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Isolation and Identification of Bioactive Compounds from the Tuber of *Brassica oleracea* var. *gongylodes*

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Abstract – *Brassica oleracea* var. *gongylodes* (red kohlrabi) is a biennial herbaceous vegetable whose edible bulbotuber-like stem and leaves are consumed globally. Sliced red kohlrabi tubers were extracted using methanol and the concentrated extract was partitioned successively with dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc), *n*-butanol (*n*-BuOH) and water (H_2O). Repeated column chromatography of EtOAc fraction through silica, sephadex LH-20 and RP-18 gel led to isolation of eleven compounds of which compound **1** was a new glycosylated indole alkaloid derivative, 1-methoxyindole 3-carboxylic acid 6-*O*-β-D-glucopyranoside. Others were known compounds namely, β-sitosterol glucoside (**4**), 5-hydroxymethyl-2-furaldehyde (**5**), methyl-1-thio-β-D-glucopyranosyl disulfide (**6**), 5-hydroxy-2-pyridinemethanol (**7**), (3*S*,4*R*)-2-deoxyribonolactone (**8**), *n*-butyl-β-D-fructopyranoside (**9**), uridine (**10**) and three fructose derivatives, D-tagatose (**11**), β-D-fructofuranose (**12**) and β-D-fructopyranose (**13**). Similarly, isolation from CH_2Cl_2 fraction gave two known indole alkaloids, indole 3-acetonitrile (**2**) and *N*-methoxyindole 3-acetonitrile (**3**). The structure elucidation and identification of these compounds were conducted with the help of ^{13}C and ^{1}H NMR, HMBC, HMQC, EIMS, HR-ESIMS and IR spectroscopic data, and TLC plate spots visualization. Compounds **2**, **3**, **4**, **5**, **6**, **7**, **8** and **9** are noted to occur in kohlrabi for the first time. Different bioactivities of these isolated compounds have been reported in literature.
Keywords – *Brassica oleracea* var. *gongylodes*, 1-methoxyindole 3-carboxylic acid 6-*O*-β-D-glucopyranoside, bioactive compounds

Introduction

Brassica oleracea var. *gongylodes* (red kohlrabi), also known as German cabbage or cabbage turnip or turnip kale, grows as an annual or a biennial crop with unbranched, shortened, swollen, sub-globose to globose, fleshy bulbo-tuber-like stem and highly branched root system. Though the cultivation of kohlrabi was reported to origin in north-western Europe, it is now produced in Europe, North America, Canada and several parts of Asia. Edible parts of the plant comprise of fleshy stem and leaves that are consumed either raw as salads or cooked as curry.[1,2]

The phytochemical investigation of kohlrabi extract by employing HPLC and GC-TOFMS identified the presence of anthocyanins, glucosinolates, phenylpropanoids, and carotenoids along with organic acids, amino acids, sugars and amines in purple and pale green kohlrabi cultivars, except for the absence of anthocyanins in pale green kohlrabi.[3,4] The metabolic profiling of kohlrabi extract has assisted in understanding the general composition of kohlrabi cultivars, however, phytochemical studies of *B. oleracea* var. *gongylodes*, involving isolation of compounds are scarce. Though numerous studies are available on the compounds derived from vegetables of *Brassica* genus, the investigations on the phytoconstituents have yet to offer the identification of more compounds. UV-treatment as well as the supplementation of radioactive L- [β-^{14}C] tryptophan and L-[$^{14}CH_3$] methionine to UV-irradiated stem tuber of kohlrabi provided six distinguishable phytoalexins in the chloroform extract of treated tuber, namely, methoxybrassitin, methoxybrassinin, cyclobrassinin, cyclobrassinon, spirobrassinin and 1-methoxyspirobrassi-nin.[5] Column chromatography (CC) of ethyl acetate faction of methanol extract resulted in the isolation of three sterols, β-sitosterol, brassicasterol and ketobrassicasterol.[6] From butanol fraction of kohlrabi sprouts, Lee *et al.* isolated four phenylpropanoids, which were 3-(3, 4, 5-

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trimethoxyphenyl)-2*E*-propenoic acid methyl ester (**a**), (*E*)-sinapic acid methyl ester, (*E*)-sinapoyl glucoside (**c**) and lawsoniaside B (**d**). Of these, three compounds (**a**, **c** and **d**) significantly inhibited nitric oxide (NO) production in raw 264.7 macrophage cells, thus indicating the anti-inflammatory effect.[7]

Our previous study demonstrated anti-diabetic, anti-inflammatory and anti-oxidant effects of the methanolic extract of the red and the green kohlrabi cultivars and on comparison, we found red kohlrabi extract to have more significant activities.[8] Likewise, kohlrabi was shown to be anti-adipogenic,[9] anti-hyperglycemic,[10] anti-hyperlipidemic,[10] anti-oxidant[10-13] and anti-proliferative.[11] Different studies have illustrated the pharmacological benefits of kohlrabi associated with the primary and secondary metabolites of the plant.[1,3]

Thus, this study aimed to explore the chemical constituents of red kohlrabi's stem tuber that are responsible for the reported bioactivities. Repeated column chromatography of the EtOAc fraction of methanolic extract of kohlrabi tuber led to the isolation of a new glycoside derivative of indole alkaloid, 1-methoxyindole 3-carboxylic acid 6-*O*-β-D-glucopyranoside (**1**), whose structure was determined using spectroscopic analyses. In addition, ten known compounds viz. β-sitosterol glucoside (**4**), 5-hydroxymethyl-2-furaldehyde (**5**), methyl-1-thio-β-D-glucopyranosyl disulfide (**6**), 5-hydroxy-2-pyridine-methanol (**7**), (3*S*,4*R*)-2-deoxyribonolactone (**8**), *n*-butyl-β-D-fructopyranoside (**9**), uridine (**10**) and three fructose derivatives, D-tagatose (**11**), β-D-fructofuranose (**12**) and β-D-fructopyranose (**13**) were isolated from EtOAc fraction whereas two known indole alkaloids viz. indole 3-acetonitrile (**2**) and *N*-methoxyindole 3-acetonitrile (**3**) were obtained from CH_2Cl_2 fraction.

Experimental

General experimental Procedures – Optical rotation was determined using P-2000 polarimeter (JASCO, Japan). UV-Vis absorption was measured using Biochrom Libra S22 UV/Vis spectrophotometer. FTIR was measured with FT-4100 (JASCO, Japan). 1D and 2D ^1H and ^{13}C NMR spectra were recorded using JEOL JNM ECP-600/400 spectrometer (Tokyo, Japan) at 150 MHz and 100 MHz using deuterated methanol (CD_3OD) and pyridine (C_5D_5N). HR-ESI-MS spectra was obtained from a JEOL JMS-700 spectrometer (Tokyo, Japan). EIMS was recorded using GCMS QP-2010 Ultra (Shimadzu, Japan). Column chromatography (CC) was conducted using silica (SiO_2) gel 60 (70-230 mesh, Merck, Darmstadt, Germany), sephadex LH-20 (20-100 μM, Sigma, St. Louis, MO, USA) and LiChroprep RP-18 (40-63 μM, Merck, Darmstadt, Germany). Thin layer chromatography (TLC) was performed with precoated Merck Kiesel gel 60 F_{254} plates and RP-18 F_{254S} plates, using 25% sulfuric acid as spray reagent for heating. TLC plates were visualized in UV chamber (UVItec, Cambridge CB4 1QB, UK) at 365 nm and 254 nm. All solvents used for CC were of reagent grade and purchased from commercial suppliers.

Plant material – Red kohlrabi tubers *(B. oleracea* var. *gongylodes*) were bought from a local retailer of Busan, S. Korea in October, 2013 and authenticated by Prof. Jae Sue Choi (Pukyong National University, Busan, South Korea). A voucher specimen 20131029 was deposited in the authorized laboratory (J.S. Choi).

Extraction and isolation – Extract of red kohlrabi tuber (754.13 g) was obtained by refluxing 5.0 kg of sliced tuber in methanol (CH_3OH) for 3 hours (5L × 2 times) and drying using rotatory vacuum evaporator at 40 °C (Laborota 4000, Heidolph, Germany). The dried methanolic extract was suspended in distilled water (H_2O) and partitioned successively with dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc) and *n*-butanol (*n*-BuOH) to yield CH_2Cl_2 (2.1 g), EtOAc (14.5 g) and *n*-BuOH (30.4 g) fractions.

EtOAc fraction (14.5 g) was subjected for normal phase silica gel column chromatography (CC) using stepwise gradient elution of CH_2Cl_2: CH_3OH: H_2O from 10:1:0.1 to 2:1:0.1 to 0:1:0, v/v/v, to give eight sub-fractions (SF). SF-2 (1.4 g) was chromatographed using SiO_2 gel with successive increase in polarity of hexane: ethyl acetate (H: E 7:1 → 1:1) solvent system and purified using sephadex LH-20 and RP-18 to obtain compounds **4** (36 mg) and **5** (120 mg). Repeated CC of SF-3 (1.15 g) through SiO_2, RP-18 and sephadex LH-20 gave **8** (120 mg). From SF-4 (1.20 g), compounds **6** (200 mg) and **7** (20 mg) were isolated by running through RP-18 gel using 50% - 0% CH_3OH and purified by using silica gel using CH_2Cl_2: CH_3OH: H_2O 10:1:0.1. SF-5 (3.13 g) was chromatographed through sephadex LH-20 using methanol and the resulting sub-fractions were run through SiO_2 gel using EtOAc: CH_3OH: H_2O 40:1:0 → 24:4:3 to separate compounds **1** (12 mg), **9** (32 mg) and **10** (10 mg), which were purified by eluting through RP-18 gel. Repeated CC using 50% - 20% CH_3OH for RP gel and EtOAc: CH_3OH: H_2O 20:1:0 → 21:5:3 of SF-6 (1.23 g) further yielded compounds **11** (85 mg) and **12** (36 mg). Likewise, SF-7 (2.61 g) when chromatographed through SiO_2 gel using EtOAc: CH_3OH: H_2O and purified by eluting through RP-18 and sephadex LH-20 with

increasing concentration of methanol gave **13** (650 mg).

CH_2Cl_2 fraction when chromatographed using SiO_2 gel with gradient elution by H: E 50:1 gave eight sub-fractions. D-SF-8 and D-SF-3 by repeated column chromatography using SiO_2 gel and sephadex LH-20 resulted in the isolation of **2** (8 mg) and **3** (6 mg) respectively.

Acid hydrolysis of compound 1 – This experiment was performed as described by Sun *et al.*[14] Briefly, compound **1** and reference sugar, D-(+)-glucose (Sigma Aldrich, Merck, USA) were dissolved in methanol, and spotted on silica gel plate. The plate was fumigated by hydrochloric acid fumes for 30 minutes, followed by expansion of the plate by EtOAc: CH_3OH: H_2O 21:4:3. The TLC spot of compound **1** was compared with that of authentic sugar, which showed the glucose in the compound to be D-glucose (*Rf* = 0.13).

1-Methoxyindole 3-carboxylic acid 6-*O*-β-D-glucopyranoside (1) – Brown solid. $[\alpha]_D^{22.5}$: -5.28° (c 0.006, CH_3OH); UV (CH_3OH) λ_{max} (logε) 228 (4.70), 288 (4.23); IR (KBr) ν_{max}, cm^{-1}: 808, 1005-1069, 1221, 1365, 1521, 1663, 2852, 2921, 3341; ^{13}C NMR and ^1H NMR see Table 1; HR-ESI-MS *m/z* 392.0953 $[M+Na]^+$ (calcd for $C_{16}H_{19}NNaO_9$, 392.0952).

Indole 3-acetonitrile (2) – White solid. UV (CH_3OH) λ_{max} 216, 244, 295; ^1H NMR (600 MHz, CD_3OD) δ: 7.57 (1H, d, *J* = 8.3 Hz, H-4), 7.37 (1H, d, *J* = 8.3 Hz, H-7), 7.23 (1H, s, H-2), 7.15 (1H, t, *J* = 7.2 Hz, H-6), 7.07 (1H, t, *J* = 7.6 Hz, H-5), 3.93 (2H, s, H-9); ^{13}C NMR (150 MHz, CD_3OD) δ: 138.2 (C-7a), 127.5 (C-3a), 124.4 (C-2), 123.0 (C-6), 120.3 (C-5) 120.0 (C-10), 118.8 (C-4), 112.6 (C-7), 105.1 (C-3), 14.2 (C-9); EI-MS *m/z* 156 $[M]^+$, 131, 101, 117, 101, 89, 77, 63.

***N*-Methoxyindole 3-acetonitrile (3)** – White solid. ^1H NMR (600 MHz, CD_3OD) δ: 7.60 (1H, d, *J* = 7.6 Hz, H-4), 7.43 (1H, d, *J* = 10.3 Hz, H-7), 7.25 (1H, t, *J* = 8.3, 15.1 Hz, H-6), 7.13 (1H, dd, *J* = 1.4, 15.1 Hz, H-5), 7.12 (1H, s, H-2), 4.07 (3H, s, N-OCH_3), 3.93 (2H, s, H-10); ^{13}C NMR (150 MHz, CD_3OD) δ: 133.8 (C-7a), 124.1 (C-7), 123.9 (C-3a), 123.2 (C-6), 121.3 (C-5), 119.6 (C-4), 119.5 (C-11), 109.5 (C-2), 102.1 (C-3), 66.5 (N-OCH_3), 14.0 (C-11); EI-MS *m/z* 186 $[M]^+$, 171, 155, 146, 128, 116, 101, 77, 63; HR-ESI-MS *m/z* 209.0689 $[M+Na]^+$.

***β*-Sitosterol glucoside (4)** – Brown powder. Confirmed by TLC comparison with reference compound.

5-Hydroxymethyl-2-furaldehyde (5) – Yellow oil. ^1H NMR (600 MHz, CD_3OD) δ: 9.25 (1H, s, -CHO), 7.38 (1H, d, *J* = 3.5 Hz, H-3), 6.58 (1H, d, *J* = 4.1 Hz, H-4), 4.60 (2H, s, H-6); ^{13}C NMR (150 MHz, CD_3OD) δ: 179.4 (C-1), 163.2 (C-5), 153.9 (C-2), 124.8 (C-3), 110.8 (C-4), 57.5 (C-6).

Methyl-1-thio-β-D-glucopyranosyl disulfide (6) – Colorless gum. ^1H NMR (C_5D_5N, 600 MHz) δ: 2.57 (3H, s, CH_3S), 5.08 (1H, d, *J* = 8.9 Hz, H-1), 4.01 (1H, m, H-2), 4.37 (1H, d, *J* = 5.4 Hz, H-3), 4.34 (1H, d, *J* = 5.4 Hz, H-4), 4.29 (1H, q, *J* = 2.1, 2.8, 3.4, 8.2 Hz, H-5), 4.57 (1H, dd, *J* = 2.7, 12.0 Hz, H-6a), 4.55 (1H, dd, *J* = 2.1, 12.0 Hz, H-6b); ^{13}C NMR (C_5D_5N, 150 MHz) δ: 93.1 (C-1), 73.4 (C-2), 80.6 (C-3), 71.9 (C-4), 83.6 (C-5), 63.4 (C-6), 25.1 (C-7); EI-MS *m/z* 163, 145, 127, 85, 73, 61, 45; ESI-MS *m/z* 265.0162 $[M+Na]^+$ (calcd for $C_7H_{14}NaO_5S_2$, 265.0175).

5-Hydroxy-2-pyridinemethanol (7) – White crystal. ^1H NMR (CH_3OD, 600 MHz) δ: 8.01 (1H, d, *J* = 3.0 Hz, H-6), 7.36 (1H, *J* = 9.0 Hz, H-3), 7.23 (1H dd, *J* = 3.6, 5.4 Hz, H-4), 4.58 (2H, s, H-7); ^{13}C NMR (CH_3OD, 150 MHz) δ: 137.2 (C-1), 154.7 (C-2), 123.3 (C-3), 125.0 (C-4), 152.5 (C-5), 35.2 (C-6); EI-MS *m/z* 124 [M]+.

(3*S*,4*R*)-2-Deoxyribonolactone (8) – Colorless semisolid. $[\alpha]_D^{25}$: +0.128° (c 0.013, CH_3OH); ^1H NMR (CH_3OD, 600 MHz) δ: 2.37 (1H, dd, *J* = 2.8, 17.9 Hz, H-2), 2.91 (1H, dd, *J* = 6.9, 17.9 Hz, H-2), 3.75 (1H, dd, *J* = 4.1, 12.4 Hz, H-5), 3.68 (1H, dd, *J* = 2.8, 12.4 Hz, H-5), 4.36 (1H, q, *J* = 3.5, 2.7, 2.4 Hz, H-3), 4.42 (1H, m, H-4); ^{13}C NMR (CH_3OD, 150 MHz) δ: 178.6 (C-1), 39.2 (C-2), 70.1 (C-3), 91.2 (C-4), 64.5 (C-5); HR-ESI-MS *m/z*: 155.0318 $[M+Na]^+$ (calcd for $C_5H_8NaO_4$, 155.0135); EI-MS *m/z* 101, 83,57,43.

***n*-Butyl-β-D-fructopyranoside (9)** – Amorphous white powder. ^1H NMR (CD_3OD, 400 MHz) δ: 3.90 (1H, d, *J* = 9.6 Hz, H-3), 3.82 (1H, q, H-5), 3.77 (1H, dd, *J* = 4.6, 3.2 Hz, H-4), 3.74 (1H, dd, *J* = 4.2, 2.1 Hz, H-6a), 3.73 (1H, d, *J* = 4.1 Hz, H-1a), 3.69 (1H, d, *J* = 11.6 Hz, H-1b), 3.64 (1H, dd, *J* = 12.0, 1.4 Hz, H-6b), 3.49 (2H, td, *J* = 9.2, 6.8 Hz, H-1'), 1.56 (2H, m, H-2'), 1.40 (2H, m, H-3'), 0.99 (3H, t, H-4'); ^{13}C NMR (CD_3OD, 100 MHz) δ: 101.6 (C-2), 71.5 (C-4), 71.1 (C-5), 70.6 (C-3), 65.2 (C-6), 63.5 (C-1), 61.6 (C-1'), 33.3 (C-2'), 20.5 (C-3'), 14.3 (C-4').

Uridine (10) – White crystal. ^1H NMR (CD_3OD, 600 MHz) δ: 7.69 (1H, d, *J* = 7.8 Hz, H-6), 5.88 (1H, d, *J* = 4.8 Hz, H-1'), 5.66 (1H, d, *J* = 7.2 Hz, H-5), 4.16 (1H, t, *J* = 4.8, 9.6 Hz, C-3'), 4.13 (1H, t, *J* = 4.8, 10.2 Hz, H-2'), 3.97 (1H, m, H-4'), 3.83 (1H, dd, *J* = 2.4, 12.3 Hz, H-5'), 3.72 (1H, dd, *J* = 3.6, 14.4 Hz, H-5'); ^{13}C NMR (CD_3OD, 150 MHz) δ: 157.1 (C-2), 172.7 (C-4), 103.0 (C-5), 141.8 (C-6), 91.5 (C-1'), 75.8 (C-2'), 71.2 (C-3'), 86.1 (C-4'), 62.4 (C-5'); EI-MS *m/z* 155, 141, 127, 113, 111, 98, 85,71, 57, 43.

D-Tagatose (11) – Colorless syrup. EIMS *m/z*: 119, 103, 86, 73, 60, 43; Confirmed by TLC comparison with

compounds **12** and **13**, and EI-MS library search.

β-D-Fructofuranose (12) – Colorless syrup. ^{1}H NMR (CH_3OD, 600 MHz) δ: 4.91 (1H, d, J = 8.3 Hz, H-3), 3.93 (1H, t, J = 15.1, 7.5 Hz, H-4), 3.73 (1H, m, H-5), 3.69 (1H, s, H-1), 3.64 (1H, s, H-1), 3.58 (1H, dd, J = 6.8, 11.6 Hz, H-6), 3.53 (1H, dd, J = 6.6, 11.3 Hz, H-6); ^{13}C NMR (CH_3OD, 150 MHz) δ: 61.5 (C-1), 105.2 (C-2), 78.7 (C-3), 77.2 (C-4), 83.5 (C-5), 64.7 (C-6).

β-D-Fructopyranose (13) – Viscous light yellow syrup. Confirmed by comparing TLC with reference.

Result and Discussion

The methanol extract of *B. oleracea* var. *gongylodes* (red kohlrabi tuber) was fractionated using CH_2Cl_2, EtOAc, *n*-BuOH and H_2O. CH_2Cl_2 fraction and EtOAc fraction were utilized for the isolation of compounds. Through repeated open column chromatography using SiO_2, sephadex LH-20 and RP-18 gel as stationary phase, a new compound (**1**) along with ten known compounds (**4 - 13**) were isolated from the EtOAc fraction while two compounds (**2**, **3**) were isolated from CH_2Cl_2 fraction, which were identified based on the spectral information, literature, and assessment of the spots developed by the compounds and their respective standard or reference on the TLC plates. The structures of isolated compounds are illustrated in Fig. 1.

Compound **1** was obtained as brown solid, soluble in methanol. The HR-ESI-MS gave a *pseudo*-molecular ion peak at *m/z* 392.0953 $[M+Na]^+$, corresponding to an elemental formula of $C_{16}H_{19}NNaO_9$, *m/z* 392.0952, with eight degree of unsaturation (Fig. 2). The UV-Vis spectrum revealed absorption bands at λ_{max} (logε) of 228 (4.70) and 288 (4.23), suggesting the indole moiety in the compound.[15,16] Optical rotation was measured as $[\alpha]_D^{22.5}$: -5.28° (c 0.006, CH_3OH). IR spectrum of **1** showed the absorption peaks indicative of different functional groups such as secondary cyclic alcohol (1005 - 1069 cm^{-1}), aryl alkyl ester (1221 cm^{-1}), aromatic amine C-N (1365 cm^{-1}), vinyl (1517 cm^{-1}), aromatic ring (1675 cm^{-1}) and methoxy (2852 cm^{-1}).

Structure of compound **1** was elucidated through the analysis of ^{1}H and ^{13}C NMR, heteronuclear correlation spectroscopy such as HMQC and HMBC, accompanied by comparison of spectral data with known indole compounds.[14-17] The NMR spectroscopic observations are summarized in Table 1 and HMBC correlation is illustrated in Fig. 2. ^{1}H NMR spectrum exhibited signals of an ABX system of aromatic protons at δ_H 8.04 (1H, d, J = 8.9 Hz, H-4), 7.01 (1H, dd, J = 8.5, 2.0 Hz, H-7) and 7.21 (1H, d,

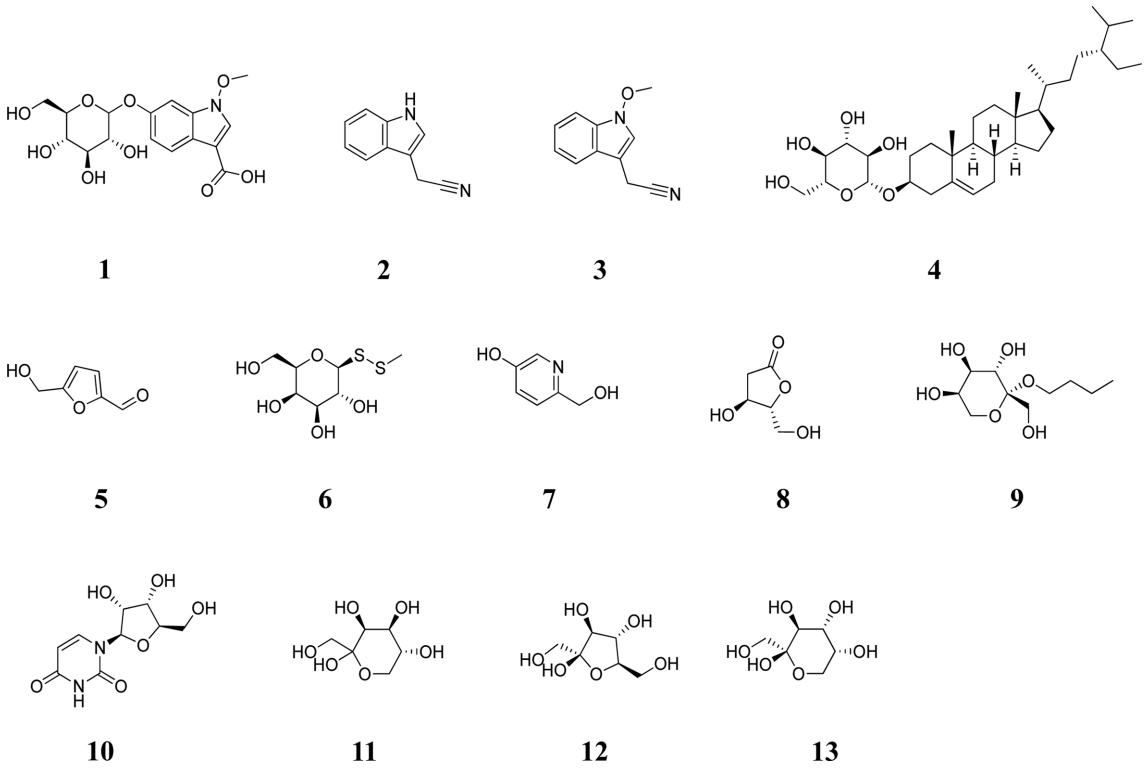

Fig. 1. Structures of **1** – **13** isolated from *Brassica oleracea* var. *gongylodes*.

Table 1. ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data of **1** with HMBC correlations

Position	δ_H (*J* in Hz)	δ_C	HMBC
	N-methoxy-indole-3-carboxylate		
N-O-CH_3	4.11 (3H, s)	66.9	
2	7.90 (1H, s)	128.8	133.90, 120.3, 108.0, 96.9, 170.9
3		108.0	
3a		120.2	
4	8.037 (1H, d, *J* = 8.9 Hz)	123.5	156.2, 133.9, 96.9, 108.0, 114.3, 120.2
5	7.01 (1 H; dd ; *J* = 8.5, 2 Hz)	114.3	120.2, 96.9, 156.2
6		156.2	
7	7.21 (1H, d, *J* = 2.1 Hz)	96.9	114.3, 120.2, 156.2, 133.9
7a		133.9	
3-COOH		170.9	
	Glucopyranoside		
1'	4.95 (1H, d, *J* = 7.6 Hz)	103.2	156.2, 78.3, 71.6
2'	3.37 (m)	78.3	78.1, 62.6, 75.0
3'	3.47 (m)	75.0	103.2, 78.3
4'	3.49 (m)	78.1	71.6, 103.2
5'	3.39 (m)	71.6	62.6
6'	3.92 (1H, dd, *J* = 12.0, 2.4 Hz) 3.69 (1H, dd, *J* = 6.0, 2.4 Hz)	62.6	71.6, 78.1

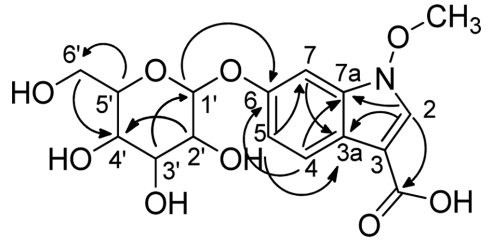

Fig. 2. Key HMBC correlations (→) in the structure of **1**.

J = 2.1 Hz, H-5), and an aromatic proton of indole ring at δ_H 7.90 (1H, s, H-2) along with a methoxy group at δ_H 4.11 (3H, s). ^{13}C NMR displayed resonance peaks at δ_C 170.9 and 66.9 indicating the presence of carboxylate and *N*-methoxy groups in the structure of compound **1** along with the eight aromatic carbons signals in the downfield chemical shifts (δ_C 96.9 to δ_C 156.2). These obtained NMR signals for aglycone part closely resembled with *N*-methoxyindole carboxylic acid, which was previously known to occur in cruciferous plants.[15] To determine definite structure of compound, 2D NMR spectroscopic analyses, HBQC and HMBC, were made which helped in the assignment of protons and carbons. From HMQC spectrum, four aromatic quaternary carbons (δ_C 156.3, 133.9, 120.2, 108.0) and one methoxy carbon (δ_C 66.9) in the structure of **1** were confirmed. The HMBC spectrum showed long range correlation of proton at δ_H 7.90 (1H, s, H-2) with carbons at δ_C 170.88 and 108.02, confirming the presence of -COOH group in C-3 position. The single bond C-H correlation spectroscopy exhibited cross-peak of methoxy proton (δ_H 4.11) with δ_C 66.9, which showed no long range coupling with other protons and carbons in HMBC spectrum, thus affirming the position of methoxy group at the *N*-position. These spectral information indicated **1** possesses *N*-methoxy indole carboxylate moiety.

Additionally, both ^{1}H and ^{13}C NMR spectra consisted of peaks attributable to glucopyranosyl moiety. Six ^{13}C NMR peaks at δ_C 103.2, 78.3, 75.0, 78.1, 71.6 and 62.6, and ^{1}H NMR chemical shifts at 4.94 (1H, d, *J* = 7.6 Hz), 3.37 (1H, m), 3.39 - 3.49 (3H, m), 3.92 (1H, dd, *J* = 12.0, 2.4 Hz) and 3.69 (1H, dd, *J* = 6.0, 2.4 Hz) suggested that the glucopyranoside is a D-glucose.[18,19] Further, acid hydrolysis of **1** showed the presence of D-glucose which was ascertained by performing co-TLC with the standard D-glucose (*Rf* = 0.13). Since the anomeric proton at δ_H 4.94 (1H, d, *J* = 7.6 Hz) showed high coupling constant in the ^{1}H NMR spectrum and anomeric carbon resonance occurred at chemical shift of δ_C 103.2, D-glucose is revealed to have β-configuration.[19-21] The glucopyranosyl ester linkage to indole moiety was determined to be at C-6 from HMBC spectrum wherein anomeric proton (δ_H 4.94) displayed bond connectivity with δ_C 156.3 (C-6). Hence, compound **1** was identified as 1-methoxyindole 3-carboxylic acid 6-*O*-β-D-glucopyranoside or *N*-methoxyindole carboxylic acid 6-*O*-β-D-glucopyranoside.

Other isolated compounds were identified to be indole

3-acetonitrile (**2**),[22] *N*-methoxyindole 3-acetonitrile (**3**),[23] 5-hydroxymethyl-2-furaldehyde (**5**),[24,25] methyl-1-thio-β-D-glucopyranosyl disulfide (**6**),[26] 5-hydroxy-2-pyridine-methanol (**7**),[27,28] (3*S*,4*R*)-2-deoxyribonolactone (**8**),[29,30] *n*-butyl-β-D-fructopyranoside (**9**),[31] uridine (**10**),[32] and β-D-fructofuranose (**12**)[33] by comparing the spectral data with the reported data in literature, while remaining were detected to be β-sitosterol glucoside (**4**), D-tagatose (**11**) and β-D-fructopyranose (**13**) by TLC comparison with reference compounds, and EI-MS library search.

In former studies, compounds **2**, **3**, **4**, **6** and **9** were found to have anti-inflammatory activity.[15,26,34,35] In a report by Yang *et al*., **2** and **3** displayed potent inhibitory action on nitric oxide (NO) production induced by lipopolysaccharide in RAW 264.7 cells. Interestingly, aglycone of **1** (1-methoxyindole 3-carboxylic acid) also exhibited significant inhibitory action on NO production.[15] Therefore, the new glucoside derivative, **1**, might have anti-inflammatory potential as its aglycone molecule. Compound **6** appeared as the major constituent in ethyl acetate fraction of red kohlrabi, since it was isolated in large quantity (>200 mg). Reported earlier to occur in *Capsella bursa-pastoris* by Cha *et al.*, it moderately inhibited LPS-induced NO production in BV2 cells, with IC_{50} value of 44.10 μM.[26] Besides anti-inflammatory action, compound **4** which is a phytosterol glucoside had demonstrated multiple bioactivities such as angiogenic,[36,37] anti-hyperglycemic,[38] and anti-proliferative on breast cancer cells.[39,40]

Compounds **5** and **7** had previously demonstrated anti-obesity effect via inhibition of lipid accumulation in adipose cells and suppression of transcriptional factors, including PPAR*γ*, C/EBP*α*, and SREBP-1c, the adipogenesis related gene (ACC), and enzymes (FAS), fatty acid binding protein-4 (FABP4) and lipoprotein lipase (LPL).[41,42] Moreover, **5** promoted the osteogenic differentiation through the increase in mRNA expression of molecular biomarkers of osteoblast such as alkaline phosphate, osteopontin (OPN), osteocalcin and collagen type 1 α1 in the rat bone mesenchymal cells.[42] Anti-fungal property against pathogenic fungi of **3** and **5** were also explored.[23,43]

Compound **8** that consists of oxa-cyclopentane ring as the prostaglandin E2 (PGE2) displayed effects related to PGE2 by inducing cFos mRNA, however, at higher concentrations, unlike PGE2, it did not enhance tetradecanoyl phorbol acetate (TPA) induced HL-60 cells differentiation but rather antagonized the TPA-induced cell differentiation in a dose-dependent manner and was found non-toxic in MTT assay, thus signifying it to be safe for normal cells at optimized concentrations.[30]

An *in vivo* study showed that dietary uridine, **10**, given along with choline and decahexanoic acid enhanced learning and memory improvement by increasing total brain phospholipids.[44] Uridine, isolated from *Pleurotus giganteus*, stimulated neurite outgrowth in vitro by inducing MEK/ ERK and PIP3K-Akt-mTOR mediated phosphorylation of cAMP response element binding protein (CREB) and expression of growth associated protein 43, thus directing its usefulness in neurological disorders.[45]

The sweet taste of *B. oleracea* var. *gongylodes* tuber can be attributed to the occurrence of the fructose derivatives, **11**, **12** and **13** in relatively large amount. **11** is a rare natural hexoketose which attains application as a low calorie sweetener and as an additive in detergents, cosmetics and pharmaceutical formulations.[46]

Overall, phytochemical investigation of red kohlrabi extract resulted in the identification of compounds, which are accountable for anti-inflammatory, anti-adipogenic and other pharmacological significances of the kohlrabi extract, which were published earlier. First and foremost, a new glycoside derivative of 1-methoxyindole 3-carboxylic acid, 1-methoxyindole 3-carboxylic acid-6-*O*-β-D-glucopyranoside was identified, along with compounds **2**, **3**, **4**, **5**, **6**, **7**, **8** and **9** being uncovered in this plant for the first time through this study.

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Conflict of interest

The authors declare that they have no competing interests.

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