plant. It also used as folk medicine including antidiuretic and mucolytic agents. Previous studies found that this plant contained steroid compounds [3-5] and had antifungal activity against *Saccharomyces cerevisiae*, *Hansenula anomala*, *Mucor mucedo*, and *Candida albicans* [6]. The research about benefits and pharmacological activity of *A. elatior* is still lacking. So, the aim of this study was to determined phytochemical constituents, antioxidant and antimicrobial activities of *A. elatior*.

MATERIALS AND METHODS

Chemicals

2,2'-azino-bis (3-ethylbenthiazoline-6-sulphonic acid (ABTS), 2, 2- diphenyl-1-picrylhydrazyl (DPPH), 2, 4, 6-tris (1-pyridyl)-5-triazine (TPTZ), 5, 5' -dithiobis-(2-nitrobenzoic acid) (DTNB), 6-hydroxy-2,5,7,8-tetramethylchromane-2 carboxylic acid (Trolox), L-ascorbic acid, Folin-Ciocalteu reagent, gallic acid, N-(1-napthyl) -ethylenediamine diahydrochloride (NED), quercetin, Sodium nitroprusside (SNP), sulfanilamide and tannic acid were purchased from Sigma- Aldrich (St. Louis, MO, USA). Another chemicals were analytical grade.

Preparation of plant extract

Dried *Aspidistra elatior* was purchased from Doi Kham Royal Project Shop, Chiang Mai, Thailand. The plants were extracted with water and 80% ethanol (1:10). After 24 h in the dark, the aqueous extract (AE) and ethanolic extract (EE) were filtrated, evaporated under vacuum and lyophilized to obtain powder extracts.

Total phenolic content

Total phenolic content was determined using Folin-Ciocalteu reagent according to Singhatong method [7]. Briefly, 50 µL of the extracts were added into 2.5 mL of Folin-Ciocalteu reagent with 2.0 mL of 7.5% (w/v) of Na_2CO_3. The reaction mixture was allowed to stand for 15 min at 45°C. The absorbance was read at 764 nm and the results were calculated as µg gallic acid equivalent per gram plant extract.

Total flavonoid content

Total flavonoid contents of AE and EE were determined by aluminium chloride colorimetric method [2]. Briefly, 0.5 mL of AE or EE, 0.1 mL of $AlCl_3$ and 0.1 mL of 1 M CH_3COONa were added in 2.8 mL of distilled water. After incubation at room temperature for 30 min, the absorbance was read at 415 nm. The total flavonoid content was calculated as µg quercetin equivalent per gram extract.

Total tannin content

Total tannin content was determined with method of Singhatong et. al. [7]. Briefly, 100 µL of each the extracts, 500 µL of Folin-Ciocalteu reagent and 1.0 mL of 0.5% (w/v) Na_2CO_3 were added in 3.4 ml distilled water tube. After incubating at room temperature for 30 min, the reaction was measured at 755 nm and the results were calculated as µg tannic acid equivalent per gram extract.

ABTS assay

ABTS assay was determined following the method of Singhatong et. al [7]. Briefly, working ABTS solution was prepared by mixing ABTS with $K_2O_8S_2$ (1:1) and kept at 4°C in dark for 16-18 h before use. 10 µL of each concentration of the extracts were added to 990 µL of working ABTS solution and read the absorbance at 734 nm. L-ascorbic acid was used as positive control and the results were expressed in concentration of L-ascorbic equivalent per gram extract.

DPPH radical scavenging assay

DPPH radical scavenging activity was estimated by the method of Singhatong et. al [7]. Briefly, 50 µL of each concentration of the extracts (0-20 mg/mL) were added to 2,950 µL of DPPH solution and then incubated in dark at room temperature for 15 min. The absorbance was measured at 515 nm. Trolox was used as positive control and the results were calculated and expressed as IC_{50}.

Hydrogen peroxide scavenging activity

Hydrogen peroxide (H_2O_2) scavenging assay was measured by Singhatong et. al [7]. The reaction mixture contained with 100 µL of various concentrations of the extracts, 80 µL of 0.003% (v/v) H_2O_2 solution, 800 µL of PBS buffer pH 6.0, 100 µL of 10 U/mL HRP and 800 µL of 0.1% (w/v) ABTS solution. Then, the mixture was mixed and incubated at 37°C for 10 min. After that, the absorbance was measured at 415 nm. L-ascorbic acid was used as positive control and the results were calculated and expressed as IC_{50}.

Nitric oxide scavenging assay

Nitric oxide (NO) scavenging assay was determined by the method of Sundararajan et. al. [8]. The reaction mixture was contained with 0.5 mL of the extracts, 2 mL of SNP and 0.5 mL of PBS pH 7.4. The mixture was mixed and incubated at 25°C for 150 min. 0.5 mL of this reaction mixture was aliquoted and added to 1 mL of sulfanilamide. After 5 min, 1 mL of NED was added and then incubated at 25°C for 30 min. The absorbance was read at 537 nm. Gallic acid was used as positive control and the results were calculated and expressed as IC_{50}.

Ferric reducing antioxidant power assay

Ferric reducing antioxidant power (FRAP) assay was determined as described by Khachitpongpanit et. al. [2]. Briefly, cocktail solution was prepared (300 mm acetate buffer: TPTZ: $FeCl_3 \cdot 6H_2O$, 10: 1: 1, 37°C). 30 µL of the *A. elatior* extracts (0-20 mg/mL) was mixed with 900 µL of cocktail solution and 90 µL of dH_2O. The reaction was rapidly read at 593 nm, then incubated at 37°C for 4 min and read again. $FeSO_4$ was used for calculated standard calibration curve and the results were expressed in concentration of $FeSO_4$ equivalent per gram extract.

Hydroxyl radical scavenging assay

Hydroxyl radical (OH•) scavenging assay was determined by Narongchai. et. al [9]. The reaction mixture composed of 35 µL of deoxyribose, 35 µL of PBS pH 7.4 and 175 µL of distilled water, 35 µL H_2O_2 solution, 35 µL EDTA, 35 µL $FeCl_3$, 35 µL *of* the *A. elatior* extracts and 35 µL ascorbic acid were added to the reaction mixture. This mixture was allowed to stand at 37°C for 60 min. After that, 350 µL of TBA and 350 µL of TCA were added. The mixture was boiled for 20 min and then cooled down on ice for 20 min. The absorbance was measured at 532 nm and used quercetin as a positive control. The results were calculated and expressed as IC_{50}.

Antimicrobial activity

Staphylococcus aureus (ATCC 25923) was cultured for 18-24 h prior to adjusted turbidity to McFarland No. 5 (1.0×10^8 CFU/mL). Antimicrobial activity was determined by Agar cup method [10]. Firstly, Mueller-Hinton Agar was prepared on plate and then placed 4 pieces of 12 mm sterile aluminium disc on the surface of MHA media. Secondly, 1 mL of *S. aureus* was mixed with 10 mL of melt-MHA and filled into the MHA and waited until solidify. Then, pulled out the disc and filled the holes with 200 µL of the *A. elatior* extracts (1.25, 2.5, 5 and 10 mg/mL). After incubated at 37°C for 24 h, the results were measured by observing the size of inhibition zone.

RESULTS AND DISCUSSION

A. elatior was extracted with water and 80% ethanol and yield extraction was 6.47 and 13.22 % , respectively. The extracts were determined phytochemical contents and the results are shown in Table 1. Total flavonoid and tannin contents were presented in EE more than AE. Several studies showed that flavonoid and tannin are dissolved in ethanol better than water. Highly extraction in ethanol might be due to the fact that it easily penetrates the cellular membrane and extracts the intracellular ingredients from the plant material [11,12]. Furthermore, the plant contained more polar and nonpolar substances. Many reports showed that alcohol give higher yield extraction than other solvents including acetone, diethyl ether, Ethylacetate and water [13].

Table 1: **Phytochemical constituents in *A. elatior* extracts**

***A. elatior* extracts**	**Total phenolic (µg GAE/g extract)**	**Total flavonoid (µg QE/g extract)**	**Total tannin (µg TAE/g extract)**
Aqueous extract (AE)	37.50 ± 0.01	7.41 ± 0.01	68.54 ± 0.01
Ethanolic extract (EE)	35.44 ± 0.01	20.08 ± 0.01	71.40 ± 0.01
Data are presented as the mean ± SD (n = 3); GAE = Gallic acid equivalent; QE = Quercetin equivalent, TAE = Tannic acid equivalent			

Phenolic and flavonoid are the most secondary metabolite that present in medicinal plants, fruits and vegetables which are responsible for scavenging free radical and biological activities. [14]. Increasing risk factors of human to disease especially non-communicate diseases (NCD), there has been a global trend toward the use of natural substance present in medicinal and dietary plants as therapeutic antioxidant.

There are many methods for testing free radical scavenging including ABTS, DPPH•, NO, H_2O_2, OH•, and FRAP methods. The results are presented in Table 2 and Figure 1. The DPPH radical scavenging activity and reduction of Fe^{3+} to Fe^{2+} in the ethanolic extract showed excellent free radical scavenging activity higher than the aqueous extract. Earlier, it has been reported that radical scavenging ability of bioactive compounds depend on number of aromatic rings. Aromatic rings are contained in Flavonoid and tannin structure then the ethanolic extract may scavenge free radical better than aqueous extract [15]. For, H_2O_2, NO, OH•, and ABTS radical scavenging, the results showed that the aqueous extract was potential to scavenge these free radicals better than the ethanolic extract. These result is indicative of the influence of total phenolic compounds [13].

Table 2: **Inhibitory concentration at 50% (IC_{50}) of antioxidant scavenging activities**

***A. elatior* extracts**	**Inhibition concentration at 50% (mg/ mL)**			
	DPPH	**H_2O_2**	**NO**	**OH•**
Aqueous extract (AE)	15.98 ± 0.13	1.17 ± 0.01	1.26 ± 0.17	2.97 ± 0.06
Ethanolic extract (EE)	14.40 ± 0.38	3.13 ± 0.01	3.74 ± 0.36	12.81 ± 0.37
Data are presented as the mean ± SD (n = 3)				

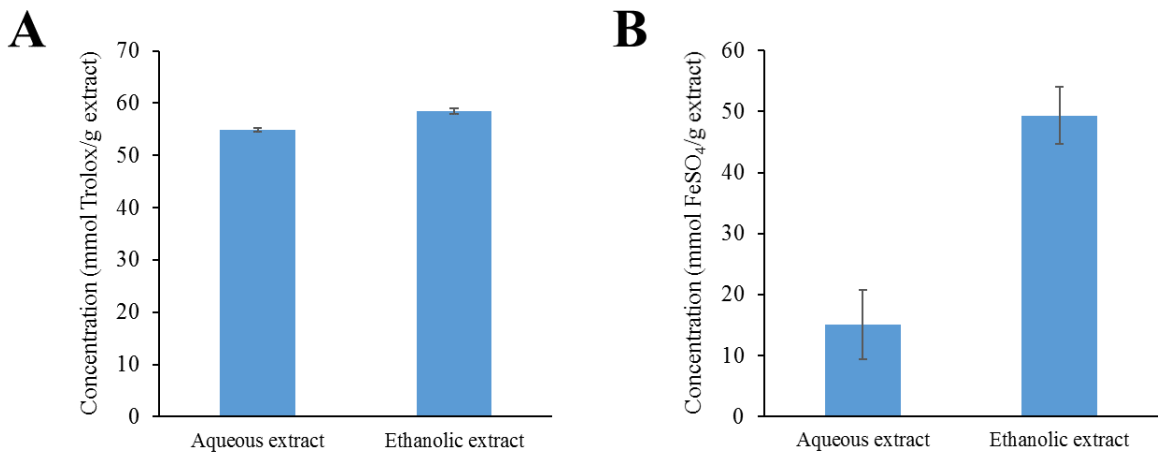

Figure 1: **antioxidant activities; (A) ABTS assay and (B) FRAP assay, data are presented as the mean ± SD (n = 3)**.

For microbial activity, this study was tested with *S. aureus*, bacterial pathogen that cause multiorgan infectious diseases [16]. The results showed that both AE and EE had no antimicrobial activity. The results may be due to the maximum concentration of *A. elatior* extracts (10 mg/mL) in this study was too low. So, it could not inhibit *S. aureus* growth or *A. elatior* extracts could be inhibited other microorganism. Therefore, it should be study antimicrobial effect against other microorganisms further.

CONCLUSION

This study showed that *A. elatior* extracted with water and ethanol also found phytochemical contents including total phenolic, flavonoid and tannin contents. Both extracted fraction had antioxidant activities but no antimicrobial activity.

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