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Research Article

Chemical Composition and α-Glucosidase Inhibitory Activity of Vietnamese *Citrus* Peels Essential Oils

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Background. Inhibition of α-glucosidase is an important factor to control postprandial hyperglycemia in type 2 diabetes mellitus. *Citrus* essential oils (CEO) are among the most widely used essential oils, and some of them exhibited promising antidiabetic effect. However, the α-glucosidase inhibition of CEO has not been investigated so far. The present work aims to evaluate the α-glucosidase inhibition of essential oils from six Vietnamese *Citrus* peels. *Methods*. The chemical composition of essential oils obtained by hydrodistillation from six *Citrus* peels was analyzed by GC-MS. All essential oils were tested for their inhibitory activity on α-glucosidase using *p*-nitrophenyl-α-D-glucopyranoside as substrate. *Results*. In Buddha's hand and lime peels, the major components were limonene (59.0–61.31%) and γ-terpinene (13.98–23.84%) while limonene (90.95–95.74%) was most abundant in pomelo, orange, tangerine, and calamondin peels. Among the essential oils, the Buddha's hand oil showed the most significant α-glucosidase inhibitory effect with the IC_{50} value of 412.2 μg/mL. The combination of the Buddha's hand essential oil and the antidiabetic drug acarbose increased the inhibitory effect. *Conclusions*. The results suggested the potential use of Buddha's hand essential oil as an alternative in treatment of type 2 diabetes mellitus.

1. Introduction

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia or increased blood glucose levels, which leads over time to serious damage to the heart, blood vessels, eyes, kidneys, and nerves. The number of diabetic sufferers worldwide is expected to rise from 415 million to 642 million individuals by 2040 [1]. It has been known that high postprandial plasma glucose affects the diabetes complications, primarily, in the macrovascular complications more severely than elevated fasting plasma glucose. Thus, it is important to control postprandial blood glucose level, so as to reduce complications and mortality [2, 3]. α-Glucosidase is a digestive enzyme that participates in glucose digestion. The inhibition of this enzyme would delay the degradation of oligosaccharides to glucose and therefore control postprandial hyperglycemia [4]. Several α-glucosidase inhibitors currently in clinical use are acarbose, miglitol, and voglibose. However, the longtime use and high-dose administration of those drugs cause adverse effects such as diarrhea, abdominal pain, flatulence, and abdominal or stomach pain [5]. Herbal medicines with antihyperglycemic activity have been used for alternative treatment of type 2 diabetes due to their low side effects and less expense when compared to synthetic hypoglycemic drugs [6]. Therefore, the search for more effective and safer hypoglycemic agents of natural origin has continued to be an important target of active research all over the world.

Citrus species (Rutaceae) are the largest fruit crop in the world and have a strong commercial value. They are produced mainly for the fresh consumption but also addressed to the food, medicinal, and cosmetic industry for their

by-products [7]. *Citrus* essential oils (CEO) are among the most widely used essential oils in the world and are used as food additives, preservatives, and flavorings [8]. Although CEO exhibited promising antidiabetic property in vitro and in animal models [9–11], however, the α-glucosidase inhibition of CEO has not been reported so far. This paper describes the investigation of chemical compositions and α-glucosidase inhibitory effect of the essential oils prepared from six Vietnamese *Citrus* peels.

2. Experimental

2.1. Materials and Isolation of Essential Oil. The mature fruits of Buddha's hand (*Citrus medica* var. *sarcodactylis*), lime (*Citrus aurantifolia*), pomelo (*Citrus grandis* L.), orange (*C. sinensis* (L.) Osbeck), tangerine (*C. reticulata* Blanco var. tangerine), and calamondin (*C. microcarpa* (Bunge.)) were freshly collected in Hanoi, Vietnam, in December 2015. The peels (each 500 g) were hydrodistilled in a Clevenger-type apparatus for 4 h, after which the essential oils were separated and dried with anhydrous $MgSO_4$. The obtained oils were stored at −5°C until use.

2.2. Essential Oil Analysis. GC/MS analysis was performed using an Agilent GC7890A apparatus coupled to a Mass Selective Detector (Agilent 5975C). A HP-5MS fused silica capillary column (60 m × 0.25 mm id. × 0.25 μm film thickness) was used. Helium was the carrier gas with a flow rate of 1.0 mL/min. The inlet temperature was 240°C and the oven temperature program was as follows: 60°C to 220°C at 4°C/min and then at 20°C/min to 240°C with an interphase temperature of 280°C. The split injection mode was 1 : 142, the detector temperature was 270°C, and the injection volume was 0.1 μL. The MS interface temperature was 270°C, MS mode, E.I. detector voltage 1300 V, and mass range 40–400 Da at 1.0 scan/s. Identification of components was achieved based on their retention indices and by comparison of their mass spectral fragmentation patterns with those stored on the MS library (NIST08, Wiley09). Component relative contents were calculated based on total ion current without standardization. Data processing was MassFinder4.0.

2.3. Assay for α-Glucosidase Inhibition. The yeast α-glucosidase (G0660, Sigma-Aldrich) inhibition assay was performed using the substrate *p*-nitrophenyl-α-*D*-glucopyranoside according to the previously described method [12]. Briefly, the sample solution (2 μL dissolved in DMSO) and 0.5 U/mL α-glucosidase (40 μL) were mixed in 120 μL of 0.1 M phosphate buffer (pH 7.0). After 5 min preincubation, 5 mM *p*-nitrophenyl-α-D-glucopyranoside solution (40 μL) was added and the solution was incubated at 37°C for 30 min. The absorbance of released 4-nitrophenol was measured at 405 nm by using a microplate reader (Xmark, Biorad, USA). Acarbose was used as positive control (IC_{50} 433.8 μg/mL).

3. Results and Discussion

The essential oils of the peels of Buddha's hand (CMSO), lime (CAO), pomelo (CGO), orange (CSO), tangerine (CRO), and calamondin (CMO) were extracted by hydrodistillation. Their chemical compositions were analyzed by GC-FID and GC/MS. The yields of the essential oils were 0.69, 0.54, 1.02, 1.30, 2.04, and 1.16% for Buddha's hand, lime, pomelo, orange, tangerine, and calamondin peels, respectively. In lime peel, a total of 27 compounds amounting to 100% were identified. 23 components were also found in Buddha's hand peel while orange peel contained only 9 volatile compounds (Table 1). Limonene (90.95–95.74%) was the most abundant in pomelo, orange, tangerine, and calamondin while limonene (59.0–61.31%) and γ-terpinene (13.98–23.84%) were the highest ones in Buddha's hand and lime. Interestingly, γ-terpinene was not detected in pomelo, orange, tangerine, and calamondin.

The inhibitory effects of six CEO against yeast α-glucosidase were evaluated using *p*-nitrophenyl-α-*D*-glucopyranoside as the substrate. Only CMSO inhibited α-glucosidase with the IC_{50} value of 412.2 μg/mL. This effect was comparable to that of the antidiabetic acarbose. Although limonene has been reported to show antidiabetic activity [13, 14], this compound might not be responsible for the inhibition of α-glucosidase even though it exists in high content in the inactive samples (CAO, CGO, CSO, CRO, and CMO). γ-Terpinene content was 1.7-fold higher in CMSO than in CAO suggesting that this monoterpene might contribute to the α-glucosidase inhibitory activity. In addition, four components nerol, neral, geraniol, and geranial might be involved in α-glucosidase inhibition since they were found in CMSO but were not detected or present at low amount in other five essential oils. These compounds also exhibited antidiabetic effect in vitro and in animal models [15–17]. Although the α-glucosidase inhibition of *Citrus limetta* peel extract has been investigated [18], the present study is the first report on the α-glucosidase inhibitory effect of CMSO. Interestingly, the hypoglycemic and antidiabetic effects of CMSO such as in vitro α-amylase inhibitory activity and in vivo insulin secretagogue and slimming effects were also reported [9, 10]. This evidence suggested that CMSO could be used in the management and/or prevention of type 2 diabetes mellitus.

It was of interest to establish whether CMSO and acarbose interact synergistically or additively in the inhibition of α-glucosidase (Figure 1). At low concentration of acarbose (10 μg/mL) and CMSO (10 and 30 μg/mL), the percentage of α-glucosidase inhibition of the mixture was equal to the sum of CMSO and acarbose, suggesting that they produced additive inhibition. The higher doses of CMSO (100 and 500 μg/mL) produced a synergistic inhibitory effect with acarbose (10 μg/mL). Acarbose is a potent α-glucosidase inhibitor and has been used in the treatment of type 2 diabetes. The lowest dose of acarbose with clinical effects is 150 mg/day, and at doses over 300 mg/day, the drug does not increase in the inhibitory effect due to the saturated binding with α-glucosidase [19]. The long-term use and high-dose administration of this drug cause severe side effects, such as diarrhea, flatulence, and abdominal or stomach pain [5, 19].

Table 1: Yield and composition of the essential oil of six *Citrus* peels.

Components	RI	CMSO	CAO	CGO	CSO	CRO	CMO
(*Z*)-Hex-3-en-1-ol	855	—	0.10	—	—	—	—
α-Thujene	932	0.60	0.45	—	—	—	—
α-Pinene	941	1.49	1.78	0.80	0.66	0.63	0.59
Sabinene	980	0.20	1.29	—	0.13	0.18	0.28
β-Pinene	986	1.62	6.72	0.24	—	0.34	1.23
Myrcene	994	1.58	1.71	—	2.33	2.55	2.11
n-Octanal	1005	—	0.38	2.33	0.40	0.51	0.42
α-Phellandrene	1012	—	—	1.17	—	—	—
α-Terpipene	1024	0.62	0.47	—	—	—	—
o-Cymene	1032	0.96	0.31	—	—	—	—
Limonene	1038	59.00	61.31	90.69	95.74	94.10	90.88
β-Phellandrene	1039	—	—	3.02	—		
Cineol	1040	—	1.94	—	—	—	—
(*E*)-*β*-Ocimene	1051	0.37	0.39	0.13	—	—	—
γ-Terpinene	1066	23.84	13.98	—	—	—	—
n-Octanol	1072	—	—	—	0.16	0.20	0.21
Trans-Linalool oxide	1080	—	—	0.24	—	—	—
Cis-Linalool oxide	1095	—	—	0.11	—	—	—
Terpinolene	1096	1.26	0.80	—	—	—	—
Linalool	1104	0.30	0.47	0.39	0.19	0.74	0.77
Nonanal	1107	—	—	—	—	—	0.23
Citronellal	1157	—	0.92	—	—	—	—
Terpinen-4-ol	1189	0.73	0.90	—	—	—	—
α-Terpineol	1201	1.48	2.14	0.40	0.17	0.42	0.41
Decanal	1209	—	—	—	0.20	0.15	0.23
Citronellol	1231	0.13	0.51	—	—	—	—
Nerol	1234	0.74	—	—	—	0.18	—
Neral	1248	1.23	0.11	0.21	—	—	—
Geraniol	1258	0.84		—	—	—	—
Geranial	1276	1.61	0.15	0.27	—	—	—
δ-Elemene	1349	—	—	—	—	—	0.11
Geranyl acetate	1385	—	—	—	—	—	0.59
β-Caryophyllene	1439	0.28	0.66	—	—	—	—
(*E*)-*α*-Bergamotene	1447	0.25	0.49	—	—	—	—
Germacrene D	1499	0.43	0.38	—	—	—	1.39
(*E*)-*α*-Farnesene	1512	—	0.70	—	—	—	—
β-Bisabolene	1518	0.43	0.82	—	—	—	—
Cadinene	1537	—	0.12	—	—	—	—
β-Eudesmol	1671	—	—	—	—	—	0.39
α-Eudesmol	1673	—	—	—	—	—	0.15
Total (%)		99.99	100.0	100.0	99.98	100.0	99.99

Therefore, lowering the required dose of acarbose may reduce its potential side effects. Our result showed that combination of a low concentration of acarbose and CMSO produced significant synergistic inhibition of *α*-glucosidase than the drug alone. As a consequence of this synergistic effect, it is possible to reduce dosage of acarbose by combining it with CMSO, resulting in a diminished adverse effect of acarbose.

Synergistic effects between acarbose and natural compounds in inhibiting *α*-glucosidase have been reported previously. Cyanidins and glycosides, natural anthocyanins widely found in various fruits such as red apple peels and berries, produced the synergistic effect with low doses of acarbose [20–22]. Satoh et al. reported that the combination of green tea extracts, green tea polyphenols, or EGCG with acarbose had a synergistic effect on *α*-glucosidase at low concentrations and the combined effect turned out to be antagonistic at high concentrations [23]. The aqueous extract of black tea leaves (JAT) is also potent *α*-glucosidase inhibitor.

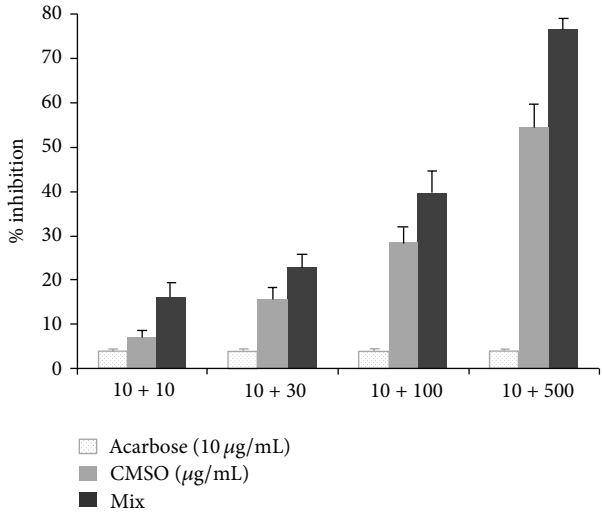

Figure 1: α-Glucosidase inhibitory effect of Buddha's hand essential oil (10–500 μg/mL) and in combination with acarbose (10 μg/mL).

The combination of acarbose and JAT exhibited mixed-type inhibition of α-glucosidase and resulted in synergistic hypoglycemic effect in vivo [24]. Buddha's hand fruit is widely cultivated over the world. In Vietnam, the fruit has been used for functional vegetable and jam. Its essential oil has been reported to possess insulin secretagogue, hypoglycemic, and anti-inflammatory activity [10, 25]. Our present study indicated that Buddha's hand essential oil might provide a significant clinical benefit in delaying postprandial hyperglycemia and minimizing the adverse effects of acarbose.

4. Conclusion

The present work reported for the first time the α-glucosidase inhibitory effect of *Citrus* essential oils. The Buddha's hand essential oil strongly inhibited α-glucosidase activity in a dose dependent manner. When it is cotreated with acarbose, this essential oil synergistically increased the enzyme inhibitory activity. The results indicated that the Buddha's hand essential oil is a promising hypoglycemic agent and can be included in antidiabetic preparations and formulations to control postprandial hyperglycemia. Further in vivo studies are needed to confirm the hypoglycemic effect of the Buddha's hand essential oil alone and in combination with acarbose.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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