



Isolation and identification of antioxidant and α -glucosidase inhibitory compounds from fruit juice of *Nitraria tangutorum*



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ABSTRACT

Nitraria tangutorum Bor., having edible berries, is valued for reputed health benefits in Qinghai-Tibet plateau. The phytochemical research on the fruit juice of *N. tangutorum* led to the isolation of twenty-six compounds including five new compounds, tangutorids A–D (**1**, **2**, **3a**, and **3b**), and (3E,5E)-7-O- β -glucosyl-4-(2-methoxy-2-oxoethyl)hepta-3,5-dienoic acid (**15**). The structures of these compounds were elucidated through comprehensive spectroscopic analyses. Tangutorids A–F were the first examples of glucose-derived β -carbolines from natural products. The biogenetic pathways of **1–8** were proposed to involve Pictet–Spengler reactions and described starting from the co-isolated tryptophan (**10**) and corresponding aldehydes. All isolates were evaluated for their antioxidant and α -glucosidase inhibitory activities. Compounds **21**, **22**, and **24** showed antioxidant activity with SC_{50} values ranging from 12.2 ± 1.9 to 30.4 ± 2.7 μ g/mL, and compound **1** showed strong α -glucosidase inhibitory effect with IC_{50} value of 63.3 ± 4.6 μ g/mL.

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1. Introduction

β -Carbolines are a kind of naturally-occurring pyridoindole alkaloids produced from tryptophan and aldehydes and widespread in commercial food and beverages (Herraiz, 2004). It has become clear that these alkaloids occur in fruits, fruits processed products (Herraiz & Galisteo, 2002), smoked foods (Papavergou & Herraiz, 2003), fermented products (Adachi et al., 1991; Herraiz, 1999; Sen, Seaman, Lau, Weber, & Lewis, 1995), and are produced during food production processing and storage (Herraiz, 2009). In addition, these alkaloids also can be found in the human biological

tissues and fluids, which illustrates that they probably come from the diet (Adachi et al., 1991). Previous studies showed that β -carboline alkaloids frequently exhibit extensive biological activities such as neuroactive, antioxidant, antimicrobial, antithrombotic, antiparasitidal, antimalarial, antiviral, and antitumor effects (Di Giorgio et al., 2004; Herraiz & Galisteo, 2015; Kusurkar & Goswami, 2004; Zhao et al., 2006). These molecules could possess both biological and toxicological effects when they are ingested from foods or beverages, which will be potentiated further if they are accumulated within body tissues (Herraiz, 2009). Therefore, the analysis, isolation and identification of β -carboline alkaloids in foodstuffs are of great interests.

Nitraria tangutorum Bobr, a shrub belonging to the *Nitraria* genus (Zygophyllaceae), is mainly grown in desert and semidesert regions of Qinghai-Tibet plateau. It is a typical desert plant with strong resistance to drought, salinity, alkalinity, and high temper-

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ature and has been used as an ideal material for studying the adaptability and response of plants to salinity stress (Yang, Wei, Shi, Fan, & An, 2010; Yang, Yang, Li, Shi, & Lu, 2013). The fruit of *N. tangutorum*, called “desert cherry”, has a high potential economic value as a source of edible berries and has been traditionally used as healthy food and folk medicine to treat abnormal menstruation, heart disease, neurasthenia, and dyspepsia in western China (Hu, Zheng, Li, & Suo, 2014). Besides, the fruit was also widely used to extract pigments as natural food colorants and make wine by locals. In previous studies, numerous compounds, such as alkaloids and flavonoids had been isolated from the plants of *Nitraria* genus, and many of them showed exert antitumor or anti-oxidative activities. (Du, Xin, & Peng, 2015) And a few β -carboline alkaloids and phenols have been identified from the leaves, seeds, and dry fruit of *N. tangutorum* (Duan, Williams, & Chen, 1999; Lu, Ouyang, Su, Ji, & Liu, 2013; Wu et al., 2014). However, there is little information about the chemical constituents of the fresh fruit juice of *N. tangutorum*. In order to identify the chemical constituents of the fresh fruit juice of *N. tangutorum*, we carried out the phytochemical investigation on it. Herein, we report the isolation and structural identification of new compounds, as well as the probable biosynthetic pathway of β -carboline alkaloids from the fruit juice of *N. tangutorum*. The antioxidant and α -glucosidase inhibitory activities of the isolates are also tested.

2. Material and methods

2.1. General experimental procedure

A Horiba SEPA-300 high-sensitive polarimeter was used to measure optical rotations. (Horiba, Kyoto, Japan) Ultraviolet absorption (UV) spectra were acquired on a Shimadzu UV2401A ultraviolet-visible spectrophotometer (Shimadzu Co., Tokyo, Japan). IR spectra were performed on a Bio-Rad FTS-135 series spectrometer (Bio-Rad, Hercules, CA, USA) with KBr pellets. 1D and 2D NMR spectra were measured on a Bruker Avance III-600 spectrometer, using tetramethylsilane as an internal standard. Chemical shifts were reported in units of δ (ppm) and coupling constants (J) were expressed in Hz. High resolution mass spectra were performed on an API QSTAR Pulsar-1 spectrometer (Advanced Biomix, Los Angeles, CA, USA) tandem an Agilent 6230 Accurate-Mass spectrometer (Agilent Technologies, Palo Alto, CA, USA). Column chromatography (CC) were carried out over D101 risen (Tianjin Agricultural Chemical Co. Ltd., Tianjin, China), silica gel (100–200 mesh) (Qingdao Haiyang Chemical Co., Qingdao, China) and MCI gel CHP 20P (75–150 μ m, Tokyo, Japan). Pre-coated silica gel plates (Qingdao Haiyang Chemical Co., Qingdao, China) were used for thin-layer chromatography (TLC). Detection was done under UV light (254 nm and 365 nm) and by spraying the plates with 10% sulfuric acid ethanol solution followed by heating. An Agilent series 1200 (Agilent Technologies, Palo Alto, CA, USA) was used for analysis HPLC. Semi-preparative HPLC was done on a Hanbon series NP7005C (Hanbon Sci & Tech, Jiangsu, China), the column used was XCharge C18 (10 μ m, 100 \AA , 250 mm \times 20 mm, Acchrom, Beijing, China). Preparative HPLC was done on a Hanbon series NP7010C equipped with a dynamic axial compression column DAC-HB50 C18 (10 μ m, 100 \AA , 400 mm \times 50 mm, Hanbon Sci & Tech, Jiangsu, China)

2.2. Plant material

The ripe fruit of *N. tangutorum* was manually collected in Qaidam Basin, Qinghai Province, People's Republic of China, and identified by Professor Lijuan Mei (Northwest Institute of Plateau Biology, Chinese Academy of Science). The fruit was washed and

ground by hydraulic laboratory-scale juice extractor. The cloudy juice obtained was filtered through degreasing cotton to remove the solid residue. According to this method, two liters of fresh fruit juice was obtained and used as experimental material.

2.3. Extraction and isolation

The fresh fruit juice (2 L) of *N. tangutorum* was subjected to D101 Risen column chromatography (CC), eluting successively with water, 40% ethanol and 90% ethanol aqueous solution (each for 4 L) to give three fractions (Fr1–3).

The 40% ethanol aqueous fraction (Fr2, 40 g) was submitted to MCI gel CHP 20P CC eluting with gradient methanol aqueous (0%, 20%, 40%, 60%, 80% and 100% of methanol, each for 2L) to afford six portions Fr21–26 by TLC analysis. Compound **13** (339 mg) was acquired by re-precipitation from Fr21 (2.1 g) in methanol and the filtrate was further separated by prep-HPLC through an XCharge C18 column (10%–30% MeCN with 0.2% formic acid, for 40 min, with a flow rate of 15 mL/min) to give compounds **16** (t_R of 12.4 min, 21 mg), **17** (t_R of 14.0 min, 13 mg), and **15** (t_R of 16.4 min, 17 mg). Fr22 (7.0 g) was subjected to a silica gel column, eluting with a CHCl_3 -MeOH- H_2O gradient system (9:1:0.1–6:4:1, v/v/v), to give three fractions (Fr221–223) based on TLC analysis. Fr221 (1.1 g) was subjected to an XCharge C18 column (5%–45% MeCN with 0.2% formic acid, for 40 min, with a flow rate of 15 mL/min) to give compound **14** (t_R of 19.1 min, 117 mg). With the same method, compound **18** (t_R of 14.3 min, 9 mg) was obtained from Fr222 (136 mg). Fr223 (3.1 g) was separated through an XCharge C18 column (5%–25% MeCN with 0.2% formic acid, for 20 min, with a flow rate of 15 mL/min) to give a Fr2231 (2.7 g) and compounds **8** (t_R of 16.7 min, 13 mg) and **11** (t_R of 18.2 min, 6 mg). Fr2231 was further subjected to an XCharge C18 column (5%–35% MeCN with 0.2% formic acid, for 60 min, with a flow rate of 15 mL/min) to yield compounds **10** (t_R of 15.7 min, 18 mg), **7** (t_R of 21.1 min, 20 mg), and **9** (t_R of 21.1 min, 73 mg). Fr23 (7.2 g) was separated by a silica column eluting with a CHCl_3 -MeOH- H_2O gradient system from 9:1:0.1 to 7:3:0.5 to yield compound **12** (5 mg) and Fr231 (3.4 g). Fr231 was separated over an XCharge C18 column (5%–55% MeCN with 0.2% formic acid, for 50 min, with a flow rate of 15 mL/min) to give compounds **23** (t_R of 17.1 min, 13 mg), **20** (t_R of 19.0 min, 88 mg), **19** (t_R of 19.9 min, 56 mg), **21** (t_R of 22.5 min, 37 mg), and **22** (t_R of 19.0 min, 113 mg). Fr24 (4.2 g) was separated through a DAC-HB50 C18 column (10%–50% MeOH with 0.2% formic acid, for 40 min, with a flow rate of 60 mL/min) to yield three fractions (Fr241–243). Fr241 (244 mg) was further purified by an XCharge C18 column (8% MeCN with 0.2% FA, for 30 min, with a flow rate of 15 mL/min) to give compounds **4a** (t_R of 18.7 min, 26 mg) and **4b** (t_R of 19.4 min, 21 mg). Fr242 (1.3 g) was purified by an XCharge C18 column (5%–35% MeCN with 0.2% FA, for 50 min, with a flow rate of 15 mL/min) to yield compounds **3a** (t_R of 21.1 min, 57 mg), **3b** (t_R of 22.2 min, 31 mg), **24** (t_R of 25.0 min, 4 mg), and **2** (t_R of 31.9 min, 12 mg). Fr25 (2.7 g) was subjected to a silica column eluting with a CHCl_3 -MeOH- H_2O gradient system (9:1:0.1–7:3:0.5, v/v/v) to yield compound **6** (67 mg) and Fr251 (1.2 g). Fr251 was further purified by an XCharge C18 column (10%–60% MeCN with 0.2% formic acid, for 50 min, with the follow rate of 15 mL/min) to give compounds **5** (t_R of 29.7 min, 77 mg) and **1** (t_R of 31.4 min, 109 mg). Fig. S43 showed the extraction and separation processes associated with the fresh fruit juice (2 L) of *N. tangutorum*.

Tangutorid A (**1**). Light brown solid. UV (MeOH) λ_{max} nm (log ϵ): 213 (4.14), 237 (4.00), 271 (4.27), 287 (3.98), 343 (3.45), 393 (3.06); IR (KBr) ν_{max} : 3426, 1716, 1694, 1670, 1625, 1366, 1305, 1276, 1135, 1021 cm^{-1} ; ^1H and ^{13}C NMR spectroscopic data see

Table 1
¹H NMR and ¹³C NMR Spectroscopic Data of Compounds **1–3b** in DMSO-*d*₆.

No.	1		2		3a		3b	
	δ_{H} , mul. (J in Hz)	δ_{C}	δ_{H} , mul. (J in Hz)	δ_{C}	δ_{H} , mul. (J in Hz)	δ_{C}	δ_{H} , mul. (J in Hz)	δ_{C}
1		134.6, C		147.8, C		146.9, C		147.9, C
3		136.7, C		135.6, C		135.3, C		135.5, C
3a		166.2, C		166.9, C		166.6, C		166.2, C
4	9.17, s	121.0, CH	8.83, s	115.8, CH	8.85, s	116.8, CH	8.83, s	115.9, CH
5		131.5, C		128.6, C		128.6, C		128.5, C
6		120.2, C		120.9, C		120.8, C		120.8, C
7	8.45, d (7.5)	122.2, CH	8.37, d (7.7)	121.8, CH	8.37, d (7.7)	121.9, CH	8.38, d (7.8)	121.8, CH
8	7.36, t (7.1)	120.9, CH	7.30, t (7.4)	120.1, CH	7.30, t (7.3)	120.1, CH	7.30, t (7.4)	120.1, CH
9	7.64, t (7.2)	129.3, CH	7.59, t (7.5)	128.5, CH	7.59, t (7.2)	128.8, CH	7.59, t (7.4)	128.4, CH
10	7.83, d (7.9)	113.4, CH	7.76, d (8.0)	112.7, CH	7.76, d (6.7)	112.8, CH	7.78, d (7.8)	112.9, CH
11		142.3, C		141.0, C		141.0, C		140.9, C
12	12.25, s		11.78, s		11.76, s		11.72, s	
13		135.0, C		134.0, C		134.3, C		133.9, C
14		201.5, C	5.11, brs	73.0, CH	5.38, brs	71.6, CH	5.37, d (8.8)	69.9, CH
15	2.94, brs	32.3, CH ₂	1.85, m 2.06, m	33.4, CH ₂	2.01, m 2.36, d (12.4, 6.3)	40.0, CH ₂	1.79, t (12.0) 2.19, t (12.0)	39.9, CH ₂
16	3.69, brs	31.5, CH ₂	1.32, m 1.72, m	30.0, CH ₂	3.53, overlapped	69.9, CH	3.86, d (6.2)	68.3, CH
17		210.2, C	3.42, brs	71.5, CH	3.35, overlapped	75.1, CH	3.39, overlapped	75.4, CH
18	4.20, s	67.8, CH ₂	3.25, dt (10.3, 7.8)	66.0, CH ₂	3.35, overlapped	63.2, CH ₂	3.40, overlapped 3.59, d (7.9)	63.5, CH ₂

Table 1; HRESIMS *m/z*: 327.0978 [M+H]⁺ (calculated for C₁₇H₁₅N₂O₅⁺ 327.0975).

Tangutorid B (**2**). Light brown solid. [α]_D^{25.3} +14.9 (c 0.27, H₂O); UV (MeOH) λ_{max} nm (log ϵ): 192 (4.12), 208 (4.04), 237 (3.96), 271 (4.18), 340 (3.31); IR (KBr) ν_{max} : 3243, 3088, 2935, 1629, 1599, 1379, 1310, 1248, 1063 cm⁻¹; ¹H and ¹³C NMR spectroscopic data see **Table 1**; HRESIMS *m/z* 331.1296 [M+H]⁺ (calculated for C₁₇H₁₉N₂O₅⁺ 331.1288).

Tangutorid C (**3a**). Light brown solid. [α]_D^{24.3} -15.7 (c 0.10, MeOH); UV (MeOH) λ_{max} nm (log ϵ): 202 (4.02), 215 (4.02), 238 (4.01), 270 (4.19), 339 (3.39); IR (KBr) ν_{max} : 3417, 2923, 1629, 1601, 1376, 1307, 1248, 1076 cm⁻¹; ¹H and ¹³C NMR spectroscopic data see **Table 1**; HRESIMS *m/z* 347.1263 [M+H]⁺ (calculated for C₁₇H₁₉N₂O₆⁺ 347.1238).

Tangutorid D (**3b**). Light brown solid. [α]_D^{25.2} -17.1 (c 0.11, H₂O); UV (MeOH) λ_{max} nm (log ϵ): 192 (3.98), 212 (3.84), 232 (3.73), 270 (3.85), 287 (3.57), 340 (3.11); IR (KBr) ν_{max} : 3440, 3232, 2922, 1639, 1600, 1376, 1322, 1232, 1077, 1051, 1029 cm⁻¹; ¹H and ¹³C NMR spectroscopic data see **Table 1**; HRESIMS *m/z* 347.1252 [M+H]⁺ (calculated for C₁₇H₁₉N₂O₆⁺ 347.1238).

Tangutorid E (**4a**). Light brown solid. [α]_D^{24.2} -3.1 (c 0.70, MeOH); UV (MeOH) λ_{max} nm (log ϵ): 211 (4.26), 238 (4.40), 289 (4.05), 340 (3.58); IR (KBr) ν_{max} : 3410, 2923, 1630, 1592, 1498, 1454, 1429, 1325, 1234, 1061, 744 cm⁻¹; ¹H and ¹³C NMR spectroscopic data see **Table 2**; HRESIMS *m/z* 303.1348 [M+H]⁺ (calculated for C₁₆H₁₉N₂O₄⁺ 303.1339).

Tangutorid F (**4b**). Light brown solid. [α]_D^{24.0} -45.2 (c 0.30, MeOH); UV (MeOH) λ_{max} nm (log ϵ): 211 (4.26), 237 (4.41), 289 (4.05), 339 (3.57); IR (KBr) ν_{max} : 3411, 2924, 1631, 1592, 1500, 1452, 1429, 1325, 1234, 1055, 745 cm⁻¹; ¹H and ¹³C NMR spectroscopic data see **Table 2**; HRESIMS *m/z* 303.1357 [M+H]⁺ (calculated for C₁₆H₁₉N₂O₄⁺ 303.1339).

(3*E*,5*E*)-7-*O*- β -glucosyl-4-(2-methoxy-2-oxoethyl)hepta-3,5-dienoic acid (**15**). White powder. [α]_D^{4.5} -45.5 (c 0.12, MeOH); UV (MeOH) λ_{max} nm (log ϵ): 203 (3.83), 223 (3.74), 278 (3.20); IR (KBr) ν_{max} : 3428, 2927, 1720, 1638, 1387, 1267, 1076, 1042 cm⁻¹; ¹H and ¹³C NMR spectroscopic data see **Table 2**; HRESIMS *m/z* 399.1247 [M+H]⁺ (calculated for C₁₆H₂₄NaO₁₀⁺ 399.1262).

2.4. Assay of ABTS and DPPH radical-scavenging

Radical scavenging activity against ABTS and DPPH was performed according to previously reported protocols (Gao, Xu, Yang, Xu, & Zhang, 2010; Liang, Xue, Kennepohl, & Kitts, 2016). Ascorbic acid (VC) was used as positive control. In DPPH radical scavenging assay, DPPH was dissolved in ethanol to a concentration of 250 μ M and allowed the mixture to stand in the dark at room temperature for 12–16 h. The DPPH stock solution was diluted with ethanol to an absorbance of 0.700 (\pm 0.020) at 517 nm before use. The absorbance reading was taken after mixing for 30 min. The optical density was measured at 517 nm on a microplate reader. In ABTS radical scavenging assay, ABTS solution

Table 2
¹H NMR and ¹³C NMR spectroscopic data of compounds **4a**, **4b**, and **15** in CD₃OD.

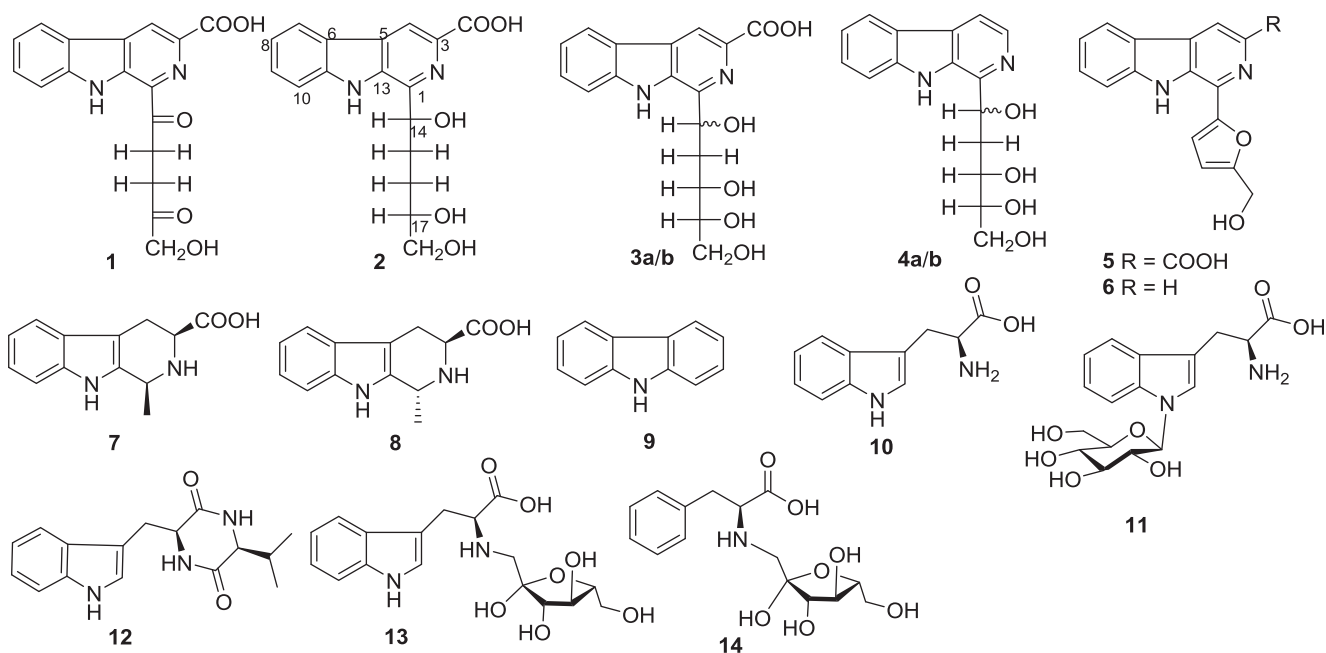
No.	4a		4b		No.	15	
	δ_{H} , mul. (J in Hz)	δ_{C}	δ_{H} , mul. (J in Hz)	δ_{C}		δ_{H} , mul. (J in Hz)	δ_{C}
1		145.5, C		145.9, C	1		175.3, C
3	8.18, brs	131.9, CH	8.18, brs	131.4, C	2	3.10, overlapped	35.0, CH ₂
4	8.13, brs	116.4, CH	8.10, brs	116.2, CH	3	5.80, t (7.4)	129.2, CH
5		133.3, C		133.9, C	4		134.2, C
6		121.2, C		121.2, C	5	6.28, d (15.9)	136.3, CH
7	8.06, d (7.4)	123.4, CH	7.98, brs	123.3, CH	6	5.70, dt (15.9, 6.1)	125.8, CH
8	7.22, brs	122.2, CH	7.18, brs	122.2, CH	7	4.12, dd (12.8, 6.5) 4.32, dd (12.3, 5.5)	70.5, CH ₂
9	7.56, overlapped	131.6, CH	7.51, overlapped	132.0, CH	8	3.25, d (1.1)	33.5, CH ₂
10	7.68, overlapped	113.7, CH	7.53, overlapped	113.5, CH	9		173.0, C
11		144.2, C		144.3, C	1'	4.20, d (7.8)	103.1, CH
13		133.9, C		132.6, C	2'	3.11, overlapped	75.1, CH
14	5.69, brs	70.1, CH	5.67, brs	67.8, CH	3'	3.25, overlapped	78.1, CH
15	2.20, brs 2.44, d (12.7)	40.4, CH ₂	2.04, brs 2.19, brs	40.7, CH ₂	4'	3.19, t (8.4)	71.7, CH
16	3.94, brs	71.1, CH	4.15, brs	70.0, CH	5'	3.16, m	78.0, CH
17	3.63, overlapped	76.4, CH	3.70, overlapped	76.6, CH	6'	3.57, overlapped 3.77, m	62.7, CH ₂
18	3.64, d (11.3) 3.74, d (9.3)	64.4, CH ₂	3.72, overlapped 3.82, brs	64.6, CH ₂	-OCH ₃	3.56, s	53.4, CH ₃

was diluted with ethanol to an absorbance of 0.700 (± 0.020) at 734 nm. 20 μL of compounds was added into each well of 96-well cell culture plates. And then 180 μL of diluted ABTS solution ($A_{734\text{nm}} = 0.700 \pm 0.020$) was added to the plates. The absorbance reading was taken exactly after initial mixing for 5 min. The optical density was measured at 734 nm on a microplate reader. The radical scavenging activity was determined by the following equation: % scavenging activity = $[A_{\text{blank control}} - A_{\text{sample}}] / A_{\text{blank control}} \times 100\%$.

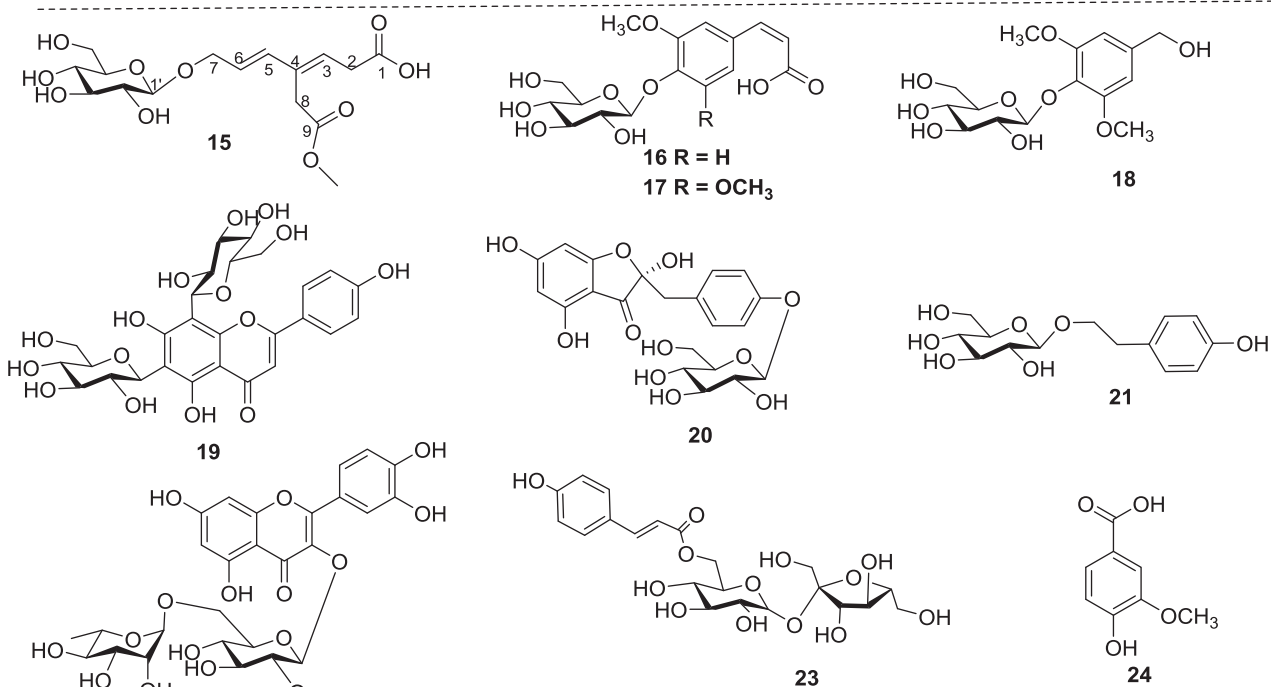
The SC_{50} value was obtained through GraphPad Prism 6.02. All determinations were carried out in triplicate.

2.5. α -Glucosidase assay

The α -glucosidase inhibitory activity assay was adapted as described previously (Yilmazer-Musa, Griffith, Michels, Schneider, & Frei, 2012). α -Glucosidase (0.26 U) from *Saccharomyces cerevisiae*



Alkaloids from *N. tangutorum*



Non-alkaloids from *N. tangutorum*

Fig. 1. Chemical structures of compounds 1–24.

were diluted in 0.1 M phosphate buffer (Na_2HPO_4 and KH_2PO_4 , pH 6.5). 25 μL of the test compound and 25 μL of α -glucosidase were preincubated in 96-well plates at 37 °C for 15 min. The reaction was initiated by adding 50 μL of 0.3125 mM pNPG (4-nitrophenyl α -D-glucopyranoside) as substrate. The plate was incubated for an additional 15 min at 37 °C, which was followed by the addition of 50 μL of 0.2 M Na_2CO_3 to stop the reaction. All test compounds were prepared in DMSO, and then diluted with the incubated buffer. Final concentrations of the tested compounds were between 6.25 and 250 $\mu\text{g}/\text{mL}$. The reaction was monitored by change of absorbance at 405 nm using a Dimension RxL Max clinical chemistry system Enspire MP150 (Siemens Healthineers).

2.6. Statistical analyses

Statistical elaborations were performed on Microsoft excel 2010 and GraphPad Prism 6.02 (GraphPad Software Inc., San Diego, CA, USA). All analyses were carried out in triplicate and the results are expressed as a mean \pm standard deviation (SD). Significant differences were determined by one-way ANOVA, using statistical package SPSS (Version 22.0, SPSS Inc., Chicago, IL, USA). The results were regarded as significantly different at $p < 0.05$.

3. Results and discussion

The fresh fruit juice of *N. tangutorum* was subjected to the column chromatography over D101, MCI gel CHP 20P, silica gel, and preparative HPLC to afford twenty-six compounds, including four new alkaloids (**1**, **2**, **3a**, and **3b**) and a new non-alkaloid component (**15**) (Fig. 1).

Tangutorid A (**1**) was isolated as a light brown solid, and its molecular formula was determined as $\text{C}_{17}\text{H}_{14}\text{O}_5$ with 12 degrees of unsaturation by HRESIMS spectrum. The UV spectrum of **1** displayed absorption maxima at 213, 237, 271, 287, and 343 nm, suggesting the presence of β -carboline-3-carboxylic acid moiety (Jiao et al., 2010). The IR absorption bands at 3426 and 1716 cm^{-1} were assignable to hydroxy and carbonyl functional groups, respectively. The ^1H NMR spectrum in $\text{DMSO}-d_6$ showed resonances of five aromatic protons between δ_{H} 9.17 and 7.36, three methylene groups [δ_{H} 4.20 (2H, s), 3.69 (3H, s) and, 2.94 (2H, brs)], and a broad NH singlet at δ_{H} 12.25. The ^{13}C NMR and HSQC spectra revealed 17

resonances between δ_{C} 210.2 and 31.5, which were attributed to nine quaternary carbons, five methines and three methylene groups. Based on the ^1H - ^1H COSY, HMBC (Fig. 2), and HSQC spectra, a set of four mutually coupled aromatic protons (δ_{H} 8.45–7.36) were assigned to the unsubstituted A ring of a β -carboline-3-carboxylic acid skeleton. The singlet proton at δ_{H} 9.17 was assigned as H-4 of a β -carboline-3-carboxylic acid by the HMBC correlations from the isolated aromatic proton to δ_{C} 166.2 (C-3a), 120.2 (C-6), and 135.0 (C-13). The above information confirmed the existence of β -carboline-3-carboxylic acid moiety. In addition, the correlations due to a $-\text{CH}_2-\text{CH}_2-$ moiety could be observed in the ^1H - ^1H COSY spectrum. Combined with 2J and 3J correlations from δ_{H} 3.69 (H₂-16) to δ_{C} 201.5 (C-14), from δ_{H} 2.94 (H-15) to δ_{C} 210.2 (C-17), and from δ_{H} 4.20 (H-18) to δ_{C} 210.2 (C-17) in HMBC experiment, a $-\text{CO}-(\text{CH}_2)_2-\text{CO}-\text{CH}_2\text{OH}$ fragment was constructed. Since the two separate moieties, β -carboline-3-carboxylic acid and $-\text{CO}-(\text{CH}_2)_2-\text{CO}-\text{CH}_2\text{OH}$ were fully defined by the NMR data, C-1 must connect with the carbonyl group (C-14), which was also confirmed through the shielded C-1 signal at δ_{C} 134.6. In conclusion, the structure of **1** was fully established as shown in Fig. 1 and named as tangutorid A.

Tangutorid B (**2**) was yielded as a light brown solid. Its molecular formula was determined as $\text{C}_{17}\text{H}_{18}\text{N}_2\text{O}_5$ on the basis of its NMR and HRESIMS spectra. It was found to possess similar UV and IR spectroscopic characteristics to those of **1**. Moreover, the ^1H and ^{13}C NMR spectra of compound **2** also resembled those of **1**, except that the resonances of two carbonyl signals (C-14 and C-17) in **1** were replaced by two oxymethines (δ_{H} 5.11, brs; δ_{C} 73.0, C-14 and δ_{H} 3.42, brs; δ_{C} 71.5, C-17) in **2**. All NMR signals of **2** can be identified by HSQC, ^1H - ^1H COSY and HMBC experiments (Table 1 and Fig. 2). Therefore, the structure of **2** was determined as shown in Fig. 1 and named as tangutorid B.

Tangutorids C and D (**3a** and **3b**) were both yielded as light brown solid and had the same molecular weight. Their molecular formulas were determined as $\text{C}_{17}\text{H}_{18}\text{N}_2\text{O}_6$ on the basis of NMR and HRESIMS spectra. Through extensive comparison of NMR spectroscopic data and molecular weight of **3a** and **3b** with those of **2**, we speculated that a methylene in **2** was substituted by a hydroxyl group in **3a** and **3b**. 2D NMR spectra confirmed that the hydroxyl group was located at C-16 (Table 1 and Fig. 2). Thus, compounds **3a** and **3b** had the same planar structure. But significant variations

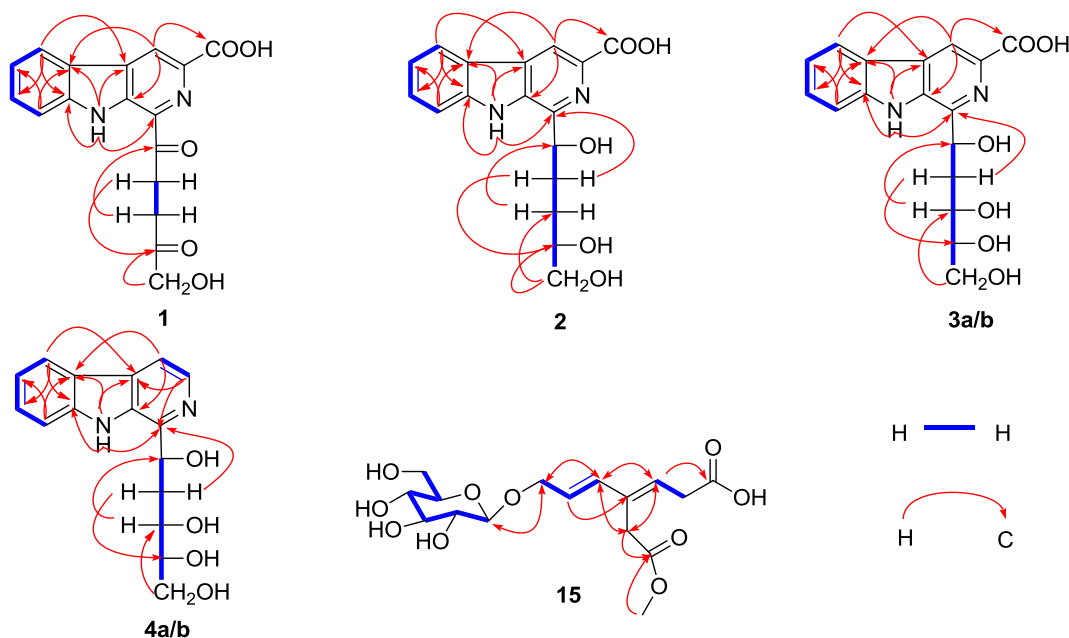


Fig. 2. Key HMBC and ^1H - ^1H COSY correlations of **1**–**4** and **15**.

Carbone, Plaza, Zampelli, & Pizza, 2002), 9H-carbazole (**9**) (Suzuki, Hirano, Satoh, & Miura, 2015), tryptophan (**10**) (Lee & Pbillips, 1992), N^1 -(β -D-glucopyranosyl- 4C_1)-L-tryptophan (**11**) (Diem, Bergmann, & Herderich, 2000), L-valyl-L-tryptophan anhydride (**12**) (Pedras, Smith, & Taylor, 1998), N -(1-deoxy- α -D-fructos-1-yl)-L-tryptophan (**13**) (Sayed et al., 2008), N -(1-deoxy-D-fructos-1-yl)-L-phenylalanine (**14**) (Röper, Röper, & Heyns, 1983), (2Z) 3-[4-(β -D-glucopyranosyloxy)-3,5-dimethoxyphenyl]-2-propenoic acid (**15**) (Uda, Ozawa, Takayama, Suzuki, & Maeda, 1998), *cis*-feruloyl-4- β -glucoside (**16**) (Park & Tomohiko, 2011), 3,5-dimethoxy-4-hydroxybenzyl alcohol 4- O - β -D-glucopyranoside (**18**) (Kitajima et al., 1998), apigenin 6,8-di- C - β -D-glucopyranoside (**19**) (Hu et al., 2006), maesopsin (**20**) (Li, Cai, & Wu, 1997), tyrosol 8- O - β -D-glucopyranoside (**21**) (Landtag et al., 2002), quercetin 3- O -(2G-rhamnosylrutinoside) (**22**) (Kazuma,

Noda, & Suzuki, 2003), 6- O -*p*-coumaroyl- β -fructofuranosyl-(2 \rightarrow 1)- α -L-glucopyranoside (**23**) (Georgopoulou, Aligiannis, Fokialakis, & Mitaku, 2005), vanillic acid (**24**) by comparison of their NMR and MS data with those reported in the literatures.

Tangutorids A–F (**1**, **2**, **3a**, **3b**, **4a**, and **4b**) are the first examples of glucose-derived β -carbolines isolated from natural product. The biosynthetic pathways for **1–8** were proposed starting from the co-isolated tryptophan (**10**) and corresponding aldehydes through Pictet–Spengler reaction. (Fig. 3) In the acidic environment of fruit, tryptophan (**10**) and acetaldehyde generate compounds **7** and **8** via formation of an iminium cation intermediate and cyclization probably through spiroindolenium species. Through the same mechanism, the intermediate **ia** was formed by the condensation between tryptophan (**10**) and glucose. Intermediate **ia** underwent dehydration reaction to produce **ib**. Then **ib** proceeded through

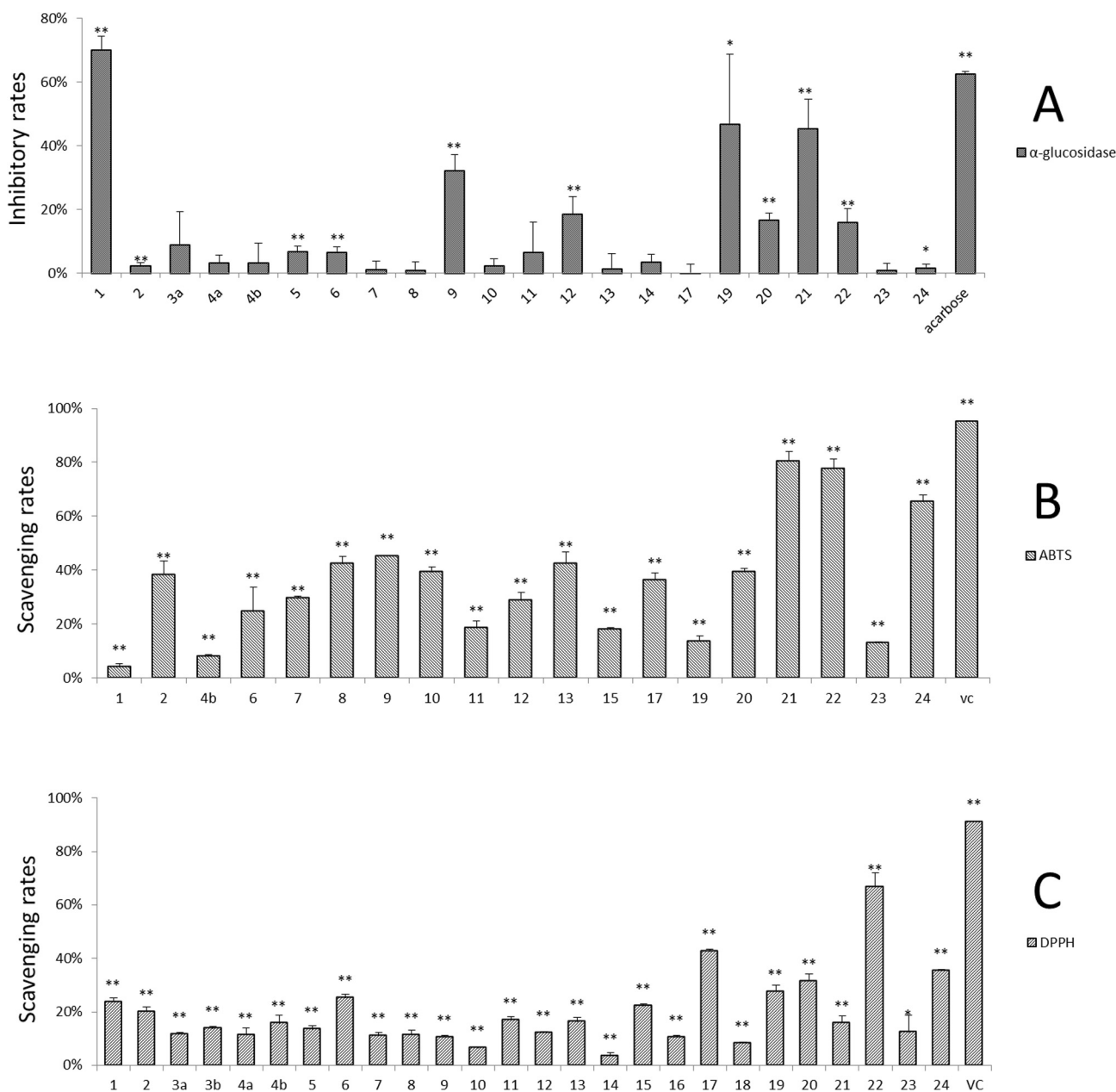


Fig. 4. (A) Inhibition rates of compounds **1–3a**, **4a–14**, **17–24**, and positive control acarbose against the α -glucosidase at a concentration of 250 μ g/mL; Compounds **3b**, **15**, and **16** showed no activity at a concentration of 250 μ g/mL; (B) ABTS free radical scavenging rates of compounds **1**, **2**, **4b**, **6–13**, **15**, **17**, **19–24** and positive control ascorbic acid (VC) at a concentration of 50 μ g/mL; Compounds **3a–4a**, **5**, **14**, **16** and **18** showed no activity at a concentration of 50 μ g/mL; (C) DPPH free radical scavenging rates of compounds **1–24** and VC at a concentration of 50 μ g/mL; * p < 0.05 and ** p < 0.01 versus blank control.

enolization to the ketone **ic**. Compounds **3a** and **3b** were generated from **ic** through reduction reaction followed by double oxidation (aromatization) reactions. Compounds **3a** and **3b** underwent decarboxylation reaction to give compounds **4a** and **4b**, respectively. On the other hand, **ib** was further dehydrated to yield another intermediate **id**. Intermediate **id** was transformed into **ie** or **if** in solution via enolization. Subsequently, compound **1** was formed through aromatization of **ie**. Compound **2** was yielded from intermediate **ie** through the hydrogenation reduction followed by aromatization reaction. Moreover, compound **5** was obtained from intermediate **if** by cyclization, dehydration and aromatization reactions in sequence. Finally, compound **5** proceeded with a decarboxylation reaction to give compound **6**. Thus, β -carboline derivatives in many fruits may be produced through Pictet–Spengler reactions using naturally occurring tryptophan (**10**) and corresponding aldehydes including glucose as precursor substance.

Nowadays, more and more people believe that the consumption of vegetables and fruits have the potential ability to protect against diseases such as cancer, cardiovascular and metabolic diseases. This is attributed to antioxidants such as vitamins C and E, carotenoids, polyphenols, and flavonoids in vegetables and fruits. (Herraiz & Galisteo, 2003) The ripe fruit of *N. tangutorum* has been used as treatment for dyspepsia and hyperglycemia by folks. Herein, all isolates from the fruit juice of *N. tangutorum* were evaluated in vitro for their antioxidant and α -glucosidase inhibitory activities with ascorbic acid and acarbose as positive control, respectively. Results of the primary screening assay showed that compounds **21**, **22** and **24** exhibited relatively better radical scavenging effect at the concentration of 50 $\mu\text{g}/\text{mL}$ and compound **1** revealed the highest inhibition rate against α -glucosidase at the concentration of 250 $\mu\text{g}/\text{mL}$. (Fig. 4) These compounds were further assayed to obtain the SC_{50} and IC_{50} values for antioxidant and α -glucosidase inhibitory activities, respectively. In the ABTS radical scavenging test, compounds **21**, **22** and **24** showed potent antioxidant activity with SC_{50} values of 12.2 ± 1.9 , 24.6 ± 1.2 and 30.4 ± 2.7 $\mu\text{g}/\text{mL}$, respectively. In the DPPH radical scavenging assay, compound **22** demonstrated strong antioxidant activity with SC_{50} value of 16.7 ± 2.3 $\mu\text{g}/\text{mL}$. And the positive control ascorbic acid showed antioxidant activity against ABTS and DPPH radical with SC_{50} values of 7.2 ± 0.5 and 6.3 ± 0.3 $\mu\text{g}/\text{mL}$, respectively. In addition, compound **1** showed strong α -glucosidase inhibitory activity with IC_{50} value of 63.3 ± 4.6 $\mu\text{g}/\text{mL}$, and acarbose was used as positive control with IC_{50} value of 118.4 ± 12.7 $\mu\text{g}/\text{mL}$. According to results, we find that the glucose-derived β -carboline derivative with ketone groups showed better α -glucosidase inhibitory activity than others and the α -glucosidase activity will decline a lot when the ketone groups were replaced by hydroxyl groups. This might indicate that the ketone group is an important functional group which could enhance the α -glucosidase inhibitory activity.

4. Conclusions

In summary, five new compounds, including four glucose-derived β -carbolines, tangutorids A–D (**1**, **2**, **3a** and **3b**), and (3E,5E)-7-O- β -glucosyl-4-(2-methoxy-2-oxoethyl)hepta-3,5-dienoic acid (**15**) were isolated from fruit juice of *N. tangutorum* together with twenty-one known ones. Tangutorids A–F stand for a class of glucose-derived β -carboline derivatives isolated from natural products. Moreover, the antioxidant and α -glucosidase inhibitory activities of all isolated compounds were evaluated, and the results showed that some of them revealed potential bioactivity. Based on the compounds isolated in this study, the biogenetic pathways of β -carboline derivatives in *N. tangutorum* were proposed to involve Pictet–Spengler reactions and described starting from tryptophan and corresponding aldehydes. In short, the

current study adds to understanding of the chemical composition and biological effects of this popularly consumed fruit juice.

Notes

The authors declare no competing financial interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.foodchem.2017.01.031>.

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