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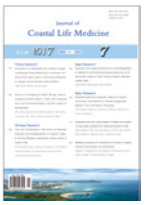

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Antioxidant and anti α-glucosidase of luteolin and luteolin 7-*O*-glucoside isolated from *Scabiosa arenaria* Forssk.

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ABSTRACT

Objective: To evaluate the antioxidant and α-glucosidase inhibitory activities of luteolin and luteolin 7-*O*-glucoside isolated from *Scabiosa arenaria* Forssk.
Methods: Antioxidant activity was measured by the 2,2-diphenyl-1-picrylhydrazyl free radical scavenging assay. The α-glucosidase inhibition was determined by colorimetric assay using the enzyme from *Aspergillus niger*, and the *p*-nitrophenyl glucopyranoside (pNPG) as substrate.
Results: The two compounds showed remarkable inhibition of α-glucosidase (IC_{50} = 0.009 2 mg/mL and 0.014 mg/mL, respectively) and they appeared to be even more potent than acarbose. A kinetic binding study indicated that the two compounds used a mechanism of non-competition to inhibit α-glucosidase. The 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay further showed that the aglycone form (luteolin) possessed the best antioxidant activity.
Conclusions: The results showed that the two flavonoids had biological activities with high potential for food and pharmacological uses.

1. Introduction

Several components derivied from natural origins with antioxidant capability have been determined as free radical or active oxygen and nitrogen fixatives. In the last decennium, a growing advantage may greatly be spotted for discovering antioxidants of natural origin, to be used in medicinal materials or foods, and the principal goal is to substitute synthetical antioxidants with those of natural ones. Natural antioxidants may delay the aging procedure in human corps and improve chronic illness like cardiovascular ailment, diabetes, obesity, arthritis, and cancer[1,2]. Diabetes mellitus is a multifactorial endocrine trouble implying disorders of protein, carbohydrate, and fat metabolism[3]. This sickness has attained epidemic level overall and about 346 million people in the whole world suffer from it and this number is assessed to double by the year 2030[4]. Of all of determined cases of diabetes, type 2 diabetes (T2D) occupied approximately 90%–95%, affecting more than 170 million people all over the world[5,6]. T2D is characterised by postprandial hyperglycemia due to resistance of cells (hepatocyte and myocyte) to the function of insulin. A long time exposure to hyperglycemic state is considered to produce reactive oxygen species continually[7]. That could modify the enzymatic activities and reduce *in vivo* antioxidant levels, apparently resulting in diabetes[8]. Therefore, plant-based compounds which are wealthy in antioxidants comprising a lot of bioactive molecules with various structures can play a key role in the remedy of diabetes.

Phenolic compounds comprising flavonoids, tocopherols, and polyphenolic components are typical natural antioxidants that may potentially afford protection versus the growth of certain oxidation-linked chronic illness[9,10]. On the other hand, phenolic components had been mentioned as glucosidase inhibitors used to decrease postprandial hyperglycemia provoked by the digestion of starch in the small intestine[11-13].

Previous researches indicated that a series of phenolic compounds were obtained from *Scabiosa arenaria* Forssk. (Dipsacaceae) (*S. arenaria*)[14-17]. Thus, the main objective of this paper was to

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evaluate the antioxidant and α-glucosidase inhibitory activities of luteolin and luteolin 7-*O*-glucoside isolated from *S. arenaria*.

2. Materials and methods

2.1. Reagents and standards

α-Glucosidase (isolated from *Aspergillus niger*), *p*-nitrophenyl-α-D-glucopyranoside (pNPG), acarbose, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and butylated hydroxytoluene (BHT) were purchased from Sigma–Aldrich (Germany).

2.2. Samples

The luteolin and the luteolin 7-*O*-glucoside used in this work were isolated from *S. arenaria* EtOAc extract and butanolic extract, respectively in our previous works.

2.3. Antioxidant activity evaluation

This activity refers to the samples capability to trap the stable radical 2,2-diphenyl-1-picryl hydrazil (DPPH) created in solution by donation of a hydrogen atom or an electron[18]. If the component has the capability to ensnare the DPPH free radical, the original blue/purple solution will be modified to yellow due to diphenyl picryl hydrazine formation. This reaction is utilized as a measurement of the samples capacity to scavenge whatever free radical. A quantity of 500 μL of every product concentration was blended by employing the similar volume of DPPH• ethanolic solution. Later, an incubation period of 30 min in away from light and at a temperature of 25 °C, absorption was read at 517 nm wave length. A concoction of 500 μL of DPPH• solution and 500 μL of ethanol was taken as a blank[19]. The reduce in absorption induced by the evaluated products was compared to that of the positive control BHT. The calculated IC_{50} values give the concentration required to snare 50% of DPPH• radicals. Results were expressed in inhibition percentage at various compounds concentrations (mg/mL). The inhibition of free radical DPPH in percentage (I%) was calculated as follows:

I% = [(A blank – A compound)/A blank] × 100

where A blank is the absorption of the control reaction (including all reagents with the exception of the test product), and A compound is the absorption of the test product.

2.4. α-Glucosidase inhibition assay

This activity was defined as before qualified by Tao *et al*.[20] with a few change as decrived by Rengasamy *et al*.[21]. The α-glucosidase reaction mixing comprising 2.5 mmol/L *p*-nitrophenyl-α-D-glucopyranoside (pNPG), 250 μL of compound (different concentrations) in dimethyl sulfoxide and 0.3 IU/mL of α-glucosidase in phosphate buffer, pH 6.9. Control trials comprising only dimethyl sulfoxide, substrate and enzyme, whereas in positive controls, acarbose substituted the pure product. Absorbance of the resulting *p*-nitrophenol (pNP) was determined at 405 nm and was considered proportional to the activity of the enzyme. The compounds of *S. arenaria* were evaluated for α-glucosidase inhibitory activity at various concentrations (1–0.0039 mg/mL). Every component was performed in triplicate. Inhibition percentages by compounds and acarbose were calculated using the following equation:

Inhibition percentage (%) = (1 – ΔDO sample/ΔDO control) × 100

where ΔDO sample = DO sample $_{(t = 15\ min)}$ – DO sample $_{(t = 0\ min)}$; ΔDO control = DO control $_{(t = 15\ min)}$ – DO control $_{(t = 0\ min)}$.

The IC_{50}, which is the concentration of the compound required to inhibit 50% of the enzyme determined for every compound. The luteolin and the luteolin 7-*O*-glucoside were compared on the basis of their IC_{50} values assessed from the dose response curves.

2.5. Kinetics essay of α-glucosidase

The luteolin and the luteolin 7-*O*-glucoside were evaluated to get the type of inhibition exerted on α-glucosidase. The reaction mixing was detailed up, with the exception, that the substrate concentration varied from 0.3 to 5.0 mmol/L, and that of the compound was preserved constant at 0.015 mg/mL. The reaction was started by the addition of enzyme, and monitored at 405 nm, at 5 min intervals in the course of 30 min. The original reaction rates were given using calibration curves constructed using different *p*-nitrophenol concentrations. The results were utilized to construct Lineweaver–Burk plots to establish the type of inhibition, Michaelis–Menten constant (K_M) and maximum velocity (Vmax) valours.

3. Results

3.1. Antioxidant activity evaluation

DPPH radical scavenging activities of the components are shown in Table 1. DPPH radical has adsorption maxima at 517 nm and a reduction in adsorption occurs when it receives an electron or a free radical species. The color of DPPH solution changes from purple to yellow depending on the radical scavenging capability of the component[22]. High activity was obtained from luteolin (IC_{50} = 0.02 mg/mL). The activity was higher compared to that of luteolin 7-*O*-glucoside (IC_{50} = 0.045 mg/mL). These two products are slightly less active than positive control BHT (IC_{50} = 0.018 mg/mL), therefore, we can deduce that the glucose takes part in the second molecule decreased the antioxidant activity.

Table 1

Antioxidant and α-glucosidase inhibition activities by luteolin and luteolin 7-*O*-glucoside.

Compounds	DPPH IC_{50} (mg/mL)	α-Glucosidase inhibition
Luteolin	0.020	0.009 2
Luteolin 7-*O*-glucoside	0.045	0.014 0
BHT	0.018	-
Acarbose	-	0.280 0

3.2. α-Glucosidase inhibition assay

According to Table 1, luteolin and luteolin 7-*O*-glucoside are more active than the reference product, acarbose (IC_{50} = 0.280 0 mg/mL). Luteolin has a very potent α-glucosidase inhibitory effect with an IC_{50} value of the order of 0.009 2 mg/mL. This product is more active than luteolin 7-*O*-glucoside (IC_{50} = 0.014 0 mg/mL), which allows us to deduce that the glycosylation of the molecule reduces the inhibitory activity of α-glucosidase. The addition of luteolin and luteolin 7-*O*-glucoside to the reaction medium resulted in a change in the Vmax velocity of hydrolysis

Table 2
The kinetic parameters of α-glucosidase in the presence and absence of luteolin and luteolin 7-*O*-glucoside.

Inhibitors (I)	Inhibition Mode	Km (mmol/L)	Vmax (DO/min)	Vmax app (DO/min)
In the absence of luteolin	-	3.33	0.333	
In the presence of luteolin	Non competitive	3.33		0.10
In the absence of luteolin-7-*O*-glucoside	-	2.17	0.333	
In the presence of luteolin-7-*O*-glucoside	Non competitive	2.17		0.15

of α-glucosidase, keeping the same Michaelinne constant (K_M). This is a noncompetitive inhibition in both cases. According to this type of inhibition, it can be suggested that these two active molecules act on the α-glucosidase without competing with its substrate which will reach the active site. The values of the kinetic parameters of α-glucosidase deduced from Figure 1 A and B in the absence and presence of these two products are summarized in Table 2. The two values of the Michaelinne (K_M) constants are comparable and range from 2.17 mmol/L for luteolin 7-*O*-glucoside to 3.33 mmol/L for luteolin. For the maximum rate of hydrolysis of α-glucosidase in the absence of inhibitor was 0.333 (DO/min). This maximal velocity decreases in the presence of luteolin (Vmax appar = 0.10 ΔDO/min) and in the presence of luteolin 7-*O*-glucoside (Vmax appar = 0.15 ΔDO/min).

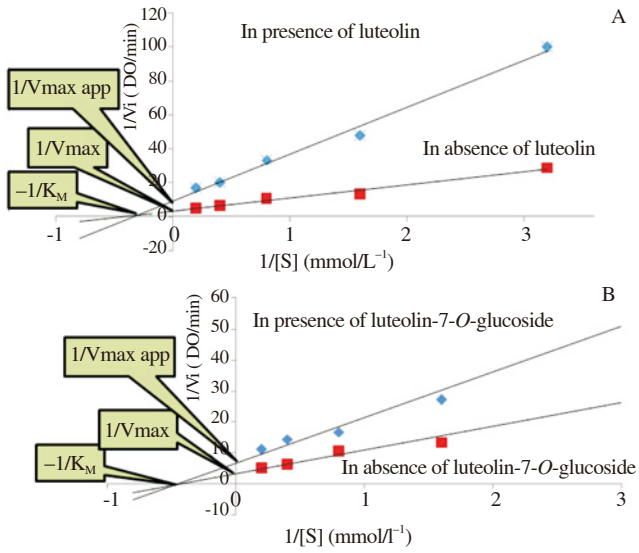

Figure 1. Double-reciprocal plot of the initial velocity (Vmax) of the hydrolysis reactions catalyzed by α-glucosidase at different substrate concentrations [S] in the presence and absence of the luteolin (A) and luteolin-7-*O*-glucoside (B). The findings indicate the average of three independent triplicate experiments.

4. Discussion

This result is confirmed by several authors such as Kumpulainen and Salonen[23] and Rice-Evans *et al.*[24] who have shown that luteolin is twice as active as the reference product, vitamin E. Igile *et al.*[25] also showed that luteolin is more active than synthetic antioxidant, BHT.

Cotelle *et al.*[26] and Cai *et al.*[27] showed that luteolin possesses very important properties in the trapping of superoxide radicals. Luteolin is also a powerful singlet oxygen sensor causing DNA alterations and chromosomal aberrations that are the main causes of cancer[28]. Luteolin inhibits *in vitro* and *in vivo* lipid peroxidation, which leads to the oxidation of polyunsaturated fatty acids and subsequent formation of free radicals causing several diseases[29-31]. This difference in previous results can be explained by the differences in the conditions of the experiment and the methods used.

At the same time, Kim *et al.*[32] have confirmed that luteolin is more active than luteolin 7-*O*-glucoside and is more active than polyglucoside (lonicerin). The IC_{50} values in the two studies are different; this may be explained by the different experimental conditions and the different enzyme sources used.

Several other researchers have demonstrated the potent inhibitory effect of aglyconic flavonoids relative to their glycosylated forms[33-39]. Thus, the decrease in enzymatic activity after glycosylation may be due to the increase in molecular size, polarity and non-planar structure of the molecule. When a hydroxyl group is substituted with a glycoside, the steric hindrance can occur, which weakens the binding interaction between flavonoids and α-glucosidase[40]. Yan *et al.*[41] also found that the type of inhibition of α-glucosidase by luteolin is uncompetitive. According to our knowledge, the type of inhibition of α-glucosidase by luteolin 7-*O*-glucoside is studied for the first time in our study.

This paper demonstrated that the two flavonoids, namely luteolin and luteolin 7-*O*-glucoside had important biological activities with interesting uses.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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