J Appl Biol Chem (2015) 58(1), 5−8
http://dx.doi.org/10.3839/jabc.2015.002

Online ISSN 2234-7941
Print ISSN 1976-0442

Original Article: Bioactive Materials

Inhibitory Effects of Quinoline Isolated from *Ruta chalepensis* and Its Structurally Related Derivatives against α-Amylase or α-Glucosidase

Jun-Hwan Park · Hoi-Seon Lee*

Received: 1 September 2014 / Accepted: 2 September 2014 / Published Online: 31 March 2015

Abstract This study was to isolate an active component of the chloroform fraction from the methanol extract of *Ruta chalepensis* leaves and to measure inhibitory effects against α-glucosidase or α-amylase. The inhibitory compound of *R. chalepensis* leaves was isolated using chromatographic methods and identified as quinoline. Quinoline and its structurally related derivatives were tested for their inhibitory activities by evaluating the IC_{50} values against α-amylase or α-glucosidase and were compared with that of acarbose. Based on the IC_{50} values, quinazoline exhibited the greatest inhibitory activity (20.5 μg/mL), followed by acarbose (66.5 μg/mL), and quinoline (80.3 μg/mL) against α-glucosidase. In case of α-amylase, quinazoline had potent inhibitory activity, followed by quinoline (179.5 μg/mL) and acarbose (180.6 μg/mL). These results indicate that *R. chalepensis* extract, quinoline, and quinazoline could be useful for inhibiting α-glucosidase or α-amylase.

Keywords α-amylase · α-glucosidase · inhibitory activity · quinoline · *Ruta chalepensis*

Introduction

Diabetes mellitus is the most serious global health problem and results in considerable morbidity and mortality (Nilubon et al., 2006). Complications of diabetes such as terminal nephritis and cardiovascular disorders are the principal cause of irreversible blindness (Perez et al., 1998; Jeong et al., 2012). Diabetes falls into two etiopathogenetic categories, types 1 and 2 (American Diabetes Association., 2005; Nilubon et al., 2006). Diabetes type 1 is resulted in absolute deficiency of insulin secretion (Nilubon et al., 2006; Frode and Medeiros, 2008). Diabetes type 2 is caused by insufficient compensatory insulin secretion and a combination of resistance to insulin action (Nilubon et al., 2006; Frode and Medeiros, 2008). Attention to herbal remedies has increased because of the side effects associated with treatment of oral hypoglycemic agents and insulin (Holman and Turner, 1991; Lee, 2005; Kim et al., 2006; Jeong et al., 2012; Lee et al., 2014).

Ruta chalepensis L. (Rutaceae) is a perennial herb that is extensively used in folk medicine. *R. chalepensis* is well-known as an alternative medical therapy (antispasmodic, antirheumatic, aphrodisiac) and a treatment for snakebites, headache, and wounds (Ghazanfar, 1994). Furthermore, this plant is a rich source of several acridones and coumarins, as well as quinoline alkaloids (Ulubelen and Guner, 1988; Ulubelen and Terem, 1988; Lee and Ahn, 1998; Lee, 2002). *R. chalepensis* exhibits insecticidal activity against pests, with no noxious effects on parasitoids (Al-mazraawi and Ateyyat, 2009) and shows antibacterial, antifungal, anthelmintic, and anthelmintic effects (Di Stasi et al., 2002; Alzoreky and Nakahara, 2003; Iauk et al., 2004; Yarnell and Abascal, 2004; Cho et al., 2005; Rigat et al., 2007; Barrera-Necha et al., 2009). However, no report on the inhibitory activity of active compound isolated from *R. chalepensis* leaves and structurally related derivatives against α-amylase or α-glucosidase is available. Therefore, we isolated an active constituent from *R. chalepensis* leaves and assessed the inhibitory effects of quinoline derivatives against α-glucosidase or α-amylase.

Materials and Methods

Isolation and identification. *R. chalepensis* leaves were collected from a market in Korea. *R. chalepensis* leaves (3.0 kg) were

J.-H. Park · H.-S. Lee
Department of Bioenvironmental Chemistry and Institute of Agricultural Science & Technology, College of Agriculture & Life Science, Chonbuk National University, Jeonju 561-756, Republic of Korea

*Corresponding author (H.-S. Lee: hoiseon@jbnu.ac.kr)

ground and extracted with methanol (11 L) at 25°C for 1.5 days. The filtrate was poured into a EYELA Autojack NAJ-100 evaporator (Japan) at 45°C, and the methanol extract (20 g) was continuously partitioned into hexane fraction (2.1 g), chloroform fraction (3.7 g), ethyl acetate fraction (2.1 g), butanol fraction (2.6 g), and water fraction (9.1 g) for subsequent bioassay. Five organic fractions were dried by rotary evaporator at 40°C, and the water fraction was freeze-dried.

Chloroform (43.8 g) fraction partitioned from the methanol extract was chromatographed on a silica gel column (70–220 mesh, Merck, USA, 540 mm i.d.×680 mm) and eluted with a stepwise gradient of chloroform/methanol (0, 10, 20, 30, 40, and 100% methanol, v/v) and petroleum ether/chloroform (10:1, v/v). The column fractions were tested by thin layer chromatography (chloroform/methanol, 10:1, v/v), and active fractions with similar patterns were collected. The active fractions were chromatographed on a silica gel column and eluted with petroleum ether/chloroform/methanol (20:15:1, v/v). The active fraction (8.4 g) was isolated by preparative high-performance liquid chromatography (HPLC) (Japan Analytical Industry Co., Ltd., Japan). The first column was a Jai gel GS Series Column (GS310 30 + GS310 50 cm) using hexane:chloroform:isopropanol (40:60:2, v/v) at a flow rate of 4.5 mL/min and detection at 291 nm. This step afforded four fractions. The active fraction (3. g) was further chromatographed on a Jaigel W Series column (W-252 50 + W-253 50 cm) using hexane:chloroform:isopropanol (40:60:2, v/v) at a flow rate of 5.1 mL/min. The active component (1.4 g) was isolated and subjected to structural determination via spectroscopic analyses. The ^{13}C-NMR and ^{1}H-NMR spectra date were studied using a Bruker AM-500 spectrometer (^{13}C-400 MHz; ^{1}H-100 MHz). Ultraviolet spectra and mass spectra were studied using a Waters 490 spectrometer and JEOL JMS-AX 302 spectrometer, respectively.

Chemicals and bioassay. Acarbose, quinazoline, and quinoxaline were supplied from Sigma-Aldrich (USA). The inhibitory effects of *R. chalepensis* extract, quinoline, and its structurally related analogs were evaluated against α-glucosidase and α-amylase. Inhibitory activity was assayed according to the procedure studied by Lee et al. (2014) and Shinde et al. (2008) with a slight modification against α-glucosidase. *p*-Nitrophenol was measured using α-glucosidase after reaction with *p*-nitrophenyl-α-D-glucopyranoside. 0.6 U Enzyme solution was made by dissolving α-glucosidase in 0.1 M phosphate buffer (pH 7.0) mixing up bovine serum albumin (2 g/L, BSA) and sodium azide (0.2 g/L). 50 μL Enzyme solution and 10 μL sample dissolved in DMSO were blended and placed in a well plate. After 15 min, 5 mM *p*-nitrophenyl-α-D-glucopyranoside (50 μL) in 0.1 M phosphate buffer was added, and the mixture was incubated for 9 min at 38°C. 0.1 M Na_2CO_3 was added to stop the reaction. The absorbance was tested at 405 nm using a Model ASYS UVM 340 microplate reader (Biochrom Ltd., England). Biological experiments were replicated three times. Inhibition percentage (%) was evaluated using the equation: Inhibition (%) = [1 – (sample/control)] × 100. The IC_{50} value was calculated by logarithmic regression analysis.

Inhibitory activity was assayed in accordance with the procedure studied by Jeong et al. (2012) and Wang et al. (2010) with some modification against α-amylase. The enzyme solution (6.30 U/mL) was made by dissolving α-amylase (Sigma Co., USA) in 0.5 M Tris buffer (pH 6.9). Starch azure (8 mg) was suspended in 0.5 M Tris buffer mixing up 0.01 M $CaCl_2$ and soaked in boiling water for 5 min followed by preincubation at 38°C for 9 min. 100 μL Enzyme solution and 100 μL sample into 50% DMSO were blended in a well plate. 50% Acetic acid (50 μL) was added to stop the reaction after 10 min. The absorbance was tested at 595 nm with a Model ASYS UVM 340 microplate reader. Biological experiments were replicated three times. Inhibition percentage (%) was evaluated using the equation: Inhibition (%) = [1 – (sample/control)] × 100.

Results and Discussion

Five fractions partitioned from methanol extracts of *R. chalepensis* leaves were assessed for inhibitory activity against α-glucosidase and α-amylase (Table 1). At 1,500 μg/mL, the chloroform fraction showed 100% inhibition against α-glucosidase and α-amylase, whereas other fractions exhibited no inhibition. Active compound was isolated by silica gel chromatography and preparative HPLC. The active compound was identified by spectroscopic methods, EI-Mass spectroscopy, ^{13}C-NMR and ^{1}H-NMR, and by comparison with an authentic reference component. The active component was characterized as quinoline (Fig. 1) based on the following evidence: quinoline (C_9H_7N, MW, 129.2); EI-MS (70 eV) m/z (% relative intensity): M^+ 129 (100), 128 (15), 102 (25), 76 (10), 51 (12); ^{1}H-NMR (CD_3OD, 400 MHz); d 8.82-8.83 (1H, *m*, *J* = 6.12 Hz, H-2), 8.34-8.36 (1H, *m*, *J* = 8.56 Hz, H-8), 8.00-8.03 (1H, *d*, 1H, *J* = 8.52 Hz, H-4), 7.92-7.94 (1H, *d*, *J* =8 .32 Hz, H-5), 7.74-7.78 (1H, *m*, *J* = 17.08 Hz, H-7), 7.58-7.62 (1H, *m*, *J* = 16.36 Hz, H-6), 7.50-7.53 (1H, *m*, *J* = 12.72 Hz, H-3); ^{13}C-NMR (CD_3OD, 100 MHz); 150.8 (C-2), 148.4 (C-9), 137.9 (C-4), 130.8 (C-7), 129.4 (C-8), 129.1 (C-10), 128.8 (C-5), 127.7 (C-6), 122.4 (C-3). The spectroscopic data of active constituent isolated from *R. chalepensis* leaves were verified to match those of quinoline (Lee and Lee, 2011).

Quinoline derivatives were selected to evaluate the changes in inhibitory activity based on the position of nitrogen atoms in the

Table 1 α-Glucosidase and α-amylase inhibitory activities of various fractions obtained from the methanol extract of *R. chalepensis* leaf

Samples[a]	Inhibitory activities (%) against α-glucosidase	Inhibitory activities (%) against α-amylase
Methanol extract	64.5±1.1	72.1±1.4
Hexane fraction	NA[b]	NA
Chloroform fraction	100	100
Ethyl acetate fraction	NA	NA
Butanol fraction	NA	NA
Water fraction	NA	NA

[a]Sample concentration, 1,500 μg/mL.
[b]NA, no activity.

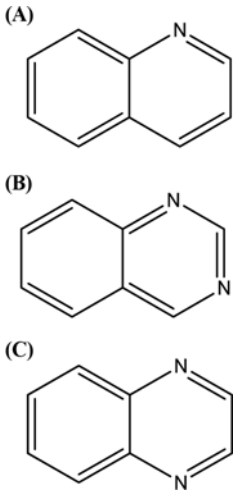

Fig. 1 Chemical structures of the quinoline derivatives. (A) Quinoline. (B) Quinazoline. (C) Quinoxaline.

Table 2 α-Glucosidase and α-amylase inhibitory activities of quinoline and IC_{50} values of its structural derivatives

Samples	α-glucosidase inhibition IC_{50} (μg/mL)[a]	α-amylase inhibition IC_{50} (μg/mL)
Quinoline	80.3±2.1	179.5±1.5
Quinazoline	20.5±1.8	55.4±1.7
Quinoxaline	NI[c]	NI
Acarbose[b]	66.5±1.5	180.6±1.3

[a]IC_{50} values calculated from regression lines, using five different concentrations in triplicate experiments.
[b]Acarbose was used as the positive control.
[c]NI, no inhibition at a concentration of 1,000 μg/mL.

pyrazine ring such as quinazoline and quinoxaline against α-glucosidase and α-amylase (Fig. 1). Quinoline, quinazoline, quinoxaline, and acarbose were tested for their inhibitory activities by measuring their IC_{50} values against α-glucosidase and α-amylase. Based on the IC_{50} values against α-glucosidase, quinazoline exhibited the greatest inhibitory activity (20.5 μg/mL), followed by acarbose (66.5 μg/mL), and quinoline (80.3 μg/mL) (Table 1). In case of the inhibitory activity against α-amylase, quinazoline had potent inhibitory activity followed by quinoline (179.5 μg/mL), and acarbose (180.6 μg/mL) (Table 2). However, quinoxaline did not exhibit any inhibitory activity against α-glucosidase or α-amylase. Compared with that of acarbose, quinazoline exhibited higher inhibitory activity against α-glucosidase than acarbose, but quinoline showed less inhibitory activity against α-glucosidase than acarbose. Quinazoline showed higher inhibitory activity against α-amylase than that of acarbose. No significant difference was observed between quinoline and acarbose against α-amylase. These results indicate that quinoline and quinazoline had the great inhibitory activity against α-glucosidase or α-amylase. Similarly, Lee and Lee (2011) reported that quinoline and quinazoline showed good relaxant effects on histamine-induced contraction in guinea pig trachea. Interestingly, quinoxaline, which has a nitrogen atom in place of a carbon atom in the pyridine ring, did not exhibit any inhibitory activity against α-glucosidase or α-amylase. In contrast, quinazoline showed the greatest inhibitory activities against α-glucosidase or α-amylase. Similarly, previous studies reported that the position of the nitrogen atom in the ring affects α- and β-glucosidase inhibitory activities (Borges de Melo et al., 2006).

Based on the Material Safety Data sheet provided by Sigma-Aldrich (2012), the oral lethal dose of quinoline (262 mg/kg) indicates moderate acute toxicity to mammals. Based on our findings, the inhibitory action of quinoline and quinazoline may be useful as an inhibitory agent. However, further work is necessary to determine toxicity to humans.

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