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Chemical Composition and Pharmacological Activities of *Plantago major* L. in Vietnam

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Plantago major L. in Vietnam was investigated for its chemical composition and also evaluated the biological activities against enzyme α-glucosidase and free radicals activities. The powder mixture of dried leave and roots of this species was extracted separately by three solvents: dichloromethane, water, water:alcohol (50:50, v:v). The chemical composition of dichloromethane extract was analyzed by GC-MS system to identify eighteen components, out of which eight biologically active compounds *viz*. 5-hydroxymethylfurfural, *n*-hecxadecanoic acid, 9,12-octadecadienoic acid (Z,Z)-methyl ester, allogibberic acid, β-tocopherol, campesterol, γ-sitosterol, lup-20(29)-en-3-ol and friedenlan-3-one were presented. The concentration of radical scavenging activity DPPH expressed by IC_{50} for water, water:alcohol (50:50, v:v) and dichloromethane with 208.7, 89.3 and 62.05 μg/mL, respectively. The dichloromethane, water and water:alcohol (50:50, v:v) extract of *Plantago major* exhibited α-glucosidase inhibitory activity with IC_{50} values of 116.4, 302.7, 195.9 μg/mL, respectively, which was comparable with acarbose (98.4 μg/mL). *Plantago major* L. in Vietnam may be effective inhibitors as the antidiabetic candidate and helpful to reduce the postprandial glucose levels.

Keywords: *Plantago major* L. Radical scavenging activity α-Glucosidase inhibitory activity, GC-MS.

INTRODUCTION

Plantago major is one of the most favourite and long-time used traditional medical herbs, belongs to the genus *Plantago* and the family of Plantaginaceae. *Plantago major* is found in a lot of various habitants such as the wetlands not only on the delta and seashore but on the highland [1]. In the Vietnamese traditional medicine, *Plantago major* L. can be used to treat some popular diseases such as chronic constipation, digestive disorders, diarrhea, piles and alleviated problems of kidney, bladder and hemorrhoids [2]. In addition, the potential modern medicine using that species have been investigated and proved to be positive treatment for wound healing activity [3,4], anti-inflammatory and analgesic activities [5,6], antiulcerogenic activity [7,8], hypoglycemic activity [9], antiviral activity [10]. Leave, seeds, roots from *P. major* contain biologically active compounds: Fatty acids in seed and leaves [11,12], alkaloid, polysaccharides, lipids, caffeic acid derivatives, flavonoids, iridoid glycosides and terpenoids [2,13-15]. According to the literature, only one species from the genus *Plantago* and the family of Plantaginaceae, *Plantago major* L. is planted popularly in Vietnam for normal vegetable supplement in everyday Vietnammese diet. Otherwise, the combination between the *Plantago major* L. and corn silk is one of the popular ingredients to boil for aqueous stock solution in the treatment of urinary tract infection. As our furthest knowledge until now, few publications from Vietnamese scientific community mentioned compounds from the species of *Plantago major* L. and their biological activities. In this report, the chemical components of *Plantago major* L. are investigated and also examined their ability against the effects of free radicals and α-glucosidase activity.

EXPERIMENTAL

Fresh plant material consist of leaves and roots was collected from Hoai Duc in the west of Hanoi in September 2018 and identified by Dr. Trieu Anh Trung, Faculty of Biology, Hanoi National University of Education. The plant sample were washed with water to completely remove soil, inorganic solid and dried by ventilation in the dark at room temperature. After drying in the oven at 50 ºC, the plant sample was ground to powder and stored in vacuum bags.

Crude extract preparation: *Plantago major* L. sample was soaked in dichloromethane/in water-ethanol (50:50, v/v) for three times, each time for 7 days at room temperature or boiled in water. The combined extracts were concentrated using rotary evaporator (Büchi, Rotavapor R215) to give the crude extracts.

DPPH radical scavenging assay: The antioxidant activity of *Plantago major* L. extract was determined the scavenging activity of DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical as described earlier [16-18]. A solution of DPPH 300 mM in methanol was prepared. Sample of *P. major* L. extract were dissolved in DMSO to prepare the concentrations range from 1 to 256 mg/mL. For DPPH radical scavenging assay, 10 µL of a different concentration of crude extract sample was added 190 µL DPPH. The reaction mixture was incubated at 37 ºC for 30 min. The absorbance of the mixture was measured at λ = 517 nm. To make a control reaction, 10 µL DMSO 0.5% was mixed with 190 µL DPPH. Quercetin was used as the comparison standard. The experiment was repeated three times. The percent of DPPH radical scavenging activity was calculated following a formula:

$$\text{DPPH radical scavenging activity } (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where, $A_{control}$ is the absorbance of the control, A_{sample} is the absorbance of the sample.

***in vitro* α-Glucosidase inhibition assay:** The inhibition of α-glucosidase activity was carried according to the reported methods [19-21]. A solution of *p*-nitrophenyl α-D-glucopyranoside (*p*-NPG) 2.5 mM and α-glucosidase 0.2 U/mL were prepared in phosphate buffer 100 mM pH 6.8. The concentration 1-256 µg/mL of extract samples were diluted in DMSO. For evaluation of α-glucosidase inhibitory activity, 10 µL sample was added to a reaction mixture consisting of 40 µL phosphate buffer 100 mM pH 6.8, 25 µL α-glucosidase 0.2 U/mL, then incubated at 37 ºC for 10 min after which 25 µL *p*-NPG was added, the reaction mixture further carried on for 20 min at 37 ºC. Sodium carbonate 100 mM (100 µL) was added to stop reaction. The absorbance of the mixture was measured at λ = 410 nm. To make a control reaction, the tested sample was replaced by 10 µL phosphate buffer 100 mM pH 6.8. Acarbose was used as the comparison standard. The experiment was repeated three times. The percent of α-glucosidase inhibitory activity was calculated following a formula:

$$\text{α-Glucosidase inhibitory activity } (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where, $A_{control}$ is the absorbance of the control, A_{sample} is the absorbance of the sample.

GC/MS chemical compound analysis: A small amount of *Plantago major* L. crude extract was dissolved in ethyl acetate and filtered by driven filter (25 mm, pore size 0.45 µm). Analyses were realized on GC/MS system (Agilent-6890N/5973i, MSD 6890; silica capillary column HP-5MS 30 m × 0.25 mm ID, 0.25 µm. The carrier gas was helium (99.999%) with a flow rate of 0.6 mL/min, the injector volume was 1 µL, the split mode in 15:1 ration; the injector and detector temperatures were 250 and 280 ºC, respectively. The oven was temperature programmed as follows: 50 ºC in 2 min, the raised by 30 ºC/min to 195 ºC, held for 2 min, raised 5 ºC/min to 250 ºC and finally held for 5 min at 280 ºC. Electronic energy was 70 eV; full scan mode, range 45-550 *m/z*. The compounds were identified by comparing their mass spectra with computer matching against commercial mass library and retention time.

RESULTS AND DISCUSSION

DPPH radical scavenging assay: Antioxidant activity is one of the most important bioactive properties of medicinal plants which is related to other properties, such as anticancer [22]. To evaluate the antioxidant property of *Plantago major* extracts, the DPPH radical scavenging assay was performed. In this study, the plant samples were extracted by using different solvents, including water, water and ethanol and dichloromethane. Quercetin was used as the standard for antioxidant activity. Dichloromethane extract of *P. major* showed higher antioxidant activity with lower IC_{50} (60.86 ± 1.11 µg/mL) compared to water and ethanol extract (IC_{50} = 86.92 ± 2.67 µg/mL), water extract (IC_{50} = 201.82 ± 6.12 µg/mL) as shown in Table-1. However, the extracts collected from different solvents in this study showed lower antioxidant activity compared to quercetin, which gave IC_{50} at 24.79 ± 0.23 µg/mL. Besides, it seemed like the less polar solvent was used in extraction, the stronger antioxidation activity of the extract possessed, which might be explained by the differences of chemical components between the extracts.

TABLE-1
DPPH RADICAL SCAVENGING AND α-GLUCOSIDASE INHIBITION ACTIVITIES OF EXTRACTS OF *P. major*

Solvent	DPPH radical scavenging IC_{50} (µg/mL)	α-Glucosidase inhibition IC_{50} (µg/mL)
Water and ethanol	86.92 ± 2.67	182.65 ± 13.26
Water	201.82 ± 6.12	301.34 ± 1.59
Dichloromethane	60.86 ± 1.11	111.33 ± 5.15
Quercetin/Acarbose	24.79 ± 0.23	94.70 ± 3.40

Previous studies about the antioxidant activity of *P. major* extracts in various solvents exhibited relatively different results (Table-2). Evaluated by using the DPPH radical scavenging assay, among listed values, the methanol-acetic acid (85:15 v/v) extract of *P. major* gave the lowest activity as the IC_{50} value 1369.31 µg/mL [23]. In contrast, the methanol extract of the plant from Dehradun, India showed the best antioxidation activity (IC_{50} = 139.19 µg/mL) [24], whereas the plant extract of which was collected in Iran had much higher IC_{50} value though using the same solvent. However, based solely on the reported IC_{50} values of DPPH radical scavenging assays in comparison with the relative values of the standard drugs, the dichloromethane and the methanol-water extracts of *P. major* in our study presented rather strong antioxidation activities, suggesting a promising potential of the *Plantago* plant found in Vietnam.

On the other hand, the results discovered by Mani *et al.* [24] seem to follow a different pattern in comparison with

TABLE-2
ANTIOXIDATION ACTIVITIES OF EXTRACTS OF SEVERAL *Plantago* PLANTS

Plant	Sampling location	Solvent	DPPH radical scavenging IC_{50} (μg/mL)		Ref.
			Plant extract	Standard	
P. major	Jiroft, Iran	Methanol-acetic acid	1369.31	22.64 (Gallic acid)	[23]
	Dehradun, India	Benzene	691.93	99.9 (Gallic acid)	[24]
		Chloroform	341.80		
		Ethanol	190.36		
		Methanol	139.19		
	Serbia	Methanol (80%)	5.35 ± 0.29	8.28 ± 0.50 (BHT)	[40]
	Gilan and Mazandaran, Iran	Methanol	320	25 (Quercetin) 400 (BHT)	[41]
P. cornuti	Bulgaria	Methanol (80%)	165.2 ± 81.42	3.15 ± 1.1 (Quercetin)	[42]
P. arenaria			55.53 ± 31.36		
Forsythia Vahl.			101.3 ± 62.56		
P. bellardii	Bandajoz, Spain	Methanol	23.70 ± 2.99	24.19 ± 4.21 (Ascobic acid)	[43]
P. argentea	Serbia	Methanol (80%)	7.38 ± 0.33	8.28 ± 0.50 (BHT)	[40]
P. holosteum			6.28 ± 0.13		
P maritima			6.79 ± 0.16		
P. media			5.77 ± 0.05		

present study results. Moreover, as compared to non-polar solvents (benzene and chloroform), the extracts in polar solvents (methanol and ethanol) likely to possess better antioxidation activities, which were also described to relate to the total phenolic contents of the extracts [24]. These dissimilarities in reported results may be due to various factors *e.g*. the differences in varieties of the samples, sampling locations, plant extract preparations, the modifications in protocols of DPPH radical scavenging assays, *etc*. This suggests further studies required about the plant extracts and their properties.

Results about antioxidation activities of several plants belonging to *Plantago* genus reported in some recent research also suggest that *P. major* and other *Plantago* plants generally possess potent capacities of antioxidation, which should be studied further.

***in vitro* α-Glucosidase inhibition assay:** α-Glucosidase inhibitory ability shows the potential in controlling hyperglycemia in type 2 diabetes mellitus patients [25]. To investigate the inhibitory effect of *P. major* extracts, an *in vitro* α-glucosidase inhibition assay was performed.

The plant extracts with different solvents, including water, water and ethanol and dichloromethane, were studied. The obtained results are shown in Table-3. Among the examined extracts, the dichloromethane extract continued to show the best enzyme inhibitory activity with an IC_{50} value 111.33 ± 5.15 mg/mL, following by the water-methanol extract (IC_{50} = 182.65 ± 13.26 mg/mL) and the water extract (IC_{50} = 301.34 ± 1.59 mg/mL). Nevertheless, acarbose, which was used as the standard reference drug for the α-glucosidase inhibitory activity (IC_{50} = 94.70 ± 3.40 mg/mL), showed slightly higher ability in comparison with the dichloromethane extract and much higher than other two sample extracts. In general, the α-glucosidase inhibitory capacity of *P. major* extracts in examined solvents presented the results that have the same pattern with the performed DPPH radical scavenging assay.

GC/MS qualitative analysis of chemical constituents of *Plantago major* L. in Vietnam: The investigation for the bioactive components of crude extracts was performed to determine the exact biological factors, which play the main role in disease treatments. The crude extracts from the mixture with dichloromethane were chosen to further researches due to their advanced bioactivities from the results of the previous biological analysis.

The constituents in crude extract after being separated in gas chromatography capillary column reached to mass spectrometry system. The compound in MS system was ionized and fragmented into smaller charged species. In the research, two spectral libraries, Wiley 7n.l and NIST spectral library were used for investigation compounds of *Plantago* extract.

The chromatography analysis of *Plantago major* L. extract enabled the identification of 18 chemical compounds, which listed in Table-4. The biologically active compounds in the

TABLE-3
α-GLUCOSIDASE INHIBITORY ACTIVITIES OF EXTRACTS OF SEVERAL *Plantago* PLANTS

Plant	Sampling location	Solvent	α-Glucosidase inhibitory activities IC_{50} (μg/mL)		Ref.
			Plant extract	Standard (Acarbose)	
P. lanceolata	Turkey	Acidified ethanol (80%)	1430 ± 40	32.34 ± 0.96	[44]
P. anatolica	Turkey	*n*-hexane	4300 ± 100	11 ± 0.1	[45]
		Chloroform	4200 ± 200	11 ± 0.1	
		Ethyl acetate	1700 ± 200	28 ± 1	
		Acetone	1900 ± 100	25 ± 1	
		Ethanol	800 ± 100	61 ± 2	
		Water	2000 ± 100	23 ± 2	

TABLE-4
VOLATILE COMPOUNDS IDENTIFIED IN DICHLOROMETHANE EXTRACT OF *Plantago major* L. IN VIETNAM

No.	Compound name	R_t	Peak area (%)
1	6-Hydroxy-4-heptenoic acid	3.179	0.35
2	Methoxyeugenol	3.516	0.73
3	5-Hydroxymethylfurfural	3.911	0.79
4	7-(1,3-Dimethylbuta-1,3- dienyl)-1,6,6-trimethyl-3, 8-dioxatricyclo[5.1.0.0(2,4)]octane	5.015	1.14
5	4-Hydroxy-4-(4-hydroxy-3-methoxyphenyl)butan-2-one	5.988	0.26
6	Melezitose	10.222	1.72
7	Isocurcumenol	12.917	3.18
8	Corymbolone	13.747	7.69
9	*n*-Hexadecanoic acid	15.984	2.39
10	1,1'-Biphenyl,3,4-diethyl	16.991	0.64
11	9,12-Octadecadienoic acid (Z,Z)-methyl ester	17.935	2.49
12	Formic acid, 3,7,11-trimethyl-1,6,10-dodecatrien-3-yl ester	18.096	0.64
13	Allogibberic acid	23.389	3.21
14	β-Tocopherol	27.394	6.33
15	Campesterol	36.784	4.81
16	γ-Sitosterol	39.793	20.31
17	Lup-20(29)-en-3-ol	41.722	15.01
18	Friedenlan-3-one	44.588	15.89

crude extract were 5-hydroxymethylfurfural (R_t: 3.911), *n*-hexadecanoic acid (R_t: 15.987), 9,12-octadecadienoic acid (Z,Z)-methyl ester (R_t: 17.935), allogibberic acid (R_t: 23.389), β-tocopherol (R_t: 27.394), campesterol (R_t: 36.784), γ-sitosterol (R_t: 39.793), lup-20(29)-en-3-ol (R_t: 41.722), friedenlan-3-one (R_t: 44.588). In extensive studies, these compounds are reported to have positive effects on human health such as 5-hydroxymethylfurfural has been proved to have antioxidative, anti-allergic, antihyperuricemic effect [26-28]; anti-inflammatory, hypocholesterolemic nematicide, 5α-reductase inhibitor, potent mosquito larvicide for hecxadecanoic acid [29-31]; anticancer effect for 9,12-octadecadienoic acid (Z,Z)-methyl ester [32]; allogibberic acid is used as a precursor in metabolic reactions into derivatives that inhibited developing cancer cells [33]; β-tocopherol for antioxidant, campesterol and γ-sitosterol for lowers blood cholesterol [34,35]. Along with its antibacterial properties, lup-20(29)-en-3-ol and fried-elan-3-one are triterpenoid compounds, has been proven to anti-inflammatory, antioxidative [36] and hepatoprotective activity and exhibit breast cancer cells growth inhibitory [37,39].

Conclusion

The GC/MS analysis of the dichloromethane *Plantago* extract helps to predict the structure of 18 chemical compounds in which the high amounts of various bioactive compounds were presented and have been proved to positive effect on human health. The dichloromethane, water, water:alcohol (50:50, v:v) were tested for radical scavenging and α-glucosidase inhibiting using complementary *in vitro* assays and compared with the strong antioxidant compounds quercetin/acarbose. There were no significant difference of activities between the dichloromethane extract and standard reference drugs, less than 2.5 and 1.17 folds, it may be related to the biologically active substances in the dichloromethane *Plantago* extract. It is increasingly medicinal plants with radical scavenging and α-glucosidase inhibiting activities effectively prevent oxidative damage, glucose metabolism and their consumption has become a strategy to address health challenges. *Plantago major* L. was used as a natural drug in traditional medicine, along with the evaluation of pharmacological activities above, leading to the conclusion that *Plantago major* L. in Vietnam could be a material candidate for developing functional foods and drugs.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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