

# LACK OF IN VITRO ANTI- $\alpha$ -GLUCOSIDASE, ANTI-LIPASE AND IMMUNOMODULATORY ACTIVITIES OF ETHANOL EXTRACT FROM FINGER-ROOT (*BOESENBERGIA ROTUNDA L.*) RHIZOME

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## Abstract

Recently, metabolic syndrome is related to development of cardiovascular diseases and diabetes mellitus. In addition, immune cells are plays role in physiological dysfunction associated with obesity and metabolic syndrome. Fingerroot (*Boesenbergia rotunda L.*) rhizome is commonly applied as food flavoring in Asian cuisines and also applied in traditional medicine to relieve commonly on gastrointestinal symptoms. Several studies had reported that flavonoids and terpenoids contained in fingerroot are exhibits wide range of biological activities and use for obesity treatment. Previously phytochemical screening of methanolic extract from *B. rotunda* rhizome had demonstrated potent  $\alpha$ -glucosidase and pancreatic lipase inhibitors. Due to Thai ethnomedicinal utilizations, traditional medicine is usually use herbs extracted with water or ethanol. Hence, this study was attempted to evaluate  $\alpha$ -glucosidase and pancreatic lipase inhibitory activities; and immunomodulatory activity of ethanol extract from fingerroot rhizome (FRE). Total phenolic content (TPC) and total flavonoid content (TFC) were  $91.51 \pm 0.76$  mg of GAE/g and  $21.05 \pm 1.53$  mg of QE/g, respectively. FRE was lack of  $\alpha$ -glucosidase and pancreatic lipase inhibitory activities when compared with acarbose (IC<sub>50</sub> =  $1.11 \pm 0.33$  mg/ml) and orlistat (IC<sub>50</sub> =  $0.29 \pm 0.08$  mg/ml). There was unable to stimulate phagocytosis of neutrophils and interpreted as lack of immunomodulatory activity when compared with lipopolysaccharide (LPS).

**Keywords:** *Boesenbergia rotunda L.*, fingerroot,  $\alpha$ -glucosidase inhibitor, anti-lipase, immunomodulation.

## INTRODUCTION

Recently, metabolic syndrome is become worldwide health aspect, which is relate to development of cardiovascular diseases and diabetes mellitus. Metabolic syndrome is characterized by occurrence of three or more metabolic abnormalities including hypertension, obesity, hypertriglyceridemia, low level of high-density lipoprotein and hyperglycemia [1, 2]. Metabolic syndrome persons are commonly occurred both of dyslipidemia and hyperglycemia [3].

Diabetes mellitus is disorder of carbohydrate metabolism defined as high level of blood glucose, hyperglycemia during fasting period. Diabetic complications are consequence of long-term hyperglycemia, which are related to mortality and morbidity occurrences [4].

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Excessive glucose can transform to glycation precursors, which are react with protein and generate advanced glycation-end products (AGEs). AGEs are lead several complications including nephropathy, neuropathy, and retinopathy [5]. Cardiovascular diseases are a major cause of death in developed countries, and hypertension is a major risk factor for cardiovascular diseases such as hyperpiesia, arrhythmia, heart failure, and atherosclerosis [6]. In addition, immune cells are plays role in physiological dysfunction associated with obesity and metabolic syndrome; and there are also related to pathogenesis and development of chronic diseases [7]. Dietary improvement and supplementation are one of most suggestion on metabolic syndrome controlling and prevent the risk of diabetes mellitus and cardiovascular diseases. Plant-based dietary supplements are suggested on prevention of metabolic syndrome via multiple mechanisms and contain bioactive compounds categorized as flavonoids, steroids, and triterpenoids [8].  $\alpha$ -glucosidase is an enzyme responsible for the catalyzing of polysaccharides and disaccharides into glucose for intestinal absorption, whereas pancreatic lipase is play role as key enzyme on lipid absorption and metabolism by hydrolyzing triglycerides into glycerol and free fatty acids. Thus, active phytochemicals, which are inhibit these two enzymes, are provided health benefits by controlling of serum sugar and fat [9].

Fingerroot (*Boesenbergia rotunda L.*) is widely used as herbal spice in Asian food i.e., Thailand, Malaysia, India, China, Indonesia and Myanmar [10, 11]. Root or rhizome is commonly applied as food flavoring in Asian cuisines including curry, sauce, and soup; and its aroma flavor is increase food appetite. Fingerroot is also applied in traditional medicine to relieve symptoms, such as febrifuge, gastrointestinal disorders, flatulence, carminative, stomachache, dyspepsia, and peptic ulcer. Fresh rhizomes are applied to heal inflammatory-related conditions i.e., dental caries, gum pyorrhea, dry cough, fever, dermatitis, wounds, and diarrhea. Aphrodisiac property of this herb is recognized by Thai ethnomedicine. Moreover, intake of this leaves can relieve food allergy and poisoning. Several flavonoids are isolated and identified from the *B. rotunda* rhizome such as flavones, flavanones, chalcones, and pimarane diterpenes [12, 13]. Several studies had reported that flavonoids and terpenoids contained in fingerroot are exhibits wide range of biological activities including, antifungal, antibacterial, antiviral, anti-inflammatory, anti-cancer, anti-osteoporosis, antioxidant, and anti-obesity activities [12, 14-17]. As fingerroot is recently use for obesity treatment [15] and preliminary phytochemical screening of methanolic extract from *B. rotunda* rhizome (100  $\mu$ g/mL) had demonstrated potent  $\alpha$ -glucosidase and pancreatic lipase inhibitors [9]. Due to metabolic syndrome and obesity controls, immunomodulatory activity of fingerroot extract is sparely report. In Thai ethnomedicinal utilizations, traditional medicine is usually use herbs extracted with water or ethanol. Hence, this study was

attempted to evaluate  $\alpha$ -glucosidase and pancreatic lipase inhibitory activities; and to evaluate immunomodulatory activity determined by In vitro stimulation of phagocytosis, which was exhibited from ethanol extract of fingerroot rhizome. This finding can clarify significance of traditional utilization from fingerroot, which is prevent risk of chronic diseases related metabolic syndrome as food supplement.

## MATERIALS AND METHODS

### Plant Collection and Extraction

*B. rotunda* is a perennial with a short stem that is replaced by pseudostems, formed by leaf sheaths growing up to 50 cm tall. There are 3-4 leaves which are 7-11 cm in width and 25-50 cm in length, which are not divided, oval or elongate shape. Rhizomes are finger appearance and light brown color. Inside of rhizomes are ovoid-globose, yellow color and strongly aromatic [18]. Rhizomes of fingerroot were purchased from local markets in Bangkok, Thailand. Botanical identification and sample voucher were done by senior physiochemist. Air-dried fingerroot rhizomes ( $\square$  45°C) were ground and extracted with 95% ethanol by maceration during 72 h. Ethanol extract (yield  $\square$  10-12%) was filtered and evaporated by vacuum-rotary evaporator. All laboratories were done as in vitro, which was exempted on approval of Ethical Committees, Suan Sunandha Rajabhat University, Bangkok, Thailand (COE. 2-392/2022).

### Chemicals and Instruments

In this study, chemical uses were ethanol (RCI Labscan, Thailand), dimethyl sulfoxide, DMSO (RCI Labscan, Thailand), Folin-Ciocalteu  $\square$ s phenol reagent (Loba Chemie, India), sodium bicarbonate (RCI Labscan, Thailand), sodium nitrite (RCI Labscan, Thailand), aluminium chloride (Loba Chemie, India), sodium hydroxide (RCI Labscan, Thailand), p-nitrophenyl- $\square$ -D-glucopyranose (Sigma, Switzerland), p-nitrophenyl-butyrate (Sigma, USA), nitroblue tetrazolium (Biobasic, USA) and basic fuchsin (Biobasic, USA). Standards chemicals were gallic acid (Sigma-Aldrich, Germany), quercetin (HWI Analytik GmbH, Germany), acarbose (Sigma-Aldrich, Germany), orlistat (Sigma-Aldrich, Germany) and lipopolysaccharide, LPS (Sigma, USA). Standard commercial enzymes were  $\alpha$ -glucosidase (Sigma, Germany) and pancreatic lipase (Sigma, USA). Instrument uses were 96-well plate (SPL Life Sciences, Korea), microplate reader (BIO-RAD, USA) and microscope (Olympus, Japan).

### Total Phenolic and Flavonoid Contents

Total phenolic content (TPC) and total flavonoid content (TFC) contained in ethanol extract of fingerroot rhizome (FRE) were evaluated according by colorimetric methods. TPC was monitored chemical reaction of phenolic compounds and Folin-Ciocalteu  $\square$ s reagent and absorbance of mixture measured at 760 nm. Gallic acid was used for

standard curve and result was reported as milligram of gallic acid equivalent (GAE) per gram. TFC was monitored chemical reaction of flavonoids and aluminium chloride reagent and absorbance of mixture measured at 420 nm. Quercetin was used for standard curve and result was reported as milligram of quercetin equivalent (QE) per gram [19, 20].

#### Evaluation of $\alpha$ -Glucosidase Inhibitory Activity

FRE concentration was adjusted with 10% (v/v) DMSO, which was 0.001, 0.01, 0.1, 1 and 10 mg/ml. Acarbose was adjusted with phosphate buffer, which was 0.0005, 0.005, 0.5 and 5 mg/ml. Enzymatic inhibitory activity was evaluated by monitored absorbance ( $\lambda_{max}$  = 415 nm) of p-nitrophenol, product from p-nitrophenyl- $\alpha$ -D-glucopyranose catalyzed by  $\alpha$ -glucosidase. Enzyme inhibition activity from FRE were calculated from triplicate measurement and reported as 50% inhibitory concentration (IC50) of FRE against  $\alpha$ -glucosidase. Positive control was acarbose [21].

#### Evaluation of Lipase Inhibitory Activity

FRE concentration was adjusted with 10% (v/v) DMSO, which was 0.001, 0.01, 0.1, 1 and 10 mg/ml. Orlistat was also adjusted with 10% (v/v) DMSO, which was 0.0005, 0.005, 0.5 and 5 mg/ml. Enzymatic inhibitory activity was evaluated by monitored absorbance ( $\lambda_{max}$  = 415 nm) of p-nitrophenol, product from p-nitrophenyl butyrate catalyzed by pancreatic lipase. Enzyme inhibition activity from FRE were calculated from triplicate measurement and reported as 50% inhibitory concentration (IC50) of FRE against pancreatic lipase. Positive control was orlistat [22].

#### Immunomodulatory Assay

This activity was determined by phagocytosis stimulation of

neutrophils. Briefly, FRE was adjusted by 10% (v/v) DMSO in phosphate buffer, which was 10 mg/ml. LPS was adjusted by serum, which was 20  $\mu$ g/ml. Venous blood was collected from (laboratory) mouse, transferred to test tube (with anti-coagulant) and centrifuged to separate neutrophils. Each cell containing tube was added FRE sample or control and then followed by nitroblue tetrazolium. Each sample mixture was prepared on glass slide, stained with basic fuchsin and washed for removing excessive color. Stained slide was dried and neutrophils magnified under microscope. Phagocytic neutrophils were counted and calculated as percent of phagocytosis. Standard LPS from Gram-negative bacteria was positive control as phagocytic stimulant [23].

#### STATISTICAL ANALYSIS

TPC, TFC, enzymatic inhibitions and immunomodulatory activity of FRE were demonstrated by descriptive analysis and compared with controls.

### RESULTS AND DISCUSSION

FRE was characterized as orange-brown color and semi-solid appearance with strongly aromatic favor. TPC and TFC contained in FRE were 91.51 $\pm$ 0.76 mg of GAE/g and 21.05 $\pm$ 1.53 mg of QE/g, respectively. FRE was lack of  $\alpha$ -glucosidase and pancreatic lipase inhibitory activities when compared with acarbose (IC50 = 1.11 $\pm$ 0.33 mg/ml) and orlistat (IC50 = 0.29 $\pm$ 0.08 mg/ml). There was unable to stimulate phagocytosis of neutrophils and interpreted as lack of immunomodulatory activity when compared with LPS (Table 1).

Table 1: Anti- $\alpha$ -glucosidase and anti-pancreatic lipase and immunomodulatory activity of fingerroot rhizome extract (FRE)

Sample / Assay	anti- $\alpha$ -glucosidase (mg/ml) <sup>a</sup>	anti-pancreatic lipase (mg/ml) <sup>a</sup>	immunomodulatory activity (%) <sup>b</sup>
FRE	NA	NA	NA
Acarbose	1.11 $\pm$ 0.33	-	-
Orlistat	-	0.29 $\pm$ 0.08	-
LPS	-	-	27.33 $\pm$ 3.06

a Enzymatic activity was represented as 50% of inhibitory concentration, IC50 (mg/ml);

b Immunomodulatory activity was represented as percentage of phagocytosis stimulation in neutrophils

NA = no activity; LPS = lipopolysaccharide

Recently,  $\alpha$ -glucosidase inhibitor drugs are effectively use on blood glucose and body weight control in type 2 diabetic patients with obesity [24]. A large number of dietary flavonoids and polyphenols originated from plants are  $\alpha$ -glucosidase inhibitors and they can synergize this activity with acarbose [25]. Previous studies had reported that *B. rotunda* root is contained flavonoid derivatives including prenylated and flavanone-coupled chalcones, which are potent  $\alpha$ -glucosidase inhibitors and can be develop to food supplements for diabetes prevention. These chalcones from *B. rotunda* root are weak lipase inhibitors, therefore, there

are exhibit anti-obesity activity through another pathway. Panduratin A isolated and identified from Indonesian fingerroot had stimulated AMP-activated protein kinase (AMPK) activity, and lead to increase of fatty acid oxidation and inhibition of lipid synthesis in animal model [9, 15, 26]. Pinostrobin, flavanone isolated from Thai fingerroot, had suppressed adipogenesis through inhibited adipocyte differentiation with non-cytotoxicity. Lipid metabolism-mediating proteins including C/EBP $\alpha$ , PPAR $\gamma$ , and SREBP-1c, as well as, cellular triglyceride in mouse pre-adipocytes treated pinostrobin had lower level when compared with untreated cells. There is also modulated the signals of

MAPK (p38 and JNK) and Akt (Akt/GSK3 $\beta$ , Akt/AMPK $\alpha$ -ACC) [27]. Thus, fingerroot is the main active ingredient in several dietary supplements for weight loss [28] due to decreasing the accumulation of visceral

fat and reducing body weight gain [9, 15, 26, 27, 29]. Several different plant species have represented anti-inflammatory activity [30]. We found that fingerroot rhizome was lack of immunomodulatory activity by unable phagocytic stimulation. This finding may be affected from its anti-inflammatory and anti-allergic activities [16, 31, 32], corresponded to Thai traditional medicine, it can use on relieve of skin disorders including dermatitis, rash and swelling. In this study, ethanol extract of fingerroot rhizome was lack of  $\alpha$ -glucosidase and pancreatic lipase inhibitory activities, while methanol extract had strongly inhibited both of enzymes as reported in previous studies. There was implied that condition of herbal extraction is very important in medicinal plant application. Use of ethanol extract was safer and more preferable rather than methanol extraction, therefore, loss of its biological activities is also need to concern on herbal preparation.

## CONCLUSION

Ethanol extract of fingerroot rhizome (FRE) was lack of  $\alpha$ -glucosidase and pancreatic lipase inhibitory activities. There was also lack of immunomodulatory activity represented by unable to stimulate phagocytosis.

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## CONFLICT OF INTEREST

The authors are declar no conflict of interest.

## REFERENCES

1. Carpentier YA, Portois L, Malaisse WJ. n-3 Fatty acids and the metabolic syndrome. *Am J Clin Nutr* 2006; 83: S1499-S1504.
2. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome: An American Heart Association/National Heart, Lung, and Blood Institute scientific statement. *Circulation* 2005; 112: 2735-2752.
3. Bardini G, Rotella CM, Giannini S. Dyslipidemia and diabetes: reciprocal impact of impaired lipid metabolism and Beta-cell dysfunction on micro- and macrovascular complications. *Rev Diabetes Stud* 2012; 9: 82-93.
4. Goh SGK, Rusli BN, Khalid BAK. Evolution of diabetes management in the 21st century: The contribution of quality-of-life measurement in Asians. *Asia Pac J Clin Nutr* 2015; 24:190-198.
5. Poulsen MW, Hedegaard RV, Andersen JM, de Courten B, Bügel S, Nielsen J, et al. Advanced glycation end-products in food and their effects on health. *Food Chem Toxicol* 2013; 60:10-37.
6. Li C, Li J, Weng X, Lan X, Chi X. Farnesoid X receptor agonist CDCA reduces blood pressure and regulates vascular tone in spontaneously hypertensive rats. *J Am Soc Hypertens* 2015; 9: 507-516.
7. Andersen CJ, Murphy KE, Fernandez ML. Impact of obesity and metabolic syndrome on immunity. *Adv Nutr* 2016; 7(1):66-75.
8. Graf BL, Raskin I, Cefalu WT, Ribnicky DM. Plant-derived therapeutics for the treatment of metabolic syndrome. *Curr Opin Investig Drugs* 2010; 11:1107-1115.
9. Chatsumpun N, Sritularak B, Likhitwitayawuid K. New biflavonoids with  $\alpha$ -glucosidase and pancreatic lipase inhibitory activities from *Boesenbergia rotunda*. *Molecules* 2017; 22(11):1862.
10. Chuakul W, Boonpleng A. Ethnomedical uses of Thai Zingiberaceae plant. *Thai Herbal J* 2003; 10:33-39.
11. Chahyadi A, Hartati R, Wirasutisna KR. *Boesenbergia pandurata* Roxb., an Indonesian medicinal plant: Phytochemistry, biological activity, plant biotechnology. *Procedia Chem* 2014; 13:13-37.
12. Eng-ChongT, Yean-Kee L, Chin-Fei C, Choon-Han H, Sher-Ming W, Li-Ping CT, et al. *Boesenbergia rotunda*: From ethnomedicine to drug discovery. *J Evid-Based Complement Altern Med* 2012; 2012: 473637.
13. Ongwisepaiboon, O.; Jiraungkoorskul, W. Fingerroot, *boesenbergia rotunda* and its aphrodisiac activity. *Pharmacogn Rev* 2017; 11: 27-30.
14. Kanjanasirirat P, Suksatu A, Manopwisedjaroen S, Munyoo B, Tuchinda P, Jearawuttanakul K, et al. High-content screening of Thai medicinal plants reveals *Boesenbergia rotunda* extract and its component Panduratin A as anti-SARS-CoV-2 agents. *Sci Rep* 2020; 10:1-12.
15. Kim D-Y, Kim M-S, Sa B-K, Kim M-B, Hwang J-K. *Boesenbergia pandurata* attenuates diet-induced obesity by activating AMP-activated protein kinase and regulating lipid metabolism. *Int J Mol Sci* 2012; 13:994-1005.
16. Isa N, Abdelwahab S, Mohan S, Abdul A, Sukari M, Taha M, et al. In vitro anti inflammatory, cytotoxic and antioxidant activities of *boesenbergia* A, a chalcone isolated from *Boesenbergia rotunda* (L.) (fingerroot). *Braz J Med Biol Res* 2012; 45:524-530.
17. Saah S, Siriwan D, Trisonthi P. Biological activities of *Boesenbergia rotunda* parts and extracting solvents in promoting osteogenic differentiation of pre-osteoblasts. *Food Biosci* 2021; 41:101011.
18. Firdouse S, Alam P. Phytochemical investigation of extract of *Amorphophallus campanulatus* tubers. *Int J Phytomed* 2011;3(1):32-35.
19. Singleton VL, Orthoffer R, Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol* 1999; 299: 152-178.
20. Biju J, Sulaiman CT, Satheesh G, Reddy VRK. Total phenolics and flavonoids in selected medicinal plants from Kerala. *Int J Pharm Pharm Sci* 2014, 6, 406-408.
21. Alam MA, Zaidul IS, Ghafoor K, Sahena F, Hakim MA, Rafii MY, et al. In vitro antioxidant and,  $\alpha$ -glucosidase inhibitory activities and comprehensive metabolite profiling of methanol extract and its fractions from *Clinacanthus nutans*. *BMC Complement Altern Med* 2017;17(1):181.
22. Bustanji Y, Al-Masri IM, Mohammad M, Hudaib M, Tawaha K, Tarazi H, Alkhatib HS. Pancreatic lipase inhibition activity of trilactone terpenes of *Ginkgo biloba*. *J Enzyme Inhib Med Chem* 2011; 26(4):453-459.
23. Chandraraj S, Prakash B, Navanath K. Immunomodulatory activities of ethyl acetate extracts of two marine sponges *Gelliodes fibrosa* and *Tedania anhelans* and brown algae *Sargassum ilicifolium* with reference to phagocytosis. *RJPBCS* 2010;1(2):302-307.
24. Sugihara H, Nagao M, Harada T, Nakajima Y, Tanimura-Inagaki K, Okajima F, et al. Comparison of three  $\alpha$ -glucosidase inhibitors for glycemic control and bodyweight reduction in Japanese patients with obese type 2 diabetes. *J Diabetes Investig* 2014; 5:206-212.
25. Zhang B-W, Li X, Sun W-l, Xing Y, Xiu Z-L, Zhuang C-L, et al.

- Dietary flavonoids and acarbose synergistically inhibit  $\alpha$ -glucosidase and lower postprandial blood glucose. *J Agric Food Chem* 2017; 65:8319-8330.
26. Kim D, Lee MS, Jo K, Lee KE, Hwang JK. Therapeutic potential of panduratin A, LKB1-dependent AMP-activated protein kinase stimulator, with activation of PPAR $\alpha$ / $\delta$  for the treatment of obesity. *Diabetes Obes Metab* 2011; 13:584-593.
  27. San HT, Khine HEE, Sritularak B, Prompetchara E, Chaatham C, Che CT, et al. Pinostrobin: an adipogenic suppressor from fingerroot (*Boesenbergia rotunda*) and its possible mechanisms. *Foods* 2022;11(19):3024.
  28. Hwang J-K, Kim DU. Use of panduratin derivative or *Boesenbergia pandurata* extract. US Patent 8653143B2, 2014.
  29. Myoung K-S, Ahn Y-T, Lee M-H, Park D-Y, Ahn Y-M, Huh C-S. Fingerroot (*Boesenbergia pandurata*) extract inhibits the accumulation of visceral fat in C57BL/6J mice. *J Korean Soc Food Sci Nutr* 2013; 42:26-32.
  30. Maione F, Russo R, Khan H, Mascolo N. Medicinal plants with anti-inflammatory activities. *Nat Prod Res* 2016; 30(12):1343-1352.
  31. Tuchinda P, Reutrakul V, Claeson P, Pongprayoon U, Sematong T, Santisuk T. Anti-inflammatory cyclo-hexenyl chalcone derivatives in *Boesenbergia pandurata*. *Phytochemistry* 2002; 59:169-173.
  32. Madaka F, Tewtrakul S. Anti allergic activity of some selected plants in the genus *Boesenbergia* and *Kaempferia*. *Songklanakarin J Sci Technol* 2011; 33:301-304.