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NOTE

Quinoline-2-carboxylic Acid Isolated from *Ephedra pachyclada* and Its Structural Derivatives Show Inhibitory Effects against α-Glucosidase and α-Amylase

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Abstract The aim of present study was to isolate a bioactive compound from the chloroform fraction of *Ephedra pachyclada* stems and to evaluate antidiabetic activities against α-glucosidase and α-amylase. According to various chromatographic and spectroscopic analyses, the bioactive compound of *E. pachyclada* was identified as quinoline-2-carboxylic acid. Based on the IC_{50} values of quinoline-2-carboxylic acid derivatives against α-glucosidase and α-amylase, quinoline-2-carboxylic acid (9.1 and 15.5 μg/mL) exhibited potent inhibitory activities, followed by quinoline-3-carboxylic acid (10.6 and 31.4 μg/mL), quinoline-4-carboxylic acid (60.2 and 152.4 μg/mL), and acarbose (66.5 and 180.6 μg/mL) against α-glucosidase and α-amylase, respectively. However, quinoline-2-carboxaldehyde, quinoline-3-carboxaldehyde, and quinoline-4-carboxaldehyde showed no inhibitory activities. Antidiabetic activity depended on the existence of a carboxyl group on quinoline for activities against α-glucosidase and α-amylase. Therefore, *E. pachyclada* and quinoline-2-carboxylic acid derivatives could be suitable as alternative synthetic antidiabetic agents.

Keywords antidiabetic activity · *Ephedra pachyclada* · quinoline-2-carboxylic acid · α-amylase · α-glucosidase

Introduction

Diabetes mellitus is a common metabolic disease characterized by hyperglycemic syndrome that causes many complications such as cardiovascular disease, diabetic neuropathy, and diabetic retinopathy (Choi et al., 2008). Hydrolysis of starch is a major process for blood glucose regulation, and α-amylase and α-glucosidase are the key enzymes related to intestinal absorption and starch breakdown, respectively (Wang et al., 2010). Inhibiting key enzymes can significantly decrease blood glucose level after a mixed carbohydrate diet (Lee, 2005; Wang et al., 2010). Therefore, the regulation of blood glucose by α-amylase and α-glucosidase can be an important strategy for managing hyperglycemia linked to type II diabetes (Lee, 2005; Wang et al., 2010). Chemical inhibitors are widely used for clinical treatment, such as acarbose and glimepiride (Cha et al., 2009). However, these inhibitors cause side effects such as abdominal cramping, diarrhea, and flatulence (Cha et al., 2009; Jeong et al., 2012).

Plant-derived materials had been used as alternative materials due to their bioactive substances (Lee and Ahn, 1998; Lee, 2002; Yang et al., 2002; Yang et al., 2003). In addition, previous studies have reported that natural products possess α-amylase and α-glucosidase inhibitory effects (Kim et al., 2009). *Ephedra pachyclada* (Ephedraceae) is one of approximately 50 *Ephedra* species worldwide and has been used as a traditional medicine for a long time (Marzieh et al., 2014). In particular, *E. pachyclada* stems have been widely used (Caveney et al., 2001; Marzieh et al., 2014). Although diverse bio-functional activities of *E. pachyclada* stems have been reported, few studies have reported the antidiabetic activity of *E. pachyclada* stems and its bioactive compound. Thus, this study was conducted to isolate a bioactive compound from *E. pachyclada* stems and determine the antidiabetic activities of *E. pachyclada* stems, the isolated compound, as well as its structural derivatives against α-amylase and α-glucosidase.

Materials and Methods

Chemicals. The chemicals used were acarbose, quinoline-3-carboxylic acid, quinoline-4-carboxylic acid, quinoline-2-

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carboxyaldehyde, quinoline-3-carboxyaldehyde, and quinoline-4-carboxyaldehyde (Sigma-Aldrich, USA). All chemicals were of reagent grade.

Materials. *E. pachyclada* stems (5 kg) were supplied from a traditional market (Korea). The *E. pachyclada* stems were cut into pieces, powdered, extracted twice with methanol (25 L) at room temperature for 2 days, and filtered using filter paper. The filtrate was vacuum-concentrated at 45°C using a rotary vacuum evaporator (EYELA, Japan), and the yield of the methanol extract from *E. pachyclada* stems was 25.5%. Subsequently, the methanol extract (20 g) of *E. pachyclada* stems was continually partitioned into hexane, chloroform, ethyl acetate, *n*-butyl alcohol, and water fractions. The organic fractions were concentrated using a rotary vacuum evaporator at 45°C, whereas the water fraction was freeze-dried.

Isolation and identification. The chloroform (10 g) fraction was loaded onto a silica gel column (70–230 mesh, Merck, Germany, 600 mm i.d. ×750 mm) and sequentially eluted with hexane and mixtures of chloroform: methylene chloride (10:1, 7:3, 5:1, and 1:1, v:v), resulting in six fractions (P1-P6), respectively. All fractions were analyzed by thin layer chromatography (chloroform: methylene chloride, 7:3, v:v). Similar fractions were combined and bioassayed. The P3 fraction exhibited α-amylase and α-glucosidase inhibitory activities; therefore, this fraction was chromatographed on a silica gel column (400 mm i.d. ×800 mm) using chloroform:methylene chloride (7:3, v:v) to obtain the active P32 fraction. The P32 fraction was isolated by preparative high-performance liquid chromatography (HPLC) (Japan Analytical Industry Co., Ltd., Japan) and was applied to a JAI gel GS Series column (JAI GS310 300 mm ×2+JAI GS310 500 mm) with a mixture of chloroform:methylene chloride (7:3, v:v) at a flow rate of 5.0 mL/min as the mobile phase. The active P321 fraction was re-chromatographed using a JAI gel W series column (W253 500 mm + W252 500 mm) with a mixture of chloroform:methylene chloride (7:3, v:v) under the same conditions. Finally, the P3212 fraction (215 mg) was isolated. The structure of the P3212 fraction was determined by spectroscopic analyses. ^{1}H- and ^{13}C-NMR spectra were obtained using a JNM-ECA600 spectrometer (JEOL Ltd, Japan; ^{1}H-600 MHz; ^{13}C-150 MHz) with $CDCl_3$.

Antidiabetic activity. The antidiabetic effects of *E. pachyclada*, the isolated compound, and its structural derivatives were assessed against α-amylase and α-glucosidase. α-amylase inhibitory activity was assayed according to the procedure described by Wang et al. (2010) with some modifications. The enzyme solution (6.25 U/mL) was prepared by dissolving α-amylase (Sigma Co., USA) in 0.5 M Tris-HCl buffer (pH 6.9). Starch azure (8 mg) was suspended in 0.5 M Tris-HCl buffer containing 0.01 M calcium chloride and soaked in boiling water for 5 min, followed by preincubation at 37°C for 10 min. The enzyme solution (100 μL) and sample (100 μL) in 50% dimethyl sulfoxide were mixed in a 96-well plate. After 10 min, 50% acetic acid (50 μL) was added to stop the reaction. The absorbance of the reactants was measured at 595 nm with a microplate reader (Model ASYS UVM 340, Biochrom Ltd., UK). Acarbose was employed as the positive control. All tests were replicated three times. Percent (%) inhibition was determined using the following equation: Inhibition (%)=[1 (sample/control)]×100. IC_{50} values were calculated by applying logarithmic regression analysis. α-Glucosidase inhibitory activity was assayed according to the procedure described by Shinde et al. (2008) with a slight modification. The formation of *p*-nitrophenol was measured using α-glucosidase after a reaction with *p*-nitrophenyl-α-D-glucopyranoside (NPG). The enzyme solution (0.6 U) was prepared by dissolving α-glucosidase in 0.1 M phosphate buffer (pH 7.0) containing 2 g/L bovine serum albumin and 0.2 g/L sodium azide. The enzyme solution (50 μL) and sample (10 μL), dissolved in dimethyl sulfoxide, were mixed and placed in a 96-well plate. After 15 min, 5 mM NPG (50 μL) in the same buffer was added, and the mixture was incubated for 10 min at 37°C. Sodium carbonate (0.1 M) was subsequently added to stop the reaction. Absorbance of the reactants was measured at 405 nm using a microplate reader (Model ASYS UVM 340, Biochrom Ltd.). Acarbose was employed as the positive control. All tests were replicated three times. Percentage (%) inhibition was determined using the following equation: Inhibition (%)=[1 (sample/control)]×100. The IC_{50} values were calculated by applying logarithmic regression analysis.

Results and Discussion

The active compound of the chloroform fraction from *E. pachyclada* stems was isolated by silica gel column chromatography and prep HPLC, and the structure of the isolated compound was determined by various spectroscopic analyses including EI-MS, ^{1}H-NMR, and ^{13}C-NMR and by direct comparison with an authentic standard compound. The active compound was identified as quinoline-2-carboxylic acid (Fig. 1) based on the following: quinoline-2-carboxylic acid ($C_{10}H_7NO_2$, MW: 173); EI-MS (70 eV) *m/z* 173 [M^+]; ^{1}H-NMR (600 MHz, CD_3OD) δ_H 10.82 (H, dd, J=6.37 Hz, H-7), 8.56 (H, d, J=6.82 Hz, H-2), 7.87 (3H, d, J=6.02 Hz, H-1, 3, 6), 7.75 (H, d, J=6.21 Hz, H-5), 7.41 (H, d, J=6.98 Hz, H-4); ^{13}C-NMR (150 MHz, CD_3OD) δ_C 167.3 (C-11), 149.2 (C-2), 148.5 (C-10), 136.1 (C-4), 130.4 (C-8), 129.5 (C-5, C-9), 128.2 (C-8), 126.9 (C-6), 121.2 (C-3). The ^{1}H-NMR and ^{13}C-NMR spectra of P3212 matched with those of quinoline-2-carboxylic acid isolated from *Ephedra* species (Starratt and Caveney, 1996; Lee and Lee, 2009).

The antidiabetic activities of quinoline-2-carboxylic acid, and its structural derivatives, including quinoline-3-carboxylic acid, quinoline-4-carboxylic acid, quinoline-2-carboxaldehyde, quinoline-3-carboxaldehyde, and quinoline-4-carboxaldehyde, as well as acarbose as a positive control were evaluated against α-amylase and α-glucosidase. Based on the IC_{50} values of quinoline-2-carboxylic acid, its structural derivatives, and acarbose against α-amylase, quinoline-2-carboxylic acid (15.5±1.9 μg/mL) had the most potent antidiabetic activity, followed by quinoline-3-carboxylic

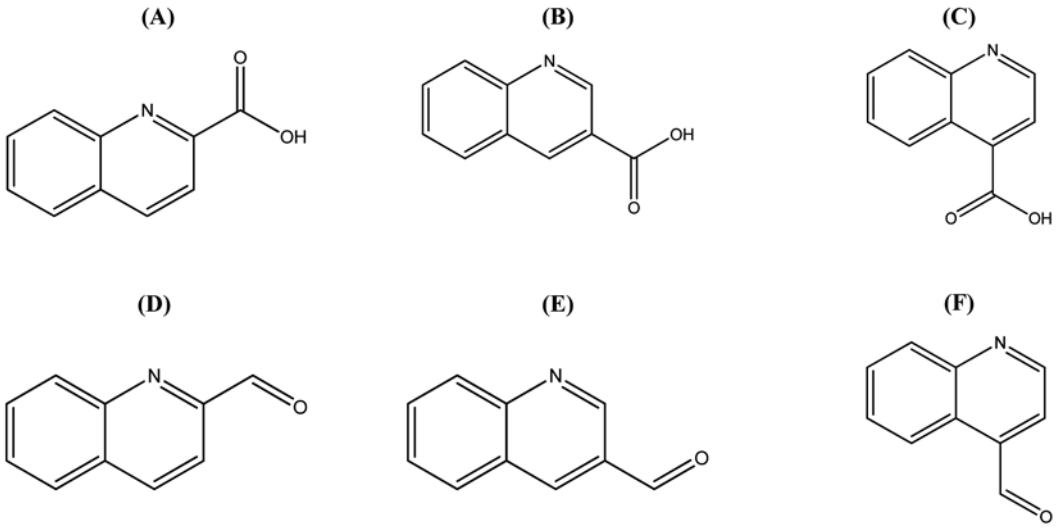

Fig. 1 Structures of quinoline-2-carboxylic acid derivatives: (A) Quinoline-2-carboxylic acid; (B) quinoline-3-carboxylic acid; (C) quinoline-4-carboxylic acid; (D) quinoline-2-carboxyaldehyde; (E) quinoline-3-carboxyaldehyde; (F) quinoline-4-carboxyaldehyde.

acid (31.4±2.2 μg/mL), quinoline-4-carboxylic acid (152.4±2.5 μg/mL), and acarbose (180.6±1.3 μg/mL). Quinoline-2-carboxylic acid (9.1±2.3 μg/mL) had the strongest antidiabetic activity in the case of α-glucosidase inhibitory activities of quinoline-2-carboxylic acid and its structural derivatives, followed by quinoline-3-carboxylic acid (10.6±1.4 μg/mL), quinoline-4-carboxylic acid (60.2±2.1 μg/mL), and acarbose (66.5±1.5 μg/mL). However, quinoline-2-carboxaldehyde, quinoline-3-carboxaldehyde, and quinoline-4-carboxaldehyde had no activity against α-amylase and α-glucosidase (Table 1). These results indicate that quinoline-2-carboxylic acid and its structural derivatives isolated from *E. pachyclada* stems have excellent inhibitory effects against α-amylase and α-glucosidase. Similarly, Chohachi et al. (1985) reported that *E. distachya* and an isolated compound had hypoglycemic activity. In addition, the antidiabetic activities of quinoline-2-carboxylic acid, quinoline-3-carboxylic acid, and quinoline-4-carboxylic acid, which contain a carboxyl group on quinoline, were more active against α-amylase and α-glucosidase than those of quinoline-2-carboxaldehyde, quinoline-3-carboxaldehyde, and quinoline-4-carboxaldehyde, with an aldehyde group on quinoline. In this regard, quinoline-2-carboxylic acid isolated from *E. pachyclada* stems and its structural derivatives (including a carboxyl group) could regulate the function of α-amylase and α-glucosidase. Matsuda et al. (1998) reported that the existence of a carboxyl group affected the antidiabetic effect. Our findings indicate that *E. pachyclada* and quinoline-2-carboxylic acid derivatives could be suitable as alternative synthetic antidiabetic agents.

Table 1 Antidiabetic activities of quinoline-2-carboxylic acid derivatives and acarbose against α-glucosidase and α-amylase

Samples	Antidiabetic activity	
	α-glucosidase inhibition IC_{50} (μg/mL)[a)]	α-amylase inhibition IC_{50} (μg/mL)
Quinoline-2-carboxylic acid	9.1±2.3	15.5±1.9
Quinoline-3-carboxylic acid	10.6±1.4	31.4±2.2
Quinoline-4-carboxylic acid	60.2±2.1	152.4±2.5
Quinoline-2-carboxaldehyde	NI[c)]	NI
Quinoline-3-carboxaldehyde	NI	NI
Quinoline-4-carboxaldehyde	NI	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 a positive control.
[c)] NI: No inhibition at a concentration of 1,000 μg/mL.

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