



E-ISSN: 2278-4136
 P-ISSN: 2349-8234
www.phytojournal.com
 JPP 2022; 11(2): 21-23
 Received: 11-01-2022
 Accepted: 14-02-2022

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Inhibitory effect of *Ammannia bacifera* leaves against lipase and angiotensin-converting enzyme

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DOI: <https://doi.org/10.22271/phyto.2022.v11.i2a.14371>

Abstract

Obesity and hypertension are multifactorial problems that are responsible for causing several life-threatening diseases. The occurrence of obesity and hypertension is increased day by day and so the remedies from natural sources are recognized as valuable tools to control obesity and hypertension with the minimum side effects. With realizing this fact, this study was designed to evaluate the inhibitory potential of *Ammannia bacifera* leaves against pancreatic lipase and angiotensin-converting enzyme (ACE). First water soluble part (WSP) of crude aqueous extract prepared from leaves of *Ammannia bacifera* was separated from water insoluble part (WIP). In lipase inhibition assay, a moderate inhibitory effect of WSP was observed against pancreatic lipase with a IC_{50} value of $4.39 \pm 0.48 \mu\text{g/mL}$ whereas WSP showed strong inhibitory effects against ACE ($14.23 \pm 0.82 \mu\text{g/mL}$) as compared with captopril ($12.54 \pm 0.85 \mu\text{g/mL}$). In case of both enzymes, WIP did not show any activity. Therefore, this study suggests that *Ammannia bacifera* leaves might be a prospective therapeutic agent for the management of obesity and hypertension.

Keywords: *Ammannia bacifera*, leaves, lipase, obesity, hypertension, ACE

Introduction

As metabolic syndromes obesity, dyslipidemia and hypertension are considered as major health and clinical challenges worldwide [1]. Both obesity and hypertension are associated with development of cardiovascular diseases. In case of obesity, diet control along with medications can help to reduce weight gain in obese persons. Pancreatic lipase plays important role in absorption of dietary fats since it catalyzes the hydrolysis of triacylglycerols into monoacylglycerols and fatty acids in small intestine [2]. On the other hand, angiotensin-converting enzyme (ACE) catalyzes the conversion of angiotensin I into angiotensin II that act as a vasoconstrictive substance to increase blood pressure. Thus, ACE controls hypertension and plays a vital role in the management of cardiovascular diseases. Therefore, the screening for inhibitors against pancreatic lipase and ACE could be an important strategy for controlling obesity and hypertension [3]. But several side effects are reported on synthetic inhibitors of both enzymes.

Thus, it is a demand of recent time to develop active ingredients from natural sources for inhibiting lipase and ACE with no or little side effects [4-5]. In previous studies, plant extracts with rich amount of phenolic content showed inhibitory effects against lipase and ACE *in vitro* experiments [6-8]. With realizing the above facts, leaves of *Ammannia bacifera* Linn. were selected here for evaluating their inhibitory effects against pancreatic lipase and ACE. From the information available so far on the beneficial effects of *Ammannia bacifera*, it is clear that most of the secondary metabolites identified in this plant are phenolic in nature and these metabolites are responsible for its various types of biological activities [9-10]. However, there have been no studies on the inhibitory effect of *Ammannia bacifera* leaves against pancreatic lipase and ACE. So, this study was aimed to evaluate *in vitro* the inhibitory effect of *Ammannia bacifera* leaves against lipase and ACE.

Materials and Methods

Chemicals and reagents

Porcine pancreatic lipase, orlistat and captopril were purchased from Merck (Darmstadt, Germany) whereas ACE inhibition kit-WST (100 tests) were collected from Dojindo EU GmbH, Germany. Methanol, dimethyl sulfoxide (DMSO) and other solvents of HPLC grade were purchased from Labscan (Thailand).

Sample collection and authentication

Leaf of *Ammannia baecifera* Linn. (Family: Lythraceae) was collected from uncultivated lands of Darusha adjacent to Rajshahi Court station area of Rajshahi district and the collection period of plant materials was January, 2021. Professor Dr. A. H. M. Mahbubur Rahman (a taxonomist), Department of Botany, University of Rajshahi, has authenticated this plant. After authentication it was deposited in the herbarium of Botany department under the specimen record number of 943. The collected leaves were cleaned and dried at room temperature. Then it was pulverized into powder and stored in air tight glass containers for further use.

Plant extract preparation

The leaf powder (20 g) was soaked in 500 mL distilled water for 48 hrs in dark condition and at 37°C temperature. After 48 hrs, the extract was filtered with through a filter paper and concentrated with a rotary evaporator. Then the water insoluble part (WIP) in concentrated extract was removed as precipitate with ethanol to have 2.3 g. Then 4.4 g water soluble part (WSP) was obtained through freeze drying. Both water soluble (WSP) and insoluble (WIP) parts were preserved in glass vials at -80°C for various analyses.

Pancreatic lipase inhibition assay

The inhibitory effect for WSP, WIP and orlistat was judged against pancreatic lipase using previously reported procedure^[11] with slight modification. In this assay, p-nitrophenyl palmitate (p-NPP) was used as a substrate. Here, the enzyme catalyzes the conversion of p-NPP into p-nitrophenol, a color agent that can be measured at 410 nm. For inhibition assay, extract and orlistat of different concentrations were prepared in DMSO. After dissolving of lipase (15 mg) in Tris-buffer (50 mM, pH 8), it was applied on stirring for 15 min and then it was centrifuged at 2000 rpm for 10 min. The clear supernatant was separated and used as enzyme solution. In a test tube, 1 mL sample (extract or orlistat) was mixed with 0.5 mL enzyme solution. It was incubated for 30 min at 37°C and then 1 mL substrate p-NPP (3 mM in 2-propanol) was added into it. This reaction mixture was incubated for 2 h at 37 °C and finally the absorbance of this mixture was measured at 410 nm using a blank. The percentage of inhibition was calculated by following equation:

$$\% \text{ inhibition of lipase} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

where A_{control} and A_{sample} are presented as absorbance of control and sample, respectively. In control all constituents were present except sample. Orlistat was used as a positive control. All the tests were done in triplicate and the mean values were used to draw graph from which the IC_{50} values ($\mu\text{g/mL}$) were determined.

Angiotensin converting enzyme (ACE) inhibition assay

The ACE inhibition assay kit was used to determine the percentage (%) inhibition of ACE by WSP, WIP and captopril. Due to the action of enzyme, the amount of 3-Hydroxybutyric acid (3HB) produced from 3-Hydroxybutyryl-Gly-Gly-Gly, was measured colorimetrically in ACE inhibition assay kit^[12-13].

Here, enzyme and indicator working solutions were prepared according to the instruction of kit. 20 μL of sample solution in DMSO was added to sample well of a microplate whereas 20 and 40 μL of deionized water were added to blank-1 and blank-2 well, respectively. Then 20 μL of substrate buffer was

added to each well. After addition of substrate buffer, 20 μL of enzyme working solution was added to sample and blank-1 using a multi-channel pipette that minimize the well-to well time lag. Microplate was incubated at 37°C for 1 hour. Finally, indicator working solution (200 μL) was added to each well. After 10 min incubation at room temperature, the absorbance was taken at 450 nm. Percentage (%) inhibition of ACE was calculated by the following equation:

$$\text{Percentage (\%)} \text{ inhibition of ACE} = [(A_{\text{blank-1}} - A_{\text{sample}}) / (A_{\text{blank-1}} - A_{\text{blank-2}})] \times 100$$

IC_{50} values ($\mu\text{g/mL}$) for ACE were determined by the same way as described in the above assays.

Statistical analysis

Experimental data were displayed as mean \pm SD (Standard Deviation) ($n=3$) and the significance of differences was compared using one-way analysis of variance (ANOVA) followed by post hoc Duncan's multiple range test at $p < 0.05$ using SPSS statistical software of 16 version. According to Duncan's multiple range test, the significance of the differences was indicated by letters (a, b) in the tables.

Results and Discussion

The percentages of inhibition caused by WSP at different doses and IC_{50} values were shown in Table 1. The percentage (%) inhibitory activity of WSP against pancreatic lipase and ACE as function of concentration caused IC_{50} values that ultimately presented the inhibitory potential of WSP against the corresponding enzyme (Table 1).

Pancreatic lipase inhibitory activity

According to the obtained results, WSP showed maximal lipase inhibitory activity at 8 $\mu\text{g/mL}$ dose (Table-1). In case of both WSP and orlistat, the percentage inhibition was increased with increasing the concentration and WSP exhibited moderate activity at various concentration levels as compared with orlistat (Table-1). The IC_{50} value for WSP was found to be $4.39 \pm 0.48 \mu\text{g/mL}$ whereas orlistat used as positive control exhibited higher inhibitory activity with lower IC_{50} value ($1.61 \pm 0.21 \mu\text{g/mL}$). Previous report also showed similar type of results for methanolic extract of *Lagenaria siceraria* fruits where orlistat showed better activity with lower IC_{50} value in respect to extract^[11]. However, in this assay, WIP did not show any inhibitory activity against lipase enzyme. The moderate pancreatic lipase inhibitory activity of WSP suggests that it may be useful to slow down the rate of formation, absorption and accumulation of fatty acids from dietary fats digestion, which can be an important approach for controlling obesity^[14].

ACE inhibitory activity

There is a link between hypertension and type-2 diabetes where hypertension itself is a risk factor of cardiovascular diseases. Use of ACE-inhibitors has been considered as a good therapeutic approach for the treatment and management of hypertension. In this investigation, WSP inhibited ACE (IC_{50} : $14.23 \pm 0.82 \mu\text{g/mL}$) as much as captopril (IC_{50} : $12.54 \pm 0.85 \mu\text{g/mL}$), a standard ACE inhibitor (Table 1). WIP did not showed any inhibitory activity against ACE. The inhibition of ACE activity by WSP revealed that leaves of *Ammannia baecifera* have the capability to control blood pressure by reducing the production of angiotensin II. The findings of previous studies confirmed that *Ammannia*

baecifera contained secondary metabolites most of which are phenolic in nature^[9-10]. So, the synergistic or additive effects of these phenolic components present in the leaves of

Ammannia baecifera may be responsible for ACE inhibitory effects WSP.

Table 1: Percentage inhibition and IC₅₀ values of WSP and corresponding standard inhibitor against pancreatic lipase and ACE.

Samples	Pancreatic lipase			ACE		
	Test concentration (µg/mL)	Percentage inhibition	IC ₅₀ (µg/mL)	Test concentration (µg/mL)	Percentage inhibition	IC ₅₀ (µg/mL)
WSP	0.5	6.0 ± 0.83	4.39 ± 0.48 ^a	2	18.7 ± 0.61	14.23 ± 0.82 ^a
	1	18.4 ± 1.02		4	27.0 ± 0.93	
	2	32.3 ± 1.31		8	45.9 ± 1.25	
	4	52.6 ± 1.80		16	59.2 ± 1.31	
	8	80.1 ± 2.01		32	79.6 ± 1.44	
Orlistat	0.25	19.0 ± 1.21	1.61 ± 0.2 ^b	-	-	-
	0.5	31.5 ± 0.91		-	-	
	1	46.5 ± 0.85		-	-	
	2	63.8 ± 1.04		-	-	
	4	84.2 ± 1.96		-	-	
Captopril	-	-	-	2	17.0 ± 2.57	12.54 ± 0.85 ^b
	-	-		4	29.5 ± 1.15	
	-	-		8	52.8 ± 1.31	
	-	-		16	67.0 ± 0.77	
	-	-		32	82.2 ± 1.50	

Experimental data were displayed as mean ± SD (Standard Deviation) (n=3). Different superscripts letters (a, b) for a given value within a column representing IC₅₀, are significantly different from each other (Duncan's significant difference multiple range post-hoc test, $P < 0.05$).

IC₅₀: Concentration of extract that inhibited enzyme activity by 50%.

ACE: Angiotensin-converting enzyme

Conclusion

The overall findings of this study suggest that water soluble part (WSP) of *Ammannia baecifera* leaves showed inhibitory effects against pancreatic lipase and angiotensin converting enzyme (ACE). However, further studies on humans and experimental animals will be useful in order to demonstrate possible therapeutic relevance of *Ammannia baecifera* leaf extract^[15].

Conflicts of interest

The authors have no conflict of interest to declare within this article.

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