SHORT REPORT

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Secondary Metabolites from *Thraustochytrium aureum* and Their Biological Activity

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Abstract: Phytochemical investigation of *Thraustochytrium aureum* led to the isolation of eleven compounds including ergosterol **(1)**, 7-dehydroporiferasterol **(2)**, (22*E*,24*R*)-ethylcholesta-7,22-dien-3*β*,5*α*,6*β*-triol (**3**), poriferasterol glucoside (**4**), perlolyrine (**5**), pyrrolezanthine-6-methyl ether (**6**), 3-(3-aminopropyl)-6-[(4-hydroxyphenyl)methyl]-2,5-piperazinedione (**7**), 5-methyluracil (**8**), 1*H*-indole-3-carboxylic acid (**9**), adenosine (**10**), and *p*-hydroxybenzoic acid (**11**). Screening for α-glucosidase inhibitory, antimicrobial and cytotoxic activities of the extracts and isolated compounds were carried out. The *n*-hexane extract was the most active, which showed strong α-glucosidase inhibitory activity with IC_{50} of 48.22 µg/mL, inhibition against *E. faecalis*, *S. aureus* and *C. albican*s microorganism strains with MIC values of 128, 64 and 64 µg/mL, respectively. Compound **6** have shown to be the most active among isolated compounds, which inhibited α-glucosidase with IC_{50} value of 7.96 µg/mL; *E. faecalis* and *C. albican*s microorganism strains with MIC values of 64 and 16 µg/mL and inhibited the growth of A549, HepG2, MCF7 and LNCaP cancer cell lines with IC_{50} values of 62.26, 41.03, 57.21 and 43.23 µg/mL, respectively.

Keywords: *Thraustochytrium aureum;* alkaloid; steroid; microalga, *α*-glucosidase.

1. Microalgal Source

The heterotrophic microalga *Thraustochytrium aureum* Goldstein 1963 (BT6 strain) used in this study, was isolated from the mangrove forest at Mui Ne, Phan Thiet city, Binh Thuan province, Vietnam (latitude 10º56'00" N, longitude 108º17'00" E) in 2010, which was deposited at Department of Algal Biotechnology, Institute of Biotechnology belonging to Vietnam Academy of Science and Technology, Vietnam.

2. Previous Studies

Microalgae have been known as the largest and most diverse group of photosynthetic organisms in freshwater and marine systems. This group are promising sources for the sustainable production of many bioactive compounds including fatty acids, phycobiliproteins, chlorophylls, carotenoids, and vitamins, which

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are widely used in pharmaceuticals, cosmetics, and food additives. The natural bioactive compounds from microalgae attract interests of many researchers due to their potential therapeutic activities such as antioxidant, antiviral, antibacterial, antifungal, anti-inflammatory, antitumor, and antimalarial effects. Surprisingly, natural products from microalgae are almost unexplored compared to those isolated from terrestrial plants although microalgae have many advantages over plants. For instance, microalgae can grow rapidly, are easier to cultivate, and do not compete directly with crops for agricultural land. These advantages highlight the importance of developing microalgae for the production of medicinally important natural products as well as for pharmaceuticals discovery and development, among other bio products [1].

Thraustochytrium aureum is one of thraustochytrids, which have potential on commercial value due to their ability to produce a large amount of lipids, especially DHA, and their heterotrophic nature, which is suitable for fermentation technology [2-3]. *T. aureum* produced DHA in a level about 50% of total lipids. All previous researches on *T. aureum* have focused on the biomass production, total lipids and DHA content [4-7]. Hitherto, there is no report in the secondary metabolites and biological activity of this species.

3. Present Study

In continuation of our research on bioactive compounds from Vietnamese marine microalgae, we focus our attention to heterotrophic microalga *Thraustochytrium aureum* because of biological activities of its extracts. Preliminary screening for the α-glucosidase inhibitory, antimicrobial and cytotoxic activities of *n*-hexane, EtOAc and aqueous extracts from *T. aureum* were carried out. *n*-hexane extract showed a good α-glucosidase inhibitory potential with IC_{50} value of 48.22 μg/mL (IC_{50} value of acarbose is 164.08 μg/mL) and inhibited *E. faecalis, S. aureus* and *C. albicans* strain with MIC values of 128, 32 and 256 μg/mL, respectively. The EtOAc extract exbibited moderate inhibition of *E. faecalis* and *C. albicans* with MIC values of 256, 128 and 128, 64 μg/mL, respectively while aqueous extract show no biological activities. No extract showed significant cytotoxicity againts any of A549, HepG2, MCF7 and LNCaP cancer cell lines. The *n*-hexane and EtOAc extracts which contain potentially bioactive compounds were further investigated for their phytochemical constituents.

Microalgal Culture: *Thraustochytrium aureum* (BT6 strain) was kept on GPY medium (glucose 0.2 %, polypeptide 0.1%, yeast extract 0.05 %, agar 1.5%, 17.5 g/L artificial sea water (ASW). The primary seed culture was carried out by taking colonies of BT6 strain culture in a petri dish in 1000 mL Erlenmeyer flask with 350 mL Bajpai liquid medium which contained 10 g/L glucose, 2.5 g/L yeast extract, and 0.5% NaCl content.

The primary seed culture flasks were incubated for 96 h at 28°C with shaking at 200 rpm. Fermentation was carried out using a 30 L fermentor, and a Bajpai medium that contained 5% glucose, 1% yeast extract, and 0.5% NaCl. The inoculum size was 3% of the total liquid volume in fermentor. Temperature was kept at 32°C-37°C. Dissolved oxygen was maintained above 10% by manually increasing the stirring speed from 250 rpm to a maximum of 450 rpm. The aeration rate was always 0.5 volume air/(vol. medium)/min after filtering through a 0.2 mm filter. Instead of antifoam, 50 mL of vegetable oil with the trade name Neptune® Gold (made by Cai Lan Vegetable Oil Limited Company, Ha Long City, Quang Ninh Province) was added to the 30 L fermentor. BT6 strain biomass was harvested after 120 h of fermentation. Under adverse culture conditions in terms of nutrient medium (5% glucose, 1% industrial yeast and 0.5% salinity) and room temperature (from 32°C -37°C) in 30 L fermentor (handmade), the growth of *T. aureum* BT6 species accounted the maximum after 120 h of culture with dry biomass, lipid and DHA contents reaching 6.98 ± 0.04 g/L; 5.5 ± 0.13% of dry biomass and 0.14 ± 0.01% of total fatty acids, respectively.

Algal biomass was harvested by centrifugation at 4,000 rpm for 10 min. The algae paste was washed three times with sterile distilled water and then dried to a constant weight in a drying oven at 50°C and stored in desiccators.

Extraction and Isolation: The dried microalga (5.0 kg) was grinded and extracted with *n*-hexane, followed by MeOH to give the respective crude extracts after solvent's evaporation under reduced pressure. The crude methanolic extract was further partitioned between EtOAc and water to get EtOAc (48.0 g) and aqueous (70.0 g) extracts after removing of total solvent *in vacuo*.

The *n*-hexane extract (120 g) was subjected on a silica gel CC, eluted with a system gradient of *n*-hexane/Me_2CO (100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 30:70, 0:100, v/v) to yield ten fractions (H1-H10).

The fraction H2 was further chromatographed on a silica gel CC, eluted with the mixture of *n*-hexane/ Me_2CO (90:10,v/v) to get five subfractions (H2.1-H2.6). The subfraction H2.5 was purified on a reverse phase silica gel (RP-18) CC with Me_2CO/H_2O (80:20, v/v) as eluent to obtain compounds **1** (5.1 mg) and **2** (4.9 mg). Compound **3** (5.2 mg) was isolated from fraction H7 by a silica gel CC with *n*-hexane/ Me_2CO (80:20, v/v) as eluent, followed by a RP-18 silica gel CC, eluted with a mixture of $MeOH/Me_2CO/H_2O$ (100:1:1, v/v/v). Compound **4** (20.5 mg) was isolated from fraction H9 by a silica gel CC eluting with *n*-hexane/Me_2CO (70:30), followed by RP-18 silica gel CC with $MeOH/H_2O$ (90:10, v/v) as eluent. The EtOAc extract (47.0 g) after repeated column chromatography on silica gel, using CH_2Cl_2/EtOAc and CH_2Cl_2/MeOH as eluents, or over Sephadex LH-20, eluting with $MeOH/CH_2Cl_2$ (90:10, v/v) and MeOH, yielded compounds **5** (4.0 mg), **6** (3.5 mg), **7** (2.5 mg), **8** (9.0 mg), **9** (3.3 mg), **10** (21.0 mg), **11** (5.5 mg).

α-Glucosidase Inhibitory Assay: The α-glucosidase inhibitory assay was carried out according to the previous method [8]. Sample was dissolved in DMSO (400 mg/mL) and phosphate buffer to final concentrations of 256.0, 128.0, 32.0, 8.0, and 2.0 mg/mL. In a 96-well plate, a reaction mixture containing sample, phosphate buffer (100 mM, pH 6.8), α-glucosidase (0.4 U/mL) were preincubated at 37 ºC for 10 min. Then, 20 µL *p*-nitrophenyl *α*-D-glucopyranoside (pNPG) (2.5 mM) was added as a substrate and incubated further at 37 ºC for 30 min. The reaction was stopped by adding 25 µL Na_2CO_3 (0.2 M). The rate of *p*-nitrophenol released from pNPG was measured at 410 nm with Elisa reader. Without test sample was set up in parallel as a control. All experiments were performed in triplicate and acarbose was used as a positive control. The results were expressed as percentage inhibition, which was calculated using the formula,

$$\text{Inhibitory activity (\%)} = (A_c - As) \times A_c \times 100$$

Where, As is the absorbance in the presence of tested sample and Ac is the absorbance of control.

The IC_{50} value is defined as the concentration of inhibitor to inhibit 50% of its activity under the assayed conditions, which was determined graphically from inhibition curves (log inhibitor concentration vs percent of inhibition).

Antimicrobial Activity Assays: Antimicrobial assays were carried out using *Enterococcus faecalis* (ATCC13124), *Staphylococcus aureus* (ATCC25923), *Bacillus cereus* (ATCC13245), *Escherichia coli* (ATCC25922), *Pseudomonas aeruginosa* (ATCC27853), *Salmonella enterica* (ATCC12228) and *Candida albicans* (ATCC1023). Stock solutions of samples were prepared in DMSO, and the antimicrobial assays were carried out in 96-well microtiter plates against the microbial strains (5×10^5 CFU/mL) using a modification of the published method [9]. After incubation for 24 h at 37°C, the absorbance at 650 nm was measured using a microplate reader. Streptomycin and cyclohexamide were used as reference compounds.

Cytotoxic Activity Assay: The cancer cell lines were maintained in Dulbecco's D-MEM medium, supplemented with 10% fetal calf serum, L-glutamine (2 mM), penicillin G (100 UI/mL), streptomycin (100 µg/mL) and gentamicin (10 µg/mL). Stock solutions of compounds were prepared in $DMSO/H_2O$ (1/9), and cytotoxicity assays were carried out in 96-well microtiter plates against A549 (ATCC, CCL-185™), HePG2 (ATCC, HB-8065™), MCF7 (ATCC, HTB-22™), and LNCaP (ATCC, CRL-1740™) cancer cell lines (3×10^3 cells/mL) using a modification of the published method [10]. After 72 h incubation at 37 ºC in air/CO_2 (95:5) with or without test compounds, cell growth was estimated by colorimetric measurement of stained living cells by neutral red. Optical density was determined at 540 nm with a Titertek Multiscan photometer. The IC_{50} value was defined as the concentration of sample necessary to inhibit the cell growth to 50% of the control. Ellipticine was used as a reference compound.

Phytochemical Studies of the Extracts: From *n*-hexane anf EtOAc extracts, eleven compounds were isolated. The isolated compounds were identified as (24*E*,24*R*)-methylcholesta-5,7,22-trien-3*β*-ol (ergosterol) **(1)** [11], (22*E*,24*R*)-ethylcholesta-5,7,22-trien-3*β*-ol (7-dehydroporiferasterol) **(2)** [11], (22*E*,24*R*)-ethylcholesta-7,22-dien-3*β*,5*α*,6*β*-triol (**3**) [12], (22*E*,24*R*)-ethylcholesta-5,22-dien-3-*O*-β-D-glucopyranoside (poriferasterol glucoside) (**4**) [13], perlolyrine (**5**) [14], pyrrolezanthine-6-methyl ether (**6**) [15], 3-(3-aminopropyl)-6-[(4-hydroxyphenyl)methyl]-2,5-piperazinedione (**7**) [16], 5-methyluracil (**8**) [17], 1*H*-indole-3-carboxylic acid (**9**) [18], adenosine (**10**) [19] and *p*-hydroxybenzoic acid (**11**) [20] by comparing their NMR data (Supporting information) to those reported in the literatures.

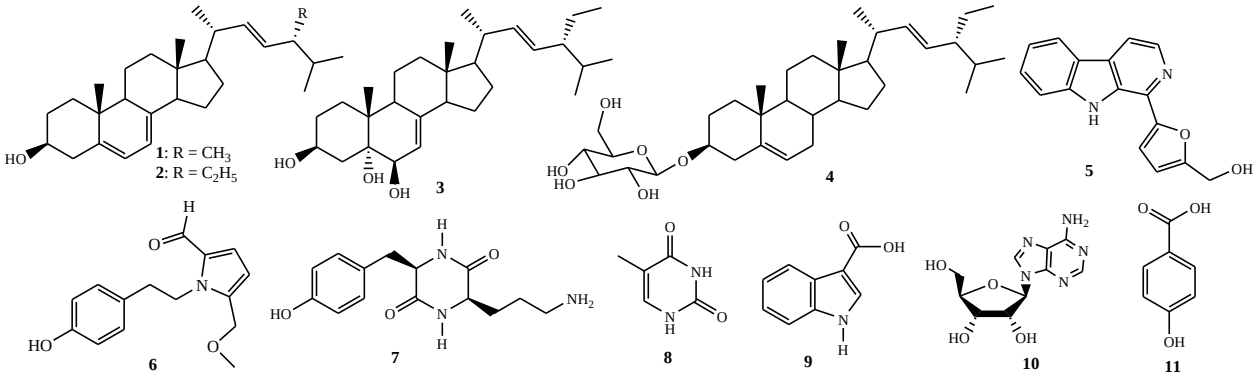

Figure 1. Chemical structure of the compounds (**1**-**11**)

Biological Activites of Isolated Compounds: In the present study, inhibitory activity test of the α-glucosidase of compounds **1**-**8** were performed *in vitro* using spectrophotometric methods. Compounds **5** and **6** were shown to be more potent α-glucosidase inhibitors with IC_{50} values of 26.86±0.29 and 7.96±0.75 μg/mL, compared with the control acarbose (IC_{50} 164.08±2.89 μg/mL). Compound **7** exhibited weak activity with IC_{50} value of 234.4±3.39 μg/mL. The α-glucosidase inhibitory activity of *p*-hydroxybenzoic acid (**11**) and β-adenosine (**10**) with IC_{50} of 56.4 μM and 9.3 mM were previously reported [21,22]. The nitrogen containing moiety in compound **5** and **6** may increase their binding affinity with target enzyme and result in lower IC_{50} values with higher therapeutic efficiency [23]. Additionally, pyrroles (**6**) and their derivatives (**5**) could act as glycosidase inhibitors, and thus can mimic oxocarbocation intermediate structure, enable tight binding and strongly inhibit the enzyme [24].

Table 1. α-glucosidase inhibitory activity of compounds **1**-**8**

Compounds	IC_{50} (μg/mL)	Compounds	IC_{50} (μg/mL)
1	>256	**5**	26.86±0.29
2	>256	**6**	7.96±0.75
3	>256	**7**	234.4±3.39
4	>256	**8**	>256
Acarbose	164.08±2.89		

In the antimicrobial assay, six compounds **1**, **4** and **6**-**9** were examined against seven microbial strains. All compounds **1**, **4** and **6**-**9** showed strong inhibition with MIC values of 16, 64, 64, 16, 64, 64 μg/mL on *E. faecalis* strain, and 128, 32, 16, 16, 16, 16 μg/mL on *C. albican*s yeast, respectively. Only compound **1** exhibited inhibition on *S. aureus* with MIC value of 64 μg/mL.

In the cytotoxic assay, only compound **6** showed the moderate cytotoxic activity against all of four tested cancer cell lines A549, HepG2, MCF7, LNCaP with IC_{50} values of 62.26, 41.03, 57.21 and 43.23 μg/mL, respectively.

Table 2. Antimicrobial activity of compounds **1**, **4**, **6-9**

Sample	MIC_{50} (μg/mL)						
	Gram (+)			Gram (-)			Yeast
	E. faecalis	*S. aureus*	*B. cereus*	*E. coli*	*P. aeruginosa*	*S. enterica*	*C. albicans*
1	16	64	>256	>256	>256	>256	128
4	64	>256	>256	>256	>256	>256	32
6	64	>256	>256	>256	>256	>256	16
7	16	>256	>256	>256	>256	>256	16
8	64	>256	>256	>256	>256	>256	16
9	64	>256	>256	>256	>256	>256	16
Streptomycin	256	256	128	32	256	128	
Cyclohexamide							32

Table 3. Cytotoxic activities of compounds **4** and **6-9**

Compound	IC_{50} (µg/mL)			
	A549	**HepG2**	**MCF7**	**LNCaP**
4	>100	>100	>100	>100
6	62.26± 3.51	41.03± 3.05	57.21± 1.50	43.23± 2.08
7	>100	>100	>100	>100
8	>100	>100	>100	>100
9	>100	>100	>100	>100
Ellipticine	**0.41± 0.04**	**0.33± 0.03**	**0.41± 0.05**	**0.36± 0.04**

Alkaloids are commonly found in terrestrial plants, but rarely in marine algae [25]. Surprisingly, pyrrole alkaloids such as **5** and **6** with significant α-glucosidase inhibitory activity and antimicrobial activity could be isolated from *T. aureum*. Among isolated compounds, **6** is the most active compound with broad spectrum of biological activities. So that, this compound could be a highly potential candidate for further study on pharmaceutical applications. Notably, only small quantities of compounds could be isolated in this study, which limit the number of biological test and the biological mechanism. The similar study with large scale of dry mass of this microalga in order to isolate other bioactive substances with low content have been currently carried out in laboratory.

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Supporting Information

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

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References

[1] W. Fu, D.R. Nelson, Z. Yi, M. Xu, B. Khraiwesh, K. Jijakli, A. Chaiboonchoe, A. Alzahmi, D. Al-Khairy, S. Brynjolfsson and K. Salehi-Ashtiani (2017). Chapter 6 Bioactive compounds from microalgae: Current development and prospects, *Studies Nat. Prod. Chem*. **54**, 199-225.

[2] K. Kalidasan, K.S. Sunil, K. Kayalvizhi and K. Kathiresan (2015). Polyunsaturated fatty acid-producing marine thraustochytrids: A potential source for antimicrobials. *J. Coast. Life Med.* **3**, 848-851.

[3] K. Kalidasan, K.S. Sunil, R. Narendran and K. Kathiresan (2015). Antioxidant activity of mangrove-derived marine thraustochytrids, *Mycosphere* **6**, 602–611.

[4] V. Ugalde, R.E. Armenta, K.-P. Azadeh; Z. Sun, K.T. Berryman and M.S. Brooks (2018). Improvement of culture conditions for cell biomass and fatty acid production by marine thraustochytrid F24-2, *J. Appl. Phycol.*. **30**, 329-339.

[5] K.H. Min, H.H. Lee, P. Anbu, B.P. Chaulagain and B.K. Hur (2012). The effects of culture condition on the growth property and docosahexaenoic acid production from *Thraustochytrium aureum* ATCC 34304, *Korean J. Chem. Eng.* **29**, 1211-1215.

[6] E. Julianti, M. Fathurohman, S. Damayanti and R. E. Kartasasmita (2018). Isolated of heterotrophic microalgae *Thraustochytrium aureum* as a potential source for docosahexaenoic acid (DHA), *Mar. Res. Indonesia*. **43**, 79-86.

[7] Y. Taoka, N. Nagano, Y. Okita, H. Izumida, S. Sugimoto and M. Hayashi (2011). Effect of Tween 80 on the growth, lipid accumulation and fatty acid composition of *Thraustochytrium aureum* ATCC 34304, *J. Biosci. Bioeng.* **111**, 420-424.

[8] N.T. Tra, N.V. Tuyen, P.V. Cuong, N.T.T. Ha, L.T. Tu Anh and N.T. Son (2021). Chemical constituents from the leaves of *Styrax argentifolius* H.L.Li and their biological activities, *Phytochem Lett*. **41**, 70 -73.

[9] F. Hadacek and H. Greger (2000). Testing of antifungal natural products: methodologies, comparability of results and assay choice, *Phytochem. Anal*. **11**, 137-147.

[10] J.P. Hughes, S. Rees, S.B. Kalindjian and K.L. Philpott (2011). Principles of early drug discovery, *Br. J. Pharmacol.* **162**, 1239-1249.

[11] F. Matteo, T. Pasquale and L. Rafael (2010). Phytosterol from *Dunaliella tertiolecta* and *Dunaliella salina*: A potentially novel industrial application, *Bioresour. Technol.* **101**, 4144-4150.

[12] M. Anna, P. Vincenzo and S. Donato (1989), New polyhydroxysterols from the dictyoceratid sponges *Hippospongia communis*, *Spongia officinalis, Ircinia variabilis*, *and Spongionella gracilis*, *J. Nat. Prod.* **52**(5), 952-961.

[13] K. Mahbuba, B. Mirajum and M. A. Quader (2012), Sterols and sterol glucoside from *Phyllanthus* species, *Dhaka Univ. J. Sci.* **60**(**1**), 5-10.

[14] B. Dassonneville, B. Witulski and H. Detert (2011). [2+2+2] Cycloadditions of Alkynylynamides - A Total synthesis of perlolyrine and the first total synthesis of "Isoperlolyrine", *Eur. J. Org. Chem.* **2011**, 2836-2844.

[15] G-H. Xu, Y-H. Kim, S-J. Choo, I-J. Ryoo, J-K. Yoo1, J-S. Ahn, and I-D. Yoo (2009). Chemical constituents from the leaves of *Ilex paraguariensis* inhibit human neutrophil elastase, *Arch. Pharm. Res.* **32**, 1215-1220.

[16] Y. Sasaki, Y. Akutsu, K. Suzuki, S. Sakurada, and K. Kisara (1981). Structure and analgesic activity relationship of Cyclo-Tyrosyl-Arginyl and its three stereoisomers, *Chem. Pharm. Bull*. **29**(11), 3403-3406.

[17] Z.-G. Ding, J.-Y. Zhao, P.-W. Yang, M.-G. Li, R. Huang, X.-L. Cui and M.-L. Wen (2009). ^{1}H and ^{13}C NMR assignments of eight nitrogen containing compounds from *Nocardia alba* sp.nov (YIM 30243T), *Magn. Reson. Chem.* **47**, 366-370.

[18] M.S. Morales-Rios, J. Espiieira and P. Joseph-Nathan (1987). ^{13}C NMR spectroscopy of indole derivatives, *Magn. Reson.Chem.* **25**, 377-395.

[19] P. Ciuffreda, S. Casati and A. Manzocchi (2007). Spectral assignments ang reference data complete ^{1}H and ^{13}C NMR spectral assignment of α- and β-adenosine, 2′-deoxyadenosine and their acetate derivatives, *Magn. Reson. Chem.* **45**, 781-784.

[20] T. Jianwen, B. Pawe, L. Jikai, S. Bernd, S. Ales and H. Klaus (2004). Universally occurring phenylpropanoid and species-specific indolic metabolites in infected and uninfected *Arabidopsis thaliana* roots and leaves, *Phytochemistry* **65**, 691-699.

[21] S.-H. Lam, J.-M. Chen, C.-J. Kang, C.-H. Chen and S.-S. Lee (2008). α-Glucosidase inhibitors from the seeds of *Syagrus romanzoffiana*, *Phytochemistry* **69**, 1173-1178.

[22] S.W. Jung, A.J. Han, H.J. Hong, M.G. Choung, K.S. Kim and S.H. Park (2006). α-Glucosidase inhibitors from the roots of *Codonnopsis lanceolata* Trauv., *Agric. Chem. Biotechnol*. **49**(**4**), 162-164.

[23] J. Zhen, Y. Dai, T. Villani, D. Giurleo, J. E. Simon and Q. Wu (2017). Synthesis of novel flavonoid alkaloids as a-glucosidase inhibitors, *Bioorg. Med. Chem.* **25**, 5355-5364.

[24] J.F. Witte and R.W. McClard (1991). Synthesis of a potent α-glucosidase inhibitor epimeric to fr 900483. *Tetrahedron Lett.* **32**, 3927-3930.

[25] K.C. Güven, A. Percot and E. Sezik (2010). Alkaloids in Marine Algae, *Mar. Drugs* **8**, 269-284.