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

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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 IC₅₀ values against α-amylase or α-glucosidase and were compared with that of acarbose. Based on the IC₅₀ 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 (Almazraawi 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
ground and extracted with methanol (11 L) at 25°C for 1.5 days. The filtrate was poured into an EYELA Autojack NAI-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 collected and examined 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 and subjected to structural determination via spectroscopic analyses. The spectroscopic data of active constituent isolated from R. chalepensis extract, quinoline, and its structurally related analogs were evaluated against α-glucosidase and α-amylase. The spectroscopic data of active constituent isolated from R. chalepensis extract, quinoline, and its structurally related analogs were evaluated against α-glucosidase and α-amylase. The spectroscopic data of active constituent isolated from R. chalepensis extract, quinoline, and its structurally related analogs were evaluated against α-glucosidase and α-amylase. The spectroscopic data of active constituent isolated from R. chalepensis extract, quinoline, and its structurally related analogs were evaluated against α-glucosidase and α-amylase.

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, 13C-NMR and 1H-NMR, and by comparison with an authentic reference component. The active component was characterized as quinoline (Fig. 1) based on the following evidence: quinoline (C9H7N, MW, 129.2); EI-MS (70 eV) m/z (% relative intensity): M+ 129 (100), 128 (15), 102 (25), 76 (10), 51 (12); 1H-NMR (CDCl3, 300 MHz; 1H-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 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 CaCl2 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.

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

| Samples  | Inhibitory activities (%) against α-glucosidase | Inhibitory activities (%) against α-amylase |
|----------|-----------------------------------------------|-------------------------------------------|
| Methanol extract | 64.5±1.1 | 72.1±1.4 |
| Hexane fraction | NA\textsuperscript{a} | NA |
| Chloroform fraction | 100 | 100 |
| Ethyl acetate fraction | NA | NA |
| Butanol fraction | NA | NA |
| Water fraction | NA | NA |

\textsuperscript{a}Sample concentration, 1,500 μg/mL.
\textsuperscript{b}NA, no activity.
Based on the IC₅₀ values against α-glucosidase inhibition of quinoline and quinazoline, acarbose were tested for their inhibitory activities by measuring their IC₅₀ values against α-glucosidase and 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).

Table 2 α-Glucosidase and α-amylase inhibitory activities of quinoline and IC₅₀ values of its structural derivatives

| Samples    | α-glucosidase inhibition (IC₅₀ (µg/mL)) | α-amylase inhibition (IC₅₀ (µg/mL)) |
|------------|-----------------------------------------|-------------------------------------|
| Quinoline  | 80.3±2.1                                 | 179.5±1.5                           |
| Quinazoline| 20.5±1.8                                 | 55.4±1.7                            |
| Quinoxaline| NI                                      | NI                                  |
| Acarbose   | 66.5±1.5                                 | 180.6±1.3                           |

*IC₅₀ values calculated from regression lines, using five different concentrations in triplicate experiments.

Based on the IC₅₀ 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 contrast, quinazoline showed less 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. Quinoxaline 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 IC₅₀ values against α-glucosidase or α-amylase, acarbose exhibited the greatest inhibitory activity (20.5 µg/mL). Interestingly, quinazoline, 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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