ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. 17:3 (2023) 501-508

records of natural products

Isolation of Undescribed Biflavonoid from *Potamogeton pusillus* L. and Antidiabetic Activity Properties

Hüseyin Akşit *

Erzincan Binali Yıldırım University, Faculty of Pharmacy, Analytical Chemistry Department

Erzincan, Türkiye

(Received January 05, 2023; Revised March 03, 2023; Accepted March 12, 2023)

Abstract: A phytochemical study on the *Potamogeton pusillus* L. led to the isolation and identification of a novel biflavonoid, pusillin A (**1**) with known seven compounds, including rosmarinic acid (**2**), luteolin-7-sulfate (**3**), eriodictyol-7-sulfate (**4**), chrysoeriol-7-sulfate (**5**), eriodictyol (**5**), luteolin (**7**) and chrysoeriol (**8**). Their structures were elucidated based on spectrometric analysis, including LC-Q-TOF/MS and NMR. All compounds were screened for *in vitro* antidiabetic activity using the spectrophotometric method. Sulfated flavones (**1**, **3**, **4**, and **5**) exhibited prominent α-glucosidase inhibitory activity, and compounds **2**, **6**, **7**, and **8** showed moderate inhibitory activities. All isolates were reported for the first time from the plant material.

Keywords: *Potamogeton pusillus* L.*;* biflavonoid*; α*-glucosidase; antidiabetic properties; sulfated flavones

1. Introduction

Diabetes mellitus (DM) *is* characterized by an absolute or relative deficiency in insulin secretion by pancreatic *β*-cells and is one of the world's most serious public health problems of the world. The International Diabetes Federation announced that there are approximately 415 million people, the majority of whom are diagnosed with type 2 (non-insulin-dependent) diabetes. It is estimated that the number of diabetic patients will reach 642 million in 2040 [1]. The major therapeutic strategy for the treatment of type 2 diabetes is the maintaining of blood glucose levels as normal as possible by the inhibition of carbohydrate hydrolyzing enzymes: α-glucosidase and α-amylase enzymes [2]. The *α*-glucosidase breaks down dietary oligosaccharides into glucose units causes to promoting blood glucose levels in the small intestine [3, 4]. Therefore, preventing or delaying the decomposition of oligosaccharides helps suppress blood sugar levels in type 2 diabetes patients by reducing the absorption of dietary carbohydrates [5, 6].

Today voglibose, acarbose, and miglitol, commercially available oral drugs, are used as α-glucosidase inhibitors [7]. These drugs have many side effects such as flatulence, diarrhea, and abdominal distention [8]. Because of these side effects, many patients abandon or reduce their regular drug use [9]. As a result, much effort must be expended in the pursuit of more potent and safer α-glucosidase inhibitors.

Aquatic plants (macrophytes) are primary producers and provide habitat and food to aquatic organisms and animals in the water. The macrophytes are important for livable water ecosystems and water quality due to their ability to filter excessive nutrients and hold heavy metals, sediments, and some environmental pollutants [10]. They can also help regulate the water flow of rivers and lakes and sedimentation properties [11]. In addition, it was reported that macrophytes contain a variety of secondary metabolites such as alkaloids [12], steroids [13, 14], phenolics [15, 16], and flavonoids [17-19]. These reports suggest that the macrophytes could be used as bioactive chemical sources.

* E-Mail: huseyinaksit@gmail.com

The article was published by ACG Publications
http://www.acgpubs.org/journal/records-of-natural-products May-June 2023 EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.385.2211.2628
Available online: March 22, 2023

Potamogetonaceae is the most prominent aquatic plant family in the world, containing about 100 species growing in various habitats [20]. *Potamogeton* has the most ecological importance of all aquatic plants [21]. The genus *Potamogeton* is represented by 72 species and 99 hybrids in Türkiye [22]. *Potamogeton pusillus* is known in Turkish under the name of "su kılı." The uses of *P. pusillus* have not been yet reported as food or traditional medicinal purposes. Furthermore, secondary metabolites isolated from macrophytes have become extremely important after recognizing their antioxidant [23, 24], antiproliferative [14, 17, 24, 25], antimicrobial [26, 27], and antifungal [28-30] activities. In this study, the secondary metabolites were isolated from the methanolic extract of *P. pusillus*, and *in vitro* α-glucosidase inhibitory properties were evaluated for the first time.

2. Materials and Methods

2.1. General

NMR and mass spectra were recorded on a Bruker Avance II 400 MHz and Agilent Technologies 600 MHz spectrometers, and a Shimadzu 5040 LC-Q-TOF/MS spectrometer, respectively. Separations were achieved using Sephadex LH-20 column chromatography (CC) and a semi-preparative Shimadzu Prominence instrument.

2.1. Plant Material

Potamogeton pusillus samples were collected from water canal Ekşisu, Erzincan, bearing mineral water, in June 2021. The plant materials were washed at the collection site with the canal water to remove epiphytes, sand, bugs, and shells, and finally, with deionized water in the laboratory. Plant material was taxonomically identified by Prof. Dr. Köksal Pabuçcu and, a voucher specimen was deposited in the herbarium of Erzincan Binali Yıldırım University (EBYU 11578), Erzincan, Türkiye.

2.2. Extraction and Isolation

Fresh biomass (700 g) was extracted with 70% methanol (5 L) at room temperature for 24 hours. The azeotropic mixture was concentrated under a vacuum to give a dark yellow slurry crude extract (62.5 g, 8.85% yield) and then the crude extract was solved in 4 L of water, and insoluble parts were removed by filtration. The filtrate was partitioned with *n*-hexane, ethyl acetate, and *n*-butanol, respectively. Each organic layer was concentrated to dryness to give 2.1 g of *n*-hexane, 12.9 g of ethyl acetate, and 22.1 g of *n*-butanol fractions. The HPLC chromatograms showed that the ethyl acetate and *n*-butanol fractions contained the highest concentrations of detectable compounds (Figure S1).

Semi-preparative HPLC was used to purify these compounds. The separations were performed on EMR C18 (250 mm × 20 mm, ID, 10 µm) column at ambient temperature. Deionized water (A) and methanol (B) were used as mobile phases. The mobile phase program was as follows: 1 min 100:0 (A: B), 29 min linear gradient from 100:0 to 0:100, and finally 5 min 0:100. Total run time was 35 minutes at a flow rate of 8 mL/min. The extracts were loaded to the column in 100 mg per injection using a 2 ml sample loop. The elution was monitored at 280 and 340 nm, and peak collection was done automatically according to absorbance at 280 nm. The separation process was repeated ten times for each extract.

The prep-HPLC elution and collection profile of EtOAc and ButOH extracts were given in Figure S1. Each collected peak was further purified over a manually packed Sephadex LH-20 column using methanol as a mobile phase. The eluants collected from Sephadex LH-20 were applied to TLC analysis. The main spots were combined according to the TLC basis and evaporated to dryness. The individual, purified metabolites were eluted from the analytical C18 (150 mm x 4.6 mm, ID, 5 µm) column and monitored at 280 nm to check purity with the same mobile phase program used in prep-separation with the 1 mL/min flow rate (Figure S2).

2.3 In vitro α-Glucosidase Inhibitory Assay

The isolated metabolites were dissolved in DMSO: phosphate buffer (0.1 M pH, 6.8) in a 1:20 ratio. The α-glucosidase inhibitory activity of isolated molecules (**1-8**) was carried out on a 96-well-based spectrophotometric method [31] with slight modification. Briefly, 50 µL buffer solution, 10 µL enzyme solution (0.1 U/mL), and 20 µL isolate solution (1, 2, 5, 10, 25, 50, 75, and 100 µM) were

mixed and incubated at 37 °C for 5 min. Then, 20 µL of *p*-nitrophenyl-*α*-glucoside (3 mM) was added, and the final solution was incubated at 37 °C for 30 min. After incubation, 50 µL Na_2CO_3 (0.1 M) was added to terminate the reaction, and the absorbance of the final mixture was measured using an Elisa reader at 405 nm. Acarbose was used as a positive control. The results were expressed as IC_{50} in µM.

3. Results and Discussion

3.1. Structure Elucidation

There are currently no published data on *P. pusillus* in the secondary metabolite profile. The preparative separations led to the isolation and characterization of a novel biflavonoid pusillin A (**1**), rosmarinic acid (**2**) (7.2 mg) [32], luteolin-7-sulfate (**3**) (5.4 mg) [33], eriodictyol-7-sulfate (**4**) (6.9 mg) [34], chrysoeriol-7-sulfate (**5**) (8.0 mg) [35], luteolin (**6**) (8.3 mg) [33] from the ButOH fraction and eriodictyol (**7**) (6.8 mg) [36] and chrysoeriol (**8**) (9.8 mg) [37] from EtOAc fraction of *P. pusillus* (Figure 1). The NMR data of all known compounds (**2**-**8**) were identical to previously published studies. Compounds **1**-**8** were for the first time isolated from *P. pusillus*, which would enrich our knowledge about phytochemical constituents of the genus.

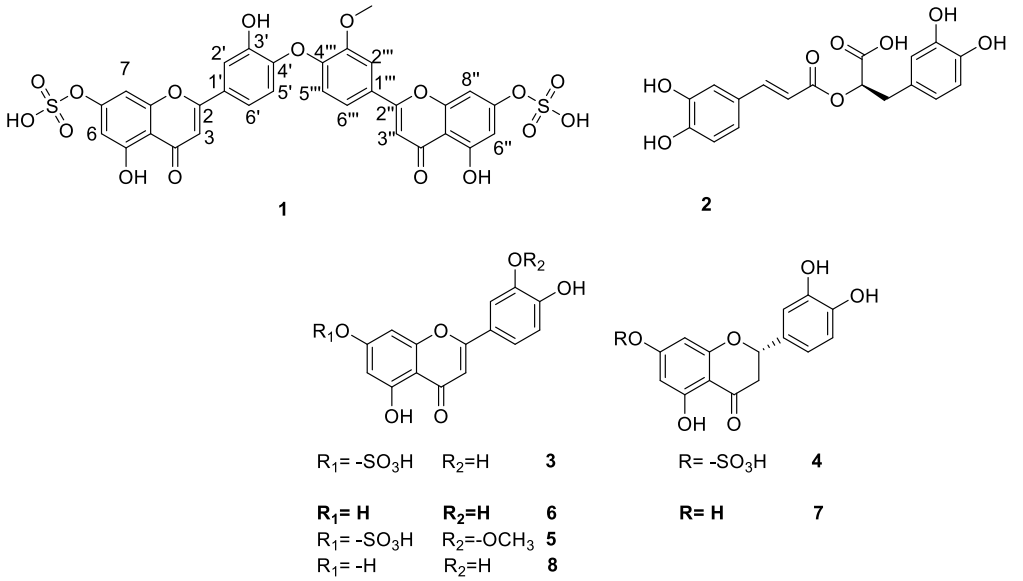

Figure 1. Chemical structures of isolated molecules (**1**-**8**).

Compound **1** (pusillin A) was obtained as a yellowish amorphous solid. Its molecular formula was established as $C_{31}H_{20}O_{17}S_2$ by the LC-Q-TOF/MS data *m/z* 729.0224 $[M+H]^+$, (calculated 729.0222 for $C_{31}H_{21}O_{17}S_2$). The IR absorption bands at 3374 cm^{-1} and 1644 cm^{-1} indicated the presence of the hydroxyl group and olefinic group, respectively. The bands revealed at 1260-1200 cm^{-1} especially attributed more information about the occurrence of sulfate groups [38]. When compared to the carbon and proton values in both luteolin (**6**) and chrysoeriol (**8**), the downfield carbon shifts for C6/C6" and C8/C8", as well as the notable downfield shifts of H6/H6" and H8/H8", strongly indicate the presence of an electron-withdrawing sulfate group at C7 and C7" of compound **1** [33]. The ^{1}H-NMR spectrum revealed the signals of two distinct ABX systems having close chemical shifts which separated each other COSY interactions corresponding to 7-sulfated chrysoeriol and luteolin moieties of **1**, [7.53 (dd, *J*=8.4, 2.2 Hz), 6.88 (d, *J*=8.4 Hz), and 7.46 (d, *J*=2.2 Hz)] and [7.40-7.29 (m), 6.77 (d, *J*=8.2 Hz, 1H)], respectively. Additionally, two AX systems corresponding to H6-H8 interactions of both moieties resonated at 6.98 (d, *J*=2.1 Hz), 6.93 (d, *J*=2.1 Hz, 1H), and the other signals of AX systems resonated as a multiplet at 6.52-6.50 ppm according to deep COSY interpretations. H-H backbone of compound 1 was established from H6 (6.52-6.50) -H8 (6.98), H2´(7.40-7.29)-H6´(7.40-7.29), H5´(6.77)-H6´(7.40-7.29), H6´(6.52-6.50)-H5´(6.98)/H2´(7,40-7.29), H6´´(6.52-6.50)-H8´´ (6.93), H2´´´(7.46)-H6´´´(7.53), H5´´´ (6.88)-H6´´´ (7.53) and H6´´´(7.53)-H5´´´ (6.88)/H2´´´(7.46) COSY interactions supporting the structure of **1**. H3 signals were observed at 6.66 and 6.85 ppm as singlets. The signal at 3.98 (s, 3H) was

linked to methoxy protons. The ^{13}C NMR spectra showed 31 carbon signals carbons containing 18 quaternary, 12 methines, and 1 methyl correspondingly to HSQC spectra contain H3 (6.85)-C3 (102.93), H6 (6.52)-C6 (102.53), H8 (6.98)-C8 (98,35), H2′ (7.40)-C2′ (113.83), H5′ (6.77)-C5′ (117.04), H6′ (7.40)-C6′ (119.52), H3′′ (6.66)-C3′′ (102.70), H6′′ (6.52)-C6′′ (102.52), H8′′ (6.93)-C8′′ (98,12), H2′′′ (7.46)-C2′′′ (110.32), H5′′′ (6.88)-C5′′′ (116.61), H6′′′(7.53)-C6′′′ (121.41), and 3.83/56.20 correlations. All ^{13}C assignments of 1 were similar to luteolin-7-sulfate (**3**) and chrysoeriol-7-sulfate (**5**) except C4′ and C4′′′. About 4 ppm up shifting of C4′ and C4′′′ prove that the esterification pattern is C4′-C4′′′. The HMBC correlation H5′-C4′′′ was the key correlation (Figure 2) of the connecting site. Thus, **1** is characterized as *O*-linked **3** and **5** at the C4′-C4′′′ position. The other HMBC correlations are given in Table 1. Additionally, two molecular ion signals in MS spectra of **1** at m/z 367.0115 and 381.0225 corresponding the protonated chrysoeriol-7-sulfate and luteolin-7-sulfate were proved esterification. Lee et al. (2015) isolated esterified flavone at the 3′-4′′′ position from *Quintinia acutifolia* leaves [39]. Moreover, acidic hydrolysis [40] of **1** gave luteolin and chrysoeriol aglycons which were corrected by TLC. R_f values were calculated as 0.3 for **1**, 0.45 for luteolin (**6**), and 0.52 for chrysoeriol (**8**) in the 95:5 EtOAc: MeOH system. TLC analysis showed an *O*-linkage pattern of (**1**) and hydrolysis of sulfate groups in acidic media.

Table 1. ^{1}H and ^{13}C-NMR assignments of **1** (600 MHz, in DMSO-d_6)

No.	^{1}H	^{13}C	HMBC	No.	^{1}H	^{13}C	HMBC
2	-	165.12		2"	-	165.41	
3	6.85 (s)	102.93	C2, C1', C4	3"	6.66 (s)	102.70	C2", C1'", C4"
4	-	182.23		4"	-	182.34	
5	-	160.96		5"	-	160.94	
6	6.52-6.50 (m)	102.53	C5, C7, C10	6"	6.52-6.50 (m)	102.52	C5", C7", C10"
7	-	159.77		7"	-	159.72	
8	6.98 (d, 2.1)	98.35	C7, C9, C6,	8"	6.93 (d, 2.2)	98.12	C7", C6", C10"
9	-	156.79		9"	-	156.73	
10	-	106.14		10"	-	106.08	
1'	-	119.62		1'"	-	119.42	
2'	7.40 – 7.29 (m)	113.83	C1', C4′	2'"	7.46 (d, 2.2)	110.32	C1'", C5'"
3'	-	147.46		3'"	-	149.06	
4′	-	154.47		4'"	-	153.66	
5'	6.77 (d, 9.0)	117.04	C4′, C3', C4'"	5'"	6.88 (d, 8.4)	116.61	C4'", C5'", C4′
6'	7.40 – 7.29 (m)	119.52	C1', C5'	6'"	7.53 (dd, 8.4, 2.2)	121.41	C1'", C4'"
				-OCH_3	3.83 (s)	56.20	C3'"

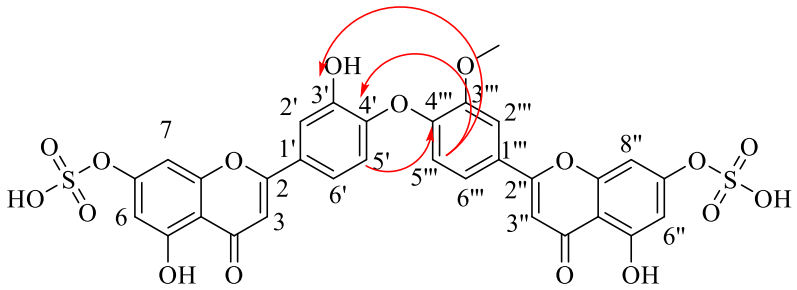

Figure 2. Key HMBC correlation of Compound **1**

3.2. α-Glucosidase Inhibitory properties

The *α*-glucosidase enzyme inhibition patterns of isolated compounds are given in Table 2. To date, no report exists that evaluated *α*-glucosidase inhibitory activities of pusillin A (**1**), luteolin-7-sulfate (**3**), eriodictyol-7-sulfate (**4**), and chrysoeriol-7-sulfate (**5**). Their activities were first reported in the current study. According to the results, the inhibition capacity of non-sulfated aglycons was found to have good activity, while sulfated ones have excellent activity ranging from 1.37 to 18.88 µM. A similar activity trend was reported by Alhassan et al. (2019) [41]. Alhasan et al. reported the sulfation of the hydroxy

group drastically increase the activity (IC_{50} in µM is 5.75 for 5,7-dihydroxyflavone-*O*-8-sulfate and is >500 for 5,7,8-trihydroxy flavone). The current study supported that the presence of sulfate was essential for inhibiting the investigated enzyme with various binding orientations on the active site of the enzyme.

The activity of **1** was found to be less than that of the two sulfated compounds (**3** and **5**) to which it was esterified. The reduction in activity can be ascribed to the extension of the molecule and the loss of a hydroxy group during esterification.

Table 2. *In vitro α*-glucosidase inhibitory activity of isolated compounds (**1-8**)

Compounds	IC_{50} (µM)
1	18.88±1.02
2	8.43±0.08
3	5.02±0.22
4	4.03±0.11
5	1.37±0.05
6	25.48±1.92
7	12.89±0.89
8	27.89±0.98
Acarbose	22.07±1.72

Several studies reported the occurrence of rosmarinic acid (**2**) in the Potamogetonaceae family [42]. **2** has a remarkable α-glucosidase inhibitory effect compared to positive control acarbose in different studies [43-46] supporting the current study.

There was limited data in the literature about *in vitro* α-glucosidase activity of sulfated flavone derivatives. The isolated compounds including rosmarinic acid (**2**) [47], luteolin (**6**) [48], eriodictyol (**7**) [47], and chrysoeriol (**8**) [49] were previously reported to have α-glucosidase inhibitory activity *in vitro* and/or *in silico*. Wang et al. (2010) reported that the free 3-position from the hydroxyl group was a critical function of flavonoids for the inhibition of the α-glucosidase enzyme [50]. In addition, the inhibitory activity was enhanced significantly with the increasing number of substitutions of the B-ring. When compared to the chemical structure of **6** and **8**, which has the same hydroxylation pattern, the methyl substitution did not significantly contribute to the activity consistent with the literature [51]. The fact that **6** and **8** possessing the 3′,4′- disubstituted exhibited good inhibitory effects on α-glucosidase was fully consistent with the literature [50]. When comparing the activity of **6** and **7** with the same substitution pattern, it was observed that 7 with a flavanone skeleton had higher activity. In contrast to our findings, Tadera et. al. (2006) showed that the 4'-hydroxylated flavone (apigenin) has greater activity than 4'-hydroxylated flavanone (naringenin) [52]. In addition, Zeng et. al. (2016) supported our findings as 3',4'-dihydroxylated flavanone (eriodictyol) has more activity than 3',4'-dihydroxylated flavone (luteolin) [53].

Chrysoeriol-7-sulfate (**5**) was the most active inhibitor among the tested molecules. The occurrence of **5** in the seagrass *Zostera marin*a [19] was previously reported. It was determined that the replacement of hydroxyl with sulfate group on the flavone skeleton drastically decreased antioxidant properties [54]. In contrast, our findings depicted that the sulfated flavones were found to be most active in inhibiting α-glucosidase compared to non-sulfated ones. To our knowledge, the α-glucosidase activity of 5 was not reported previously. Moreover, the enzyme inhibition activities of sulfated flavones, such as aldose reductase [55] and AChE [56] were previously reported.

Sulfated flavonoids are usually described in plants found in slurry or near aquatic areas. It is assumed that there was a strong correlation between plants growing rich in mineral salts and the biosynthesis of sulfated flavonoids for ecological adaptation to the environment. The production of sulfated flavonoids and other sulfated secondary metabolites from 3′-phosphoadenosine-5′-phosphosulphate and specific precursors can catalyze by the cytosolic sulfotransferases [57].

Sulfation of flavonoids can enhance the interaction with many biological targets by improving water solubility and the negative charge. Thus, it was suggested that the sulfated flavonoids can be a promising option in discovering novel medications for the treatment of type 2 diabetes. Further research

using more sulfated naturally occurring or semi-synthetic sulfated flavone derivatives is needed to understand the structure-activity relationships of sulfated flavonoids as α-glucosidase enzyme inhibitors.

Acknowledgments

The author special thanks to Prof. Dr. Köksal Pabuçcu for the authentication of macrophyte samples.

Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

ORCID
Hüseyin Akşit: 0000-0002-1509-851X

References

[1] A. Salsali and M. Nathan (2006). A review of types 1 and 2 diabetes mellitus and their treatment with insulin, *Am. J. Ther.* **13**, 349-361.
[2] N. Cheng, W.-B. Yi, Q.-Q. Wang, S.-M. Peng and X.-Q. Zou (2014). Synthesis and α-glucosidase inhibitory activity of chrysin, diosmetin, apigenin, and luteolin derivatives, *Chin. Chem. Let.* **25**, 1094-1098.
[3] J. Xiao and P. Hogger (2015). Dietary polyphenols and type 2 diabetes: current insights and future perspectives, *Curr. Med. Chem.* **22**, 23-38.
[4] H. Özbek, H. Yuca, S. Gözcü, B. Dursunoğlu, N. Özenver, Z. Güvenalp, C. Kazaz, M. Önal and L. Ö. Demirezer (2019). Phenolic compounds from *Cotinus coggygria* Scop. with alpha glucosidase inhibition, *FABAD J. Pharm. Sci.* **44**, 127-132.
[5] S. R. Joshi, E. Standl, N. Tong, P. Shah, S. Kalra and R. Rathod (2015). Therapeutic potential of α-glucosidase inhibitors in type 2 diabetes mellitus: an evidence-based review, *Exp. Op. Pharmacother.* **16**, 1959-1981.
[6] S. Karakaya, S. Gözcü, Z. Güvenalp, H. Özbek, H. Yuca, B. Dursunoğlu, C. Kazaz and C. S. Kılıç (2018). The α-amylase and α-glucosidase inhibitory activities of the dichloromethane extracts and constituents of Ferulago bracteata roots, *Pharm. Biol.* **56**, 18-24.
[7] R. P. Dash, R. J. Babu and N. R. Srinivas (2018). Reappraisal and perspectives of clinical drug–drug interaction potential of α-glucosidase inhibitors such as acarbose, voglibose and miglitol in the treatment of type 2 diabetes mellitus, *Xenobiotica* **48**, 89-108.
[8] K. Aoki, T. Muraoka, Y. Ito, Y. Togashi and Y. Terauchi (2010). Comparison of adverse gastrointestinal effects of acarbose and miglitol in healthy men: a crossover study, *Intern. Med.* **49**, 1085-1087.
[9] L. Zhang, Q. Chen, L. Li, J. S. Kwong, P. Jia, P. Zhao, W. Wang, X. Zhou, M. Zhang and X. Sun (2016). Alpha-glucosidase inhibitors and hepatotoxicity in type 2 diabetes: a systematic review and meta-analysis, *Sci. Rep.* **6**, 1-8.
[10] M. V. Monferrán, M. L. Pignata and D. A. Wunderlin (2012). Enhanced phytoextraction of chromium by the aquatic macrophyte Potamogeton pusillus in presence of copper, *Environ. Poll.* **161**, 15-22.
[11] L.-H. Kim, E. Choi and M. K. Stenstrom (2003). Sediment characteristics, phosphorus types and phosphorus release rates between river and lake sediments, *Chemosphere* **50**, 53-61.
[12] M. Ostrofsky and E. Zettler (1986). Chemical defences in aquatic plants, *J. Ecol.* **74**, 279-287.
[13] R. Malathy and S. A. Stanley (2015). Studies on the potential therapeutic effects on the aquatic macrophytes namely Cabomba aquatica, Ceratophyllum demersum and Hygrophila corymbosa, *J. Chem. Pharm. Res.* **7**, 479-483.
[14] M. Kendel, G. Wielgosz-Collin, S. Bertrand, C. Roussakis, N. Bourgougnon and G. Bedoux (2015). Lipid composition, fatty acids and sterols in the seaweeds Ulva armoricana, and Solieria chordalis from Brittany (France): An analysis from nutritional, chemotaxonomic, and antiproliferative activity perspectives, *Mar. Drugs* **13**, 5606-5628.
[15] D. F. Spencer and G. G. Ksander (1999). Phenolic acids and nutrient content for aquatic macrophytes from Fall River, California, *J. Freshw. Ecol.* **14**, 197-209.

[16] G. Mayora, B. Schneider and M. F. Gutierrez (2022). Phenolic content of aquatic macrophytes of the middle paraná river Floodplain, *Wetlands* **42**, 1-10.

[17] J. r. Conrad, B. Förster-Fromme, M.-A. Constantin, V. Ondrus, S. Mika, F. Mert-Balci, I. Klaiber, J. Pfannstiel, W. Möller and H. Rösner (2009). Flavonoid glucuronides and a chromone from the aquatic macrophyte *Stratiotes aloides*, *J. Nat. Prod.* **72**, 835-840.

[18] A. Y. Ugya, T. S. Imam and J. Ma (2019). Mini-review on the efficacy of aquatic macrophytes as mosquito larvicide, *J. Appl.Bot. Food Qual.* **92**, 320-326.

[19] K. H. Enerstvedt, M. Jordheim and Ø. M. Andersen (2016). Isolation and identification of flavonoids found in Zostera marina collected in Norwegian coastal waters, *Am. J. Plant Sci*. **7**, 1163-1172.

[20] M. Kothari (2001). A revision of Family Potamogetonaceae of India, *Nelumbo* **43**, 151-194.

[21] R. R. Haynes (1985). A revision of the clasping-leaved Potamogeton (Potamogetonaceae), *SIDA, Contr. Bot.* **11**, 173-188.

[22] Z. Kaplan and J. Fehrer (2013). Molecular identification of hybrids from a former hot spot of Potamogeton hybrid diversity, *Aquat. Bot.* **105**, 34-40.

[23] L. Cavas and K. Yurdakoc (2005). A comparative study: assessment of the antioxidant system in the invasive green alga *Caulerpa racemosa* and some macrophytes from the Mediterranean, *Ecology* **321**, 35-41.

[24] T.-T. Chai, K.-F. Ooh, Y. Quah and F.-C. Wong (2015). Edible freshwater macrophytes: a source of anticancer and antioxidative natural products—a mini-review, *Phytochem. Rev.* **14**, 443-457.

[25] A. Dellai, M. Deghrigue and A. Bouraoui (2013). Evaluation of the antiproliferative activity of methanol extract and its fractions from the mediterranean seaweed, *Halurus equisetifolius*, *Int. J. Pharm. Pharm. Sci*, **5**, 148-152.

[26] M. N. Manvar (2018). Antibacterial activity of leaves and flowers of I*pomoea aquatica* forsk.(Convolvulacea), *Asian J. Pharm. Res.* **8**, 94-98.

[27] J. H. Chandra and M. Shamli (2015). Antibacterial, antioxidant and in silico study of Ipomoea aquatica Forsk, *J. Pure App. Microbiol.* **9**, 1371-1376.

[28] E. Ballesteros, D. Martin and M. J. Uriz (1992). Biological activity of extracts from some Mediterranean macrophytes, *Bot. Mar.* **35**, 481-485.

[29] G. D. de Alkimin, J. Santos, A. M. Soares and B. Nunes (2020). Ecotoxicological effects of the azole antifungal agent clotrimazole on the macrophyte species Lemna minor and Lemna gibba, *Comp. Biochem. Physiol. Part - C: Toxicol. Pharmacol.* **237**, 108835.

[30] A. M. Haroon (2006). Effect of some macrophytes extracts on growth of *Aspergillus parasiticus*, *Egyptian J. Aquatic Res*, **32**, 301-313.

[31] B. Asghari, P. Salehi, M. M. Farimani and S. N. Ebrahimi (2015). α-Glucosidase Inhibitors from Fruits of Rosa canina L., *Rec. of Nat. Prod*. **9**, 276-283.

[32] H. Aksit, S. M. Çelik, Ö. Sen, R. Erenler, I. Demirtas, İ. Telci and M. Elmastas (2014). Complete isolation and characterization of polar portion of *Mentha dumetorum* water extract, *Rec. Nat. Prod*. **8**, 277-282.

[33] S. W. Lee, J. H. Kim, H. Song, J. K. Seok, S. S. Hong and Y. C. Boo (2019). Luteolin 7-Sulfate attenuates melanin synthesis through inhibition of CREB-and MITF-mediated tyrosinase expression, *Antioxidants* **8**, 87.

[34] C. Noleto-Dias, C. Harflett, M. H. Beale and J. L. Ward (2020). Sulfated flavanones and dihydroflavonols from willow, *Phytochem. Lett.* **35**, 88-93.

[35] K. H. Enerstvedt, M. Jordheim and Ø. M. Andersen (2016). Isolation and identification of flavonoids found in Zostera marina collected in Norwegian coastal waters, *American J. Plant Sci.*, **7**, 1163-1172.

[36] J. A. Cortes-Morales, A. Olmedo-Juárez, M. González-Cortazar, A. Zamilpa, M. A. López-Arellano, E. A. Ble-González, D. Tapia-Maruri, G. Flores-Franco and D. O. Salinas-Sánchez (2022). In vitro ovicidal activity of Brongniartia montalvoana against small ruminant gastrointestinal nematodes, *Exp. Parasitol.* **240**, 108336.

[37] Y. Park, B. H. Moon, H. Yang, Y. Lee, E. Lee and Y. Lim (2007). Complete assignments of NMR data of 13 hydroxymethoxyflavones, *Magn. Res. Chem.* **45**, 1072-1075.

[38] F. Cabassi, B. Casu and A. S. Perlin (1978). Infrared absorption and Raman scattering of sulfate groups of heparin and related glycosaminoglycans in aqueous solution, *Carbohydr. Res.* **63**, 1-11.

[39] J.-S. Lee, H.-J. Choi, M.-J. Kim and J.-S. Park (2015). *In vitro* Anti-cancer effect of wellness-compound (Ochnaflavone), *J. Digit Conver.* **13**, 337-344.

[40] T. T. Minh, N. T. V. Thanh and V. D. Hoang (2022). A New Iridoid Glucoside from the Stems of Myoporum bontioides (Sieb. et Zucc.) A. Gray, *Rec. Nat. Prod.* **16**, 426-432.

[41] A. M. Alhassan, Q. U. Ahmed, J. Latip and S. A. A. Shah (2019). A new sulphated flavone and other phytoconstituents from the leaves of Tetracera indica Merr. and their alpha-glucosidase inhibitory activity, *Nat. Prod. Res.* **33**, 1-8.

[42] M. F. Mahmoud, S. Rezq, A. E. Alsemeh, M. A. Abdelfattah, A. M. El-Shazly, R. Daoud, M. A. El Raey and M. Sobeh (2021). *Potamogeton Perfoliatus* L. Extract attenuates neuroinflammation and neuropathic pain in sciatic nerve chronic constriction injury-induced peripheral neuropathy in rats, *Front. Pharmacol.* **12**, 799444-799452.

[43] M. B. Chanu, R. K. Labala, Y. Sheikh, J. C. Borah, S. K. Ghosh, D. Sahoo, O. J. Singh, A. Shakya and B. Thongam (2018). Bioassay guided isolation of alpha-glucosidase inhibitory compound, in vivo postprandial anti hyperglycemia and docking study of the isolated compound from the leaves of the methanolic extract of *Quercus serrata*, *Biosci. Biotech. Res. Commun.* **11**, 647-657.

[44] L. Lin, Y. Dong, H. Zhao, L. Wen, B. Yang and M. Zhao (2011). Comparative evaluation of rosmarinic acid, methyl rosmarinate and pedalitin isolated from *Rabdosia serra* (MAXIM.) HARA as inhibitors of tyrosinase and α-glucosidase, *Food Chem.* **129**, 884-889.

[45] F. Zhu, J. Wang, H. Takano, Z. Xu, H. Nishiwaki, L. Yonekura, R. Yang and H. Tamura (2019). Rosmarinic acid and its ester derivatives for enhancing antibacterial, α-glucosidase inhibitory, and lipid accumulation suppression activities, *J. Food Biochem.* **43**, e12719.

[46] Y. L. Ngo and L. S. Chua (2018). Anti-diabetic activity of rosmarinic acid rich fractions from *Orthosiphon stamineus*, *Curr. Enz. Inhibit.* **14**, 97-103.

[47] M. Tolmie, M. J. Bester and Z. Apostolides (2021). Inhibition of α‐glucosidase and α‐amylase by herbal compounds for the treatment of type 2 diabetes: a validation of in silico reverse docking with in vitro enzyme assays, *J. Diabetes* **13**, 779-791.

[48] R. Sangeetha (2019). Luteolin in the management of type 2 diabetes mellitus, *Curr. Res. Nutr. Food Sci. J.* **7**, 393-398.

[49] B. J. Oso and I. F. Olaoye (2020). Antiglycaemic potentials and molecular docking studies of the extracts of Cassia alata L, *Beni-Suef Uni. J. Basic Appl. Sci.* **9**, 1-8.

[50] H. Wang, Y.-J. Du and H.-C. Song (2010). α-Glucosidase and α-amylase inhibitory activities of guava leaves, *Food Chem.* **123**, 6-13.

[51] C. Zhang, J. Yu, Q. Tu, F. Yan, Z. Hu, Y. Zhang and C. Song (2022). Antioxidant Capacities and Enzymatic Inhibitory Effects of Different Solvent Fractions and Major Flavones from Celery Seeds Produced in Different Geographic Areas in China, *Antioxidants* **11**, 1542.

[52] K. Tadera, Y. Minami, K. Takamatsu and T. Matsuoka (2006). Inhibition of *α*-glucosidase and *α*-amylase by flavonoids, *J. Nutr. Sci. Vitaminol.* **52**, 149-153.

[53] B. Zeng, K. Chen, P. Du, S. S. Wang, B. Ren, Y. L. Ren, H. S. Yan, Y. Liang and F. H. Wu (2016). Phenolic Compounds from *Clinopodium chinense* (Benth.) O. Kuntze and Their inhibitory effects on *α*-glucosidase and vascular endothelial cells injury, *Chem. Biodiver.* **13**, 596-601.

[54] O. Beck, G. Cartier, G. David, M. Dijoux-Franca and A. Mariotte (2003). Antioxidant flavonoids and phenolic acids from leaves of *Leea guinensis* G. Don (Leeaceae), *Phytother. Res*, **17**, 345-347.

[55] H. Haraguchi, I. Ohmi, S. Sakai, A. Fukuda, Y. Toihara, T. Fujimoto, N. Okamura and A. Yagi (1996). Effect of *Polygonum hydropiper* sulfated flavonoids on lens aldose reductase and related enzymes, *J. Nat. Prod.* **59**, 443-445.

[56] V. Cavallaro, A. Estévez Braun, A. Gutiérrez Ravelo and A. P. Murray. In *17th International Electronic Conference on Synthetic Organic Chemistry*: Basel, Switzerland, **2013**.

[57] Y. C. Teles, M. S. R. Souza and M. d. F. V. d. Souza (2018). Sulphated flavonoids: biosynthesis, structures, and biological activities, *Molecules* **23**, 480.