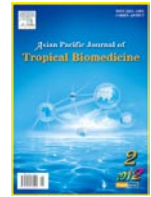




Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb



Document heading doi:10.1016/S2221-1691(12)60301-3 ©2012 by the Asian Pacific Journal of Tropical Biomedicine. All rights reserved.

In vitro prevention of chick pancreatic lipase activity by *Abroma augusta* extract

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ARTICLE INFO

Article history:

Received 25 June 2012

Received in revised form 5 July 2012

Accepted 7 August 2012

Available online 28 August 2012

Keywords:

Abroma augusta

Anti-lipase

Alkaloids

Flavonoids

ABSTRACT

Objective: To investigate chick pancreatic lipase inhibitory activities of the *Abroma augusta* (*A. augusta*). **Methods:** *A. augusta* was first extracted with methanol and subjected to phytochemical screenings. Quantitative estimation of flavonoids, phenolics and alkaloids was done. Pancreatic lipase from chick pancreas was isolated and used as substrate for anti-lipase studies. **Results:** *A. augusta* extract effectively inhibited concentration dependent lipase activity, whereby extract at concentration 100 μ g/mL inhibited 88.6% enzyme activity. **Conclusions:** From these results, it could be concluded that *A. augusta* can be used as a potential source anti-lipase agents.

1. Introduction

Hyperlipidemia, one of the metabolic disorder, is associated with abnormal lipid metabolism whereby increase in plasma lipid-cholesterol and triglycerides is observed. Increase in occurrence of hyperlipidemia may also predispose to condition like coronary cardiac diseases and neuropathy [1, 2]. Other complication associated with hyperlipidemia includes fatty liver, diabetes mellitus and hypertension[2]. Some of the currently available hypolipidemic agents aim at clearing lipids from circulation[3], but are still not effective[4]. Thus, prevention of digestion of digestive lipids could be an effective strategy for preventing systemic absorption of lipids, ultimately preventing further complications.

From time immortal, plants have been used to cure a number of diseases. Folk remedies are eternal part of modern and complimentary system. In African subcontinent, phytotherapy is still utilized for treatment of diseases. The low cost of traditional medicine along with its

effectiveness and less side effects is also the reason behind its widespread use [5]. A large number of plants are known for their medicinal and therapeutic activity worldwide.

Abroma augusta (Ulatkambal, Family: Malvaceae) (*A. augusta*) is one of the widely found plant all over in India and Australia. The plant is reported to show hypoglycemic and hypolipidemic effect [6]. The aim of current work was to extrapolate the effect of *A. augusta* on enzyme lipase.

2. Materials and methods

2.1. Collection of chick pancreas

Pancreas of freshly slaughtered chicken (*Gallus domesticus*) was collected and immediately transferred in normal saline at temperature 0–4 °C and immediately transported to laboratory (within 10 min).

2.2. Chemicals

All the chemicals unless otherwise stated were purchased from CDH, India.

2.3. Collection and identification of *A. augusta*

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Foundation Project: This study is supported by Rewa Shiksha Samiti (Support No.RSS/SRITP/MPY/101).

The whole plant of *A. augusta* (Ulatkambal) was collected in the month of January from the local forest of Jabalpur, identified and authenticated by Dr. A.B. Tiwari, Sr. Scientist, Jawaharlal Nehru Krishi Vishwavidyalaya Jabalpur M.P., India.

2.4. Preparation of methanol extract

The plant was dried under shade. After drying, the plant material was powdered and extracted by soxhlet apparatus using methanol as solvent. The extract was filtered using Whatmann filter paper No.1 and concentrated at 40 °C under reduced pressure.

2.5. Phytochemical analysis

The extract obtained after solvent evaporation was subjected to standard tests for detection of phytoconstituents [7, 8].

Total soluble phenolic compounds in the extract were determined with Folin–Ciocalteu reagent according to the method of Slinkard and Singleton [9] using pyrocatechol as a standard phenolic compound. The total concentration of phenolic compounds in the extract determined as microgram of pyrocatechol equivalent by using an equation that was obtained from standard pyrocatechol graph:

$$\text{Absorbance} = 0.0056 \times \text{total phenols [pyrocatechol equivalent (}\mu\text{g)]} - 0.0057.$$

Total flavonoid content was determined using the method given elsewhere [10, 11]. The concentrations of flavonoid compounds were calculated according to the following equation that was obtained from the standard quercetin graph:

$$\text{Absorbance} = 0.0342 \text{ quercetin (}\mu\text{g)} - 0.0003; R^2 = 0.9994.$$

The total alkaloidal content in extract was determined by a spectrophotometric method, in which the alkaloids were precipitated by Dragendorff's reagent, following a described procedure [12, 13] and results were expressed as mg/kg dry basis.

2.6. Anti-lipase activity of methanolic extract of *A. augusta*

2.6.1. Extraction of lipase from chicken (*Gallus domesticus*) pancreas

Pancreas of freshly slaughtered chickens was collected, washed and placed in ice cold sucrose solution (0.01M). The pancreas was homogenized in 0.01M sucrose, centrifuged; supernatant was separated and subjected to ammonium sulphate precipitation (50% saturation). The pellet obtained after centrifugation was dissolved in sucrose solution and again saturated to 50% ammonium sulphate saturation and

centrifuged. The pellet obtained was dissolved in phosphate buffer and used as enzyme source [14].

2.6.2. Determination of chicken pancreatic lipase activity

The activity of lipase was determined by incubating an emulsion containing 8 mL of olive oil, 0.4 mL of phosphate buffer and 1ml of chicken pancreatic lipase for an hour in rotary shaker, followed by stopping the reaction by addition of 1.5 mL of a mixture solution containing acetone and 95% ethanol (1:1). The liberated fatty acids were determined by titrating the solution against 0.02M NaOH (standardized by 0.01M oxalic acid) using phenolphthalein as an indicator [15, 16].

2.6.3. Lipase Inhibitory activity of methanol extract of *Abroma augusta*

Lipase inhibitory activity of different concentrations of methanol extract was tested by mixing 100 μ L of each concentration of methanol extract, 8 mL of oil emulsion and 1 mL of chicken pancreatic lipase followed by incubation of 60 minutes. The reaction was stopped by adding 1.5 mL of a mixture solution containing acetone and 95% ethanol (1:1). The liberated fatty acids were determined by titrating the solution against 0.02M NaOH (standardized by 0.01M oxalic acid) using phenolphthalein as an indicator [17].

Percentage inhibition of lipase activity was calculated using the formula:

$$\text{Lipase inhibition} = A - B/A \times 100,$$

Where A is lipase activity, B is activity of lipase when incubated with the extract.

3. Results

3.1. Phytochemical screening

Preliminary phytochemical analysis of methanolic extract of *A. augusta* was determined by chemical tests. Phytoconstituents namely alkaloids, glycosides, flavonoids, tannins, amino acids, protein and carbohydrates were detected in the extract.

The preliminary phytochemical analysis of *A. augusta* extract showed presence of alkaloids, flavonoids, tannins, phenols. The total amount of phenolic content present in extract was found to be (689.25 \pm 3.65) mg PE (pyrocatechol equivalent)/100 g. Using the standard curve of quercetin ($R^2 = 0.9993$), the total flavonoid content of extract was found to be (175.34 \pm 2.35) mg QE (quercetin equivalent) /100 g. Total alkaloidal content in the extract was found to be 18.47 mg/kg dry basis.

3.2. Lipase inhibitory activity of methanolic extract of *A. augusta*

Inhibitory activity on chicken pancreatic lipase on methanolic extract of *A. augusta* was determined using olive oil as the substrate. The activity of pancreatic lipase was checked. It was found that the activity of lipase was affected when incubated with the methanol extract. A dose dependent inhibition of lipase activity was observed. At concentration of 100 mg/kg percent inhibition of lipase was found to be 88.6% (Table 1).

Table 1
Antilipase activity of *A. augusta* extract against chicken pancreatic lipase.

Concentration of extract (μ g/mL)	Antilipase activity of extract	% inhibition
25	1.24 \pm 0.03	66.5
50	0.89 \pm 0.06	75.9
75	0.65 \pm 0.04	82.4
100	0.42 \pm 0.02	88.6

All data were expressed as mean \pm SD, n= 3.

4. Discussion

Lipid metabolism plays an important role in energy dissipation and hence responsible to maintain a steady state in body^[18]. Disruption in the metabolism of lipids may lead to life threatening situations like obesity, hypercholesterolemia, atherosclerosis, heart blockade etc ^[19]. Thus prevention of lipid absorption could be an alternate strategy to treat obesity^[20].

From the eternal era, man has been using plants for medicinal purpose and in current times, phytochemicals from plants serve as lead for many pharmaceuticals^[21]. *A. augusta* is one of the evergreen quick growing shrub widely is almost all parts of central India. In this work, phytochemical screening and phytoanalytical studies confirmed the presence of flavonoids and alkaloids in the extracts.

Plant flavonoids are unique phytochemicals with wide range of therapeutic activities. Flavonoid intake could be inversely correlated with death due to coronary heart disease^[22]. Plant flavonoid quercetin is reported to show anti-lipase activity^[23]. In an independent experiment, quercetin has showed to increase energy expenditure ^[24], whereas in another study, quercetin along with resveratrol inhibited production of fat cells^[25]. Plant alkaloids are also known to be reputed lipase inhibitors^[26]. Alkaloids like berberine and sanguinarine^[27] inhibited lipase in experimental studies. In the current studies, flavonoids, polyphenols and alkaloids were quantified, which might be responsible for inhibition of activity of lipase.

In this study we determined that *A. augusta* extract inhibited activity of pancreatic lipase which indicates its protective role of against prevention of obesity and unlock an access for isolation and characterization of active compounds responsible for it.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

We gratefully acknowledge Dr. A.B. Tiwari, Sr. Scientist, Jawaharlal Nehru Krishi Vishwavidyalaya Jabalpur, M.P., India for authenticating samples of *Abroma augusta*. Authors also kindly acknowledge the support from Rewa Shiksha Samiti (Support No.RSS/SRITP/MPY/101) during studies.

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