Full Length Research Article
Advancements in Life Sciences – International Quarterly Journal of Biological Sciences

ARTICLE INFO

Date Received: 10/03/2022;
Date Revised: 10/06/2022;
Date Published Online: 31/07/2022;

Authors' Affiliation:
1. Department of Organic Chemistry, School of Chemistry, University of the Punjab, Quaid-e-Azam Campus, Lahore- Pakistan.
2. Government Hospital Samanabad, Lahore - Pakistan
3. Department of Plant Pathology, Faculty of Agricultural Sciences, University of the Punjab, Quaid-e-Azam Campus, Lahore - Pakistan

***Corresponding Author:**
Shabnam Javed
Email: shabnam.chem@pu.edu.pk

How to Cite:
Javed S, Javed I, Shoaib A, Parveen S, (2022). Oleanolic acid (pentacyclic triterpenes) as a potential candidate for α-glycosidase inhibition activity. Adv. Life Sci. 9(2): 219-223.

Keywords:
Diabetes mellitus; Enzyme inhibition; Medicinal plants; Pentacyclic triterpenes

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Oleanolic acid (pentacyclic triterpenes) as a potential candidate for α-glycosidase inhibition activity

Shabnam Javed[1]*, Iqra Javaid[2], Amna Shoaib[3], Shagufta Perveen[3]

Abstract

Background: Diabetes mellitus is a common health dilemma worldwide and is characterized by hyperglycemia. Inhibition in the activity of one of the digestive tract enzymes α-glucosidase is one of the therapeutic approaches to hydrolyze carbohydrates into glucose using natural agents. Many natural compounds with α-glucosidase inhibitory activity have transpired to be secondary metabolites. Monotheca buxifolia, native to Pakistan is a major medicinal tree, which has been known for its extensive pharmacological activities.

Methods: α-glucosidase activity of ten isolated compounds (lupeol, lupeol acetate, betulin, β-sitosterol, β-amyrin, oleanolic acid, vanillic acid, protocatechuic acid, kaempferol and quercetin) from lipophilic hexane fraction of M. buxifolia (stem and leaves) was assessed against α-glucosidase enzyme using acarbose as a control.

Results: All ten compounds hold α-glucosidase inhibition potential (91-99%). However, IC50 (half-maximal inhibitory concentration) values of oleanolic acid (5 µM) were 8-fold lower than that of acarbose. Moreover, inhibition potencies of lupeol (15.87 µM), β-amyrin (18.14 µM) betulin (21.49 µM), quercetin (23.47 µM), and lupeol acetate (29.45 µM) were much stronger than the inhibitory effect obtained from acarbose (38.25 µM).

Conclusion: Oleanolic acid of M. buxifolia exhibited a potent inhibitory effect against α-glucosidase, therefore, oleanolic acid may be utilized in medicinal formulations against diabetic disorders.

Introduction

Diabetes mellitus (DM) is a chronic condition caused by hyperglycemia resulting from defects in insulin secretion and/or insulin action [1], hence leading to dysfunctioning and failure of different organs (e.g. eyes, kidneys, nerves, heart, and blood vessels) along with the development of macro- and microvascular diseases [2]. In recent times, DM is ranked as the leading cause of death [3], while its prevalence has risen from 4.7 to 8.5% from 2014 to 2019 [4] and is expected to target 5.4% of the world's population by 2025 [5]. An inexorable rise in DM has decreased the quality of life despite the promises of a wide range of antidiabetic drugs [6]. Alternatively, the α-glucosidase inhibition may be a promising aspect to reduce the postprandial blood glucose level. The enzyme α-glucosidase secreted from intestinal chorionic epithelium help in the reduction of glycosylated hemoglobin and postprandial insulin levels by delaying carbohydrate absorption [7]. The α-glucosidase gained importance as a new class of antidiabetic drug in 1980, later it has been endorsed as the first line of treatment for lowering postprandial hyperglycemia by the Third Asia-Pacific Region Diabetes Treatment Guidelines [8].

Medicinal plants have many polyphenolic compounds that aid in prevention of fast breakdown of sugar, thereby controlling the blood sugar level by competitively inhibiting α-glucosidase activity [9]. Several natural α-glucosidase inhibitors e.g. acarbose and voglibose have been used clinically against DM, while only a limited are available commercially [10]. Therefore, more natural sources of α-glucosidase inhibitor need to be explored.

Monotheca buxifolia (Falc.) A. DC. of the family Sapotaceae is native to Pakistan and is locally known as Gwargwara is found in barren hilly areas. The plant is well-known for its medicinal values including antioxidant, digestive, hematinic, laxative, purgative, vermicidal, anthelmintic, antipyretic, etc. in South-Asia and Middle-East [11-13]. Its leaves and stem are enriched with anticancerous and antidiabetic compounds including flavonoids, phenolics, and terpenoids derivatives [11, 12]. In earlier studies, prominent α-glucosidase inhibitory activity has been documented in M. buxifolia extracts due to the presence of many important bioactive compounds and antioxidant activity [9]. Likewise, many plant-based compounds like betulinic acid, quercetin, quercitrin, α-amyrin have shown antioxidant, antiinflammatory and antidiabetic activity [14]. Znag et al., [15] reported 35.6 µM as IC50 value of oleanolic acid. Nguyen et al., [16], found IC50 values of 17 and 35 µM values for lupeol, and betulinic acid derivatives, respectively. Recently, Phan et al., [17] also explored substantial α-glucosidase inhibitory and cytotoxic activities (IC50: 20-29 µM) of lupeol derivatives containing a benzylidene chain. The present study is an extension of a previous study conducted by Javed et al., [11] where 10 compounds (viz., lupeol, lupeol acetate, betulin, β-sitosterol, β-amyrin, oleanolic acid, vanillic acid, protocatechuic acid, kaempferol, and quercetin) isolated from lipophilic hexane fraction of M. buxifolia were assessed for their antifungal activity. In the current study, all these 10 compounds were analyzed for their α-glucosidase activity.

Methods

Briefly, the lipophilic hexane fraction of aerial parts (stem and leaves) of M. buxifolia was subjected to vacuum liquid chromatography over silica gel and eluted with increasing order of solvent polarity as hexane-EtOAc (0 → 10) to isolate lupeol (1) and lupeol acetate (2). The three main sub-fractions of chloroform resulted in the purification of botulin (3), botulin (4), β-amyrin (5), and oleanolic acid (6). The EtOAc fraction was chromatographed using silica gel and eluted with a solvent system of increasing polarity n-hexane, n-hexane:DCM and DCM:MeOH to attain vanillic acid (7), protocatechuic acid (8), kaempferol (9), and quercetin (10). The structures of all isolated compounds are presented in Fig. 1 [11].

The isolated compounds (lupeol, lupeol acetate, betulin, β-sitosterol, β-amyrin, oleanolic acid, vanillic acid, protocatechuic acid, kaempferol, and quercetin) were screened against α-glucosidase enzyme by a modified method described earlier [18]. Briefly, a mixture of 70 µL phosphate buffer (50 mM; pH 6.8) was mixed thoroughly with 10 µL test compound (dissolve in DMSO) and α-glucosidase enzyme (0.0234 units, 10 µL) in a 96-well plate and incubation at 37 ºC for 10 min. After incubation, each well was further added with 10 µL of 0.5 mM pNPG (p-nitrophenyl glucopyranoside). The sample-loaded 96-well plate was incubated again at 37 ºC for 10 min. Finally, 80 µL of Na2CO3 solution was added to terminate the reaction. The control and blank were prepared by adding DMSO, while acarbose was taken as a positive control. The absorbance of the samples was determined at 405 nm using a microplate reader (Synergy HT BioTek, USA). The α-glucosidase inhibitory activity was expressed as the IC50 according to the percentage inhibition.

Results

The α-glucosidase inhibitory effects of all 10 compounds and their IC50 values are presented in Table 1. All compounds exhibited substantial α-glucosidase inhibition capabilities. However, four compounds viz.,

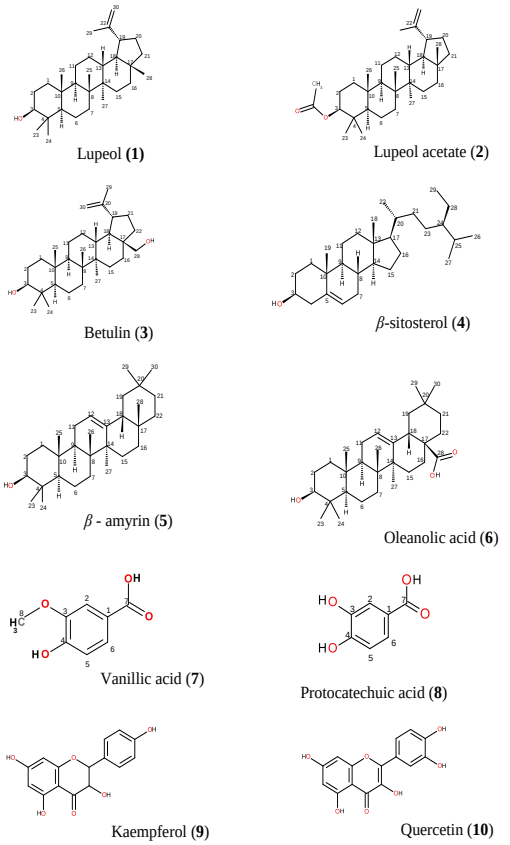

Figure 1: Structures of compounds isolated from n-hexane (1 and 2), chloroform (3-6) and ethyl acetate (7-10) fraction of methanolic extract of Monotheca buxifolia [11].

oleanolic acid, β-amyrin, quercetin and lupeol exhibited significantly greater (⩾ 97%) inhibitory potential as compared to acarbose. The inhibitory potential of remaining 6 compounds viz., lupeol acetate, betulin, β-sitosterol, vanillic acid, protocatechuic acid, and kaempferol was recorded in the range of 91-95%, which was statistically similar to acarbose. These compounds also exhibited variable IC50 values as compared to the control. β-sitosterol, and protocatechuic acid showed significantly greater inhibitor affinity (IC50) values of 65.31 and 54.75 µM as compared to acarbose (38.25 µM). However, IC50 values of vanillic acid and kaempferol were insignificantly different than acarbose. Moreover, the IC50 values of oleanolic acid (5 µM) followed by lupeol (15.87 µM), β-amyrin (18.14 µM) betulin (21.49 µM), quercetin (23.47 µM), and lupeol acetate (29.45 µM) were significantly lower than that of acarbose, especially oleanolic acid, with IC50value being 8-fold lower than that of acarbose.

Discussion

Ten compounds isolated from the organic fraction of M. buxifolia exhibited various physical characteristics and spectroscopic data [11]. The α-glucosidase activity was exhibited by all ten compounds to a variable extent. Many times greater IC50 values for the α-glucosidase activity of lupeol derivatives [17] betulin [19], lupeol and β-amyrin [20], quercetin [21], and oleanolic acid [22] have already been reported in the literature. Oleanolic acid (pentacyclic triterpenoid) has been known for multiple biological potentials [23], and its antidiabetic action might occur through multiple unique mechanisms including a reduction in lipid peroxidation, inhibition in α-glucosidase activity, acceleration in glucose metabolism, secretion and sensitization of insulin [23-25]. Such antidiabetic potentials of oleanolic acid highlighted the essentiality of further exploration through clinical trials for its role in the treatment of diabetes. Likewise, lupeol (triterpene) isolated from a number of medicinal plants has shown a potential role as an anti-inflammatory, antidiabetic, and cytoprotective agent [26, 27], which by ameliorating the antioxidative profile of the body can significantly inhibit α-glucosidase activity [26]. Moreover, it has been found that lupeol-treated cell helped in impairing wound healing in diabetic patients through activation of mitogen-activated protein kinase (MAPK p38), the proliferation of fibroblasts, and improving angiogenesis [28].

Compound	Inhibition (%) at 0.5 mM	IC_{50} (µM)
(**1**) Lupeol	97.42±1.52 ab	15.87±1.16 **g**
(**2**) Lupeol acetate	95.28±1.13 a-c	29.45±0.37 **e**
(**3**) Betulin	94.13±0.97 bc	21.49±0.51 **f**
(**4**) *β*-Sitosterol	91.18±1.34 d	65.31±0.96 **a**
(**5**) *β*-Amyrin	97.62±0.78 ab	18.14±0.27 **g**
(**6**) Oleanolic acid	98.73±0.14 a	5.18±0.02 **h**
(**7**) Vanillic acid	92.85±1.12 cd	36.54±0.95 **d**
(**8**) Protocatechuic acid	94.34±0.86 bc	54.75±0.42 **b**
(**9**) Kaempferol	92.17±0.68 cd	39.12±0.19 **c**
(**10**) Quercetin	97.59±1.84 ab	23.47±1.12 **f**
Control (Acarbose)	92.23±0.14 cd	38.25±1.12 **cd**

Table 1: α-Glucosidase inhibition of various isolated compounds from Monotheca buxifolia.

Betulinic acid is also a lupane-type triterpenoid abundant in plants, and it has been found to be antidiabetic, anti-malarial, anti-tumor, anti-retroviral, anti-inflammatory, anti-cancer and anti-obesity activity. Betulinic acid has been recommended to be developed into an antidiabetic drug like metformin, which has both anticancer and antidiabetic properties [29]. Quercetin is plant-based polyphenol and has been regarded as a powerful antioxidant, anti-cancer, and anti-inflammatory agent, which is also active against neurodegenerative and cardiovascular diseases. The antidiabetic action of quercetin may occur through lipid peroxidation, inhibition in absorption of intestinal glucose, insulin secretion, improved glucose uptake and inhibition of α-glucosidase [30].

The gram-negative bacteria were the major cause of infections in the ICU. The commonest isolates were *S.aureus, Acinetobacter, Enterobacteriaceae, and Pseudomonas Aeruginosa*. The best empirical therapy should include vancomycin, Tigecycline, cefepime, and polymyxin B can be used as first-line drugs with carbapenems as second-line agents. The high frequency of multidrug resistance bacteria in ICU suggests that we need to prescribe broad-spectrum antibiotics more wisely to reduce pressure on sensitive strains. This could be beneficial for saving ICU patients and preventing the spread of resistant isolates in critical wards.

Competing Interest

The author declares that there is no conflict of interest regarding the publication of this paper.

Authors' Contribution

All authors contributed equally.

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