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Phenolics from Durian Exert Pronounced NO Inhibitory and Antioxidant Activities

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ABSTACT

Durian, known as the king of fruits, is native to Southeast Asia and popular in many countries. Bioactivity-guided fractionation of the peel of durian was applied to determine its bioactive constituents. Four novel phenolics, along with sixteen known, were purified and identified. Four novel phenolics were elucidated to be durianol A (**1**), durianol B (**2**), durianol C (**3**) and 5′-methoxy-7′-epi-jatrorin A (**4**), respectively. The novel compounds were elucidated to be durianol A (**1**), durianol B (**2**), durianol C (**3**) and 5′-methoxy-7′-epi-jatrorin A (**4**), respectively. The antioxidant and NO inhibitory activities were evaluated for the isolated phenolics. Some phenolics showed significant antioxidant activity in DPPH and superoxide anion radical scavenging capacity assay. Most of the phenolics revealed pronounced inhibitory effects on NO production in murine RAW 264.7 cells induced by LPS, which showed more potent NO inhibitory activity compared to indomethacin. The results strongly demonstrated that the phenolics may be partially responsible for durian's NO Inhibitory activity.

KEYWORDS: *durian, Durio zibethinus, phenolics, antioxidant, NO inhibitory activity*

INTRODUCTION

Known as the king of fruits, durian is native to Southeast Asia and popular in many countries including China, Thailand, and Malaysia. Several kinds of wild *Durio* species produce edible fruits, and only *D. zibethinus* Murr. is cultivated in large quantities in Southeast Asia and China. The durian fruit is in round or oval shape, about the size of a coconut. The peel of them is thorny, with a color from yellowish to dark yellow.[1] Durian is an argumentative fruit and some people regard the durian as having a pleasantly sweet fragrance, others find the aroma overpowering and revolting. However, durian fruit is rich in nutrients of carbohydrates, protein and also contains sufficient vitamins B and C. In addition to eaten raw, its attractive flesh is frozen or dried for a popular snack. Previous chemical studies on *Durio* have resulted in the identification of triterpenoids, phenolics, lignans, coumarins, flavonoids, sulphur-containing compounds and some uncommon esters.[2-5] In spite of the abundant secondary metabolites in durian, few research has been concerned with the bioactive constituent in durian, especially in its peel that has currently been treated as agricultural waste.

Studies of epidemiology have revealed that high fruit consumption was beneficial to prevent chronic diseases of atherosclerosis, cancer, diabetes and coronary heart diseases.[6] Anti-cytokines and antioxidants in fruits showed the potential to reduce the probability of above diseases to improve human health.[7] In the process of seeking effective bioactive components from medicinal plants, fruits and vegetables [8-10], detailed phytochemical characterization of phenolics in the peel of durian were

conducted, and four new along with sixteen known phenolics were isolated and identified in this study. The potent anti-inflammatory and antioxidant activities of the isolated components were also evaluated *in vitro*.

MATERIALS AND METHODS

General Apparatus and Chemicals. PerkinElmer 100 IR spectrometer (Perkin Elmer Inc., Waltham, MA, USA) was used to scan IR spectra with KBr and Bruker AVANCE III-500 NMR spectrometer (Bruker Inc., Fällanden, Switzerland) was applied in obtaining NMR spectra. High-resolution ESI-MS (HR-ESI-MS) data were acquired on a Waters AQUITY UPLC/Q-TOF mass spectrometer (Milford, MA, USA). Preparative HPLC was performed with a Rainin HPLC system (Rainin Instrument Co. Inc., Woburn, MA, USA), including a Rainin pump, a Rainin UV Absorbance detector of Model UV-D II and a Cosmosil HPLC column (5C18-MS-II, 10ID×250 mm, Nacalai Tesque, Kyoto, Japan). Silica gel (200-300 mesh and 300-400 mesh) was obtained from Anhui Liangchen Silicon Material Co. Ltd. (Lu′an, China). ODS (40-60 μm, Merck KGaA, Darastadt, Germany) was used for medium pressure liquid chromatography (MPLC). Methanol for HPLC was purchased from Oceanpak Chemical Co. (Gothenburg, Sweden). (*S*)-(+)-2-Methylbutyric acid (Acoros organics, Belgium) were used for HPLC analysis, which was applied with Waters 600 and Waters 996 Photodiode Array Detector (Milford, MA, USA). Sugar reagents for GC/MS analysis were product of Sigma (St. Louis, MO, USA). A Varian CP-3800 GC (Varian Inc., Palo Alto, CA, USA) was applied for sugar analysis.

Plant Material. The peels of durian were collected in Sep. 2014 from the local fruit markets of Guangzhou (Guangzhou, China) and the species was identified by Prof. X. J. He of Guangdong Pharmaceutical University. A voucher specimen (No. GDPU-NPR-201403) was deposited in the Lead Compounds Laboratory, School of Pharmacy, Guangdong Pharmaceutical University.

Extraction and Isolation Procedure. The clean, air-dry peels of durian (20 kg) were extracted with 70% EtOH for four times at reflux, and the combined filtrate was concentrated under vacuum at 55 °C. The obtained extract was suspended in 15 L distilled water and extracted with cyclohexane, chloroform, ethyl acetate and n-butanol successively. All fractions were tested in DPPH radical-scavenging capacity assay and NO inhibitory assay. The chloroform and ethyl acetate fractions were found to exhibit preferable activities with IC_{50} of 195.43±17.10 and 230.38±15.13 μg/ml in DPPH assay, and with IC_{50} of 32.98±2.85 and 28.70±1.35 μg/ml in NO inhibitory assay. Therefore, the fractions of chloroform and ethyl acetate were selected for further purification. The chloroform fraction (50 g) was divided to ten fractions (A1-A10) by a preliminary isolation of a silica gel column ($CHCl_3$/MeOH, 100:0 to 0:100, v/v). The part of A6 (8 g) was isolated by silica gel ($CHCl_3$/MeOH, 80:20 to 0:100, v/v), ODS MPLC (MeOH/H_2O, 10:90 to 100:0, v/v), Sephadex LH-20 (cyclohexane/$CHCl_3$/MeOH, 5:5:1, v/v/v) column chromatography to get compounds **1** (3 mg), **8** (23 mg) and **9** (7 mg). Fraction A9 (5 g) was subjected to an ODS MPLC and preparative HPLC (MeOH/H_2O, 60:40, v/v) to obtain compounds **2** (11 mg), **7** (6

mg), and **14** (3 mg). Fraction A5 (8 g) was separated by silica gel ($CHCl_3$/ CH_3COCH_3, 80:20 to 0:100, v/v), ODS MPLC (MeOH/H_2O, 10:90 to 100:0, v/v), Sephadex LH-20 (C_6H_{12}/$CHCl_3$/MeOH, 5:5:1, v/v/v) column chromatography, and finally subjected to a preparative HPLC to get compound **10** (33 mg). Compounds **6** (4.3 mg, MeOH/H_2O, 50:50, v/v) and **11** (5 mg, MeOH/H_2O, 70:30, v/v) were obtained from Fraction A4 through preparative HPLC. Compounds **12** (1.8 mg) and **15** (3 mg) were purified by preparative HPLC from Fraction A9 using 30% and 70% methanol as mobile phase, respectively.

The ethyl acetate fraction (68 g) was subjected to a silica gel column ($CHCl_3$/MeOH, 100:0 to 0:100, v/v) to give 8 fractions (B1-B8). Fraction B6 was isolated by ODS column (MeOH/H_2O, 10:90 to 100:0, v/v), and compounds **3** (4 mg), **4** (2 mg), **18** (6 mg), and **20** (5.7 mg) were obtained by preparative HPLC (MeOH/H_2O, 80:20, v/v). Compound **5** (3 mg) was purified from fraction B3 (2.9 g). Compounds **16** (13 mg) and **17** (22 mg) were obtained by preparative thin layer chromatography (pTLC) ($CHCl_3$/CH_3COCH_3/MeOH, 6:2:1, v:v:v) from fraction B7. Compound **19** (3 mg) was isolated by silica gel, Sephadex LH-20, ODS MPLC and preparative HPLC from fraction B4 using 70% methanol as mobile phase.

Durianol A (**1**): $[\alpha]_D^{15}$: −13.9 (c 0.1, MeOH); UV (MeOH) λ_{max} 216, 290, 340 nm; IR (KBr) ν_{max} 3483, 2975, 2940, 2883, 1724, 1616, 1569, 1510, 1454, 1425, 1380, 1276, 1243, 1194, 1123, 1072, 1013, 920, 881, 833, 755, 682, 624, 581, 511 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HR-ESI-MS m/z 461.1407 $[M+Na]^+$ (calcd. for $C_{21}H_{26}O_{10}Na$, 461.1424).

Durianol B (**2**): $[\alpha]_D^{15}$: −6.8 (c 0.1, MeOH); UV (MeOH) λ_{max} 204, 249, 289 nm; IR (KBr) ν_{max} 3368, 2963, 2676, 1729, 1705, 1606, 1520, 1463, 1432, 1384, 1283, 1188, 1066, 1023, 880, 762, 723, 615, 519 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HR-ESI-MS m/z 437.1443 $[M+Na]^+$ (calcd. for $C_{19}H_{26}O_{10}Na$, 437.1424).

Durianol C (**3**): $[\alpha]_D^{15}$: −7.0 (c 0.1, MeOH); UV (MeCN) λ_{max} 240, 286 nm; IR (KBr) ν_{max} 3413, 2971, 1694, 1604, 1511, 1425, 1242, 1181, 1074, 1024, 832, 631 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HR-ESI-MS m/z 411.1684 $[M + H]^+$ (calcd. for $C_{20}H_{27}O_9$, 411.1655).

5′-Methoxy-7′-epi-jatrorin A (**4**): $[\alpha]_D^{15}$: −10.2 (c 0.12, MeOH); UV (MeOH) λ_{max} 210, 309 nm; IR (KBr) ν_{max} 3427, 2925, 2853, 1685, 1619, 1573, 1524, 1500, 1468, 1425, 1384, 1325, 1208, 1207, 842, 802, 724, 602 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HR-ESI-MS m/z 425.0854 $[M + Na]^+$ (calcd. for $C_{20}H_{18}O_9Na$, 425.0849).

DPPH Radical-Scavenging Capacity Assay. DPPH assay were applied to evaluate antioxidant activities. The procedure of which were determined by reported method.[11] Briefly, DPPH solution (in methanol, 50 mg/L, 995 μL) was mixed with 5 μL of each of tested sample. The samples were dissolved in DMSO. The solution system was shaken and reacted at room temperature in the dark for 30 min. The absorbance was measured at 517 nm. Gallic acid and ascorbic acid were used as positive control.

Superoxide Anion Radical Scavenging Capacity Assay. The antioxidant of superoxide anion scavenging capacity of the isolated phenolics were evaluated

according to a described procedure with some modifification.[12] The 1000 μL reaction mixture contained 445 μL Tris-HCl (pH 8.1, 50 mM), 250 μL NADH (0.15 mM), 50 μL PMS (0.03 mM), 250 μL NBT (0.10 mM) and 5 μL tested sample. All tested samples were dissolved in Tris-HCl (50 mM, pH 8.1). The reaction was conducted for 5 min at 37 °C, and initiated by the addition of PMS. Gallic acid and ascorbic acid were used as positive control. The radical scavenging capacity was calculated in the way: scavenging activity (%) = $(B_1 - B_2)/B_1 \times 100$， B_1, B_2 are the absorbance of the blank and sample at 546 nm.

Nitric Oxide Inhibitory Assay. Macrophage RAW264.7 cells were cultured in DMEM containing 10% FBS, 100 units/mL penicillin and 100 μg/mL streptomycin at 37 °C with a humid atmosphere of 5% CO_2/95% air. NO produced in the medium was measured by assaying the levels of NO_2^- via the Griess reaction.[13]

Acid Hydrolysis and GC Analysis of Sugars. The acid hydrolysis and GC analysis were applied to determine the chirality of the sugars of new phenolic glycosides. Compounds **1**–**3** (1.5 mg) were heated for 3 h at 90 °C in ampoule with 5 mL 2 M HCl. Then the residue was evaporated under vacuum at 55 °C after extracted with EtOAc. The residue was dissolved in 600 μL pyridine and 5 mg $NH_2OH \cdot HCl$ was added. Then the system was heated at 90 °C for half hour. 300 μL Ac_2O was added to the mixture when the system cooled to room temperature. After homogenized, the mixtures were heated at 90 °C for 1 h. The reaction mixtures were

149 analyzed by GC using standard aldononitrile peracetates as reference samples after
150 cooling.
151
152 **Identification of (*2S*)-Methyl Butanoic Acid.** The unit of (2*S*)-methyl butanoic
153 acid in molecule was confirmed by a described procedure.[14] Compounds **1, 2, 3** (2.0
154 mg) were dissolved in 2 mL 5% KOH-H_2O and refluxed for 3 h. The reactant was
155 acidified to pH 4.0 with 4.0 M HCl and then extracted with $CHCl_3$ for three times.
156 Then 2.0 mg *N*, *N*-dimethyl-4-aminopyridine was added after the aqueous fraction
157 was washed, dried in vacuum. The mixture was dissolved in 1.0 mL $CHCl_3$. Then
158 10.0 μL (*R*)-1-phenylethanol and 15.0 mg *N, N′*-dicyclohexylcarbodiimide were
159 added after the mixtures cooled to 0 °C in ice bath. The system was reacted at 0 °C for
160 20 min and at room temperature for 15 h, and then filtrated. The filtrate was washed
161 with water (4 × 1 mL) and the reactant was analyzed by HPLC at following
162 chromatographic conditions: column RP-18e (5 μm), detector wavelength at 210 nm,
163 column temperature at 35 °C, with mobile phase of 65% MeOH-H_2O at flow rate of
164 1.0 mL/min. The retention time of (*R*)-1-phenylethyl-(*S*)-2-methylbutanoate was
165 17.90 min, which was the same with standard of
166 (*R*)-1-phenylethyl-(*S*)-2-methylbutanoate (t_R 17.90 min). Reference substance was
167 obtained from (*R*)-1-phenylethanol and (*S*)-2-methylbutanoic acid by the same
168 method.

RESULTS AND DISCUSSION

Structural Elucidation of Phenolics. In this study, peels of durian were collected, dried and extracted with 70% ethanol. The obtained extract was then successively partitioned with different solvents and repeatedly chromatographed on silica gel, Sephadex LH-20, ODS MPLC and RP-HPLC to give four new phenolics, as well as sixteen known congeners (Figure 1).

Compound **1** was purified as a bright white powder. The molecular formula of $C_{21}H_{26}O_{10}$ was deduced according to its HR-ESI-MS at m/z 461.1407 $[M+Na]^+$ (calcd. for $C_{21}H_{26}O_{10}Na$, 461.1424), which could be confirmed through its ^{1}H-NMR and ^{13}C-NMR. The maximum absorption peak at 340 and 216 nm in UV, and the strong absorption at 1724 cm^{-1} in IR, indicated that compound **1** was a coumarin derivative. The deduction was further verified by the proton signals at δ_H 6.32 (1H, d, J = 9.5 Hz, H-3) and 7.95 (1H, d, J = 9.5 Hz, H-4) in ^{1}H-NMR, which coincided with the character of coumarin. Two characteristic singlet signals at δ_H 7.14 (1H, s) and 7.30 (1H, s) suggested the positions of C-6 and C-7 were substituted in coumarin. Moreover, the ^{1}H-NMR spectrum revealed a glucosyl anomeric proton at δ_H 5.18 (1H, d, J = 7.3 Hz), a methoxyl group at δ_H 3.78 (3H, s) and the protons of 2-methylbutyric acid in the higher field. The deduced fragments were linked by the correlations in HMBC, which were showed in Figure 2. The sugars were determined as D-glucose by GC analysis of their chiral derivatives after acid hydrolysis. In the NOESY spectrum, the main correlations approved the relative configuration of compound **1**. The

absolute configuration of (*S*)-2-methyl butyrate was determined by the standard substance after derivatization according to the protocol described in the experimental section.[14] Based on above analyses, compound **1** was elucidated to be 6-methoxy-7-O-*β*-D- [6-(*S*)-2-methylbutanoyl-glucopyranosyl]-coumarin, and named as durianol A. The full assignments of its NMR data were shown in Table 1.

Compound **2** was obtained as bright needle crystals. The molecular formula of $C_{19}H_{26}O_{10}$ was deduced on the basis of HR-ESI-MS m/z 437.1443 $[M+Na]^+$ (calcd. for $C_{19}H_{26}O_{10}Na$, 437.1424). The IR spectrum suggested the presence of hydroxyl (3368 cm^{-1}), alkyl (2963 cm^{-1}), carbonyl (1705 cm^{-1}), and aromatic ring (1606 and 1520 cm^{-1}) functionalities. The ^{1}H-NMR spectrum showed typical signals for 1, 3, 4-trisubstituted benzene at δ_H 7.48 (1H, d, J = 9.1 Hz), 7.47 (1H, br. s) and 7.13 (1H, d, J = 9.1 Hz) with a typical ABX spin system. The signal at δ_H 3.80 (3H, s) was a methoxyl linked to an aromatic ring. The NMR spectrum of **2** showed an anomeric proton signal at δ_H 5.08 (1H, d, J = 7.5 Hz), corresponding to an anomeric carbon at δ_C 99.2 in the spectrum of HSQC. That indicated the presence of a sugar moiety which was identified as D-glucose by above GC analysis. And its coupling constants (J = 7.5 Hz) exposed the *β*-anomeric configuration. In the higher filed of ^{1}H-NMR, the isobutyl signal was evident, which was further inferred as 2-methyl butyric acid on the basis of signal of carbonyl in the ^{13}C-NMR. Therefore, the molecule of compound **2** was consisted of three parts, a *β*-glucopyranosyl, a 3-hydroxyanisic acid and a 2-methyl butyric acid. The 2D-NMR, including HSQC and HMBC, were applied to elucidate the structure of **2**. In HMBC, the correlations from H-1′ to C-4,

213 from the two protons of H-6′ to C-1″ provided the mutual connection positions of
214 three fragments, which the key correlations were shown in Figure 2. In the NOESY
215 spectrum, the main correlations approved the relative configuration of compound **2**.
216 The absolute configurations of D-glucose and (*S*)-2-methyl butyrate were determined
217 by GC and HPLC analyses of their relevant derivatives. Combined with the above
218 analyses, compound **2** was elucidated to be 3-methoxy-4-O-*β*-D-[6-(*S*)-2-
219 methylbutanoyl-glucopyranosl] benzoic acid, and named as durianol B. The full
220 assignments of its NMR data were shown in Table 1.

221 The IR spectrum compound **3** suggested the presence of hydroxyl (3413 cm^{-1}),
222 alkyl (2971 cm^{-1}), carbonyl (1694 cm^{-1}), and aromatic ring (1604 and 1511 cm^{-1})
223 functionalities. The molecular formula of $C_{20}H_{26}O_9$ was verified on the basis of
224 HR-ESI-MS m/z 411.1684 $[M+H]^+$ (calcd. for $C_{20}H_{27}O_9$, 411.1655). The ^{1}H-NMR
225 spectrum showed characteristic proton signals for 1, 4-disubstitued benzene at δ_H 7.61
226 (2H, d, J = 8.3 Hz) and 7.03 (2H, d, J = 8.3 Hz) in a typical A_2B_2 spinning system.
227 The signals at δ_H 7.54 (H, d, J = 15.9 Hz) and 6.40 (H, d, J = 15.9 Hz) were a pair of
228 oleafic group in *trans* configuration. The sugar obtained from the hydrolysate was
229 identified as D-glucose by GC analysis of their chiral derivatives. The anomeric
230 configuration of glucose was determined to be *β*-configuration by the coupling
231 constants of the anomeric proton at δ_H 4.99 (1H, d, J = 7.4 Hz). In the high filed of
232 ^{1}H-NMR, the isobutyl signal was evident and it was further inferred as 2-methyl
233 butyric acid on the basis of signal of carbonyl in the ^{13}C-NMR. Therefore, three
234 fragments could be deduced in the molecule, which were *β*-D-glucopyranosyl, a

trans-cinnamic acid and a 2-methyl butyric acid. The 2D-NMR including HSQC and HMBC data were applied to prove the connections of the deduced fragments. The correlations from H-1′ to C-4, from the two protons of H-6′ to C-1″ confirmed the mutual connection position of three fragments in HMBC. Other key correlations in HMBC were showed in Figure 2. In the NOESY spectrum, the main correlations approved the relative configuration of compound **3**. The fragment of (*S*)-2-methyl butyrate was confirmed by standard substance after derivatives. From these results, compound **3** was established as 4-O-*β*-D-[6-(*S*)-2-methylbutanoyl]-glucopyranosyl cinnamic acid, and named as durianol C.

Compound **4** was isolated as a pale-yellow oily matter. The molecular formula of $C_{20}H_{18}O_9$ was verified on the basis of HR-ESI-MS at m/z 425.0854 $[M+Na]^+$ (calcd. for $C_{20}H_{18}O_9Na$, 425.0849), which could also be confirmed through ^{1}H- and ^{13}C-NMR. The UV maximum absorption at 309 and 210 nm, and the absorption of IR at 1685 cm^{-1} suggested that it was a coumarin derivative. The characteristic was confirmed by the obvious signals δ_H 6.27 (H-3) and 7.91 (H-4) with coupling constants of 9.5 Hz in the ^{1}H-NMR spectrum. Meanwhile, there was found two coincident proton signals at δ_H 6.76 indicating a symmetric benzene ring when combined its ^{13}C-NMR data. The deduction was determined by the long-range correlations between H-2′/6′ and C-3′/5′, C-1′, C-4′ in spectrum of HMBC. The other signals were a fragment with three *O*-bearing carbons. The above data were coincident with the reported data of cleomiscosins.[15] The structure of compound **4** was deduced by its ^{1}H-^{1}H COSY and HMBC experiment (Figure 2). Its relative

257 configuration was determined using the NOESY spectrum. The correlations in
258 NOESY (Figure 3) were observed for H-7′/H-8′ with coupling constants of 7.9 Hz in
259 the ^{1}H-NMR spectrum suggested that these protons lie on one face of the molecule
260 with a *trans* configuration. In view of the optical activity $[\alpha]_{D}^{15} = -10.2$ (c 0.12, MeOH)
261 of compound **4** , it was concluded to be a single enantiomer and its absolute
262 configuration was opposite with 8′-epi-cleomiscosin.[16] Owing to these observations
263 and ^{1}H-NMR, ^{13}C-NMR data shown in the Table 1, compound **4** was elucidated to be
264 5′-methoxy-7′-epi-jatrorin A. The long-range correlations between H-7′ and C-7 were
265 extreme weak in HMBC. Fortunately, it existed a pattern that C-7 shifted to high field
266 and C-8 shifted to low field when the connection mode converted from C-7′– C-7 to
267 C-8′–C-7.[17] Based on above analyses, compound **4** was elucidated to be
268 5′-methoxy-7′-epi-jatrorin A, which was a novel compound as far as we knew.
269 The sixteen known compounds were confirmed as propacin (**5**),[18] jatrocin A (**6**),
270 cleomiscosin A (**7**),[19] cleomiscosin B (**8**),[19] propacin isomer (**9**),[20] 7-hydroxycoumarin
271 (**10**),[21] scopoletin (**11**),[21] fraxetin (**12**),[22] fraxidin (**13**),[22] 3,4-dihydroxybenzoic acid
272 (**14**),[23] 4-hydroxy-3-methoxybenzoic acid (**15**),[23] ethyl protocatechuate (**16**),[24]
273 3,4-dihydroxybenzaldehyde (**17**),[25] evofolin-B (**18**),[26] 2-hydroxy-8α-
274 hydroxycalamenene (**19**),[27] α-conidendrin (**20**) [28] by comparing spectroscopic data
275 with the reported compounds in literature.
276
277 **Antioxidant Activity.** The DPPH and PMS/NADH-NBT assays were applied to
278 evaluate the antioxidant activities. Ascorbic acid and gallic acid were used as positive

279 control for their efficient antioxidant and widely used in food and medicinal industry.
280 In the DPPH assays (Table 2), compound **13** exhibited stronger activity with IC_{50} of
281 7.83±1.00 μM than Gallic acid (19.10±2.23 μM) and ascorbic acid (10.21±1.45 μM).
282 Other phenolics showed weaker antioxidant activity by comparing with the controls.
283 It was noteworthy that compound **16** (ethyl protocatechuate), showed more potent
284 antioxidant capacity. Only few compounds (such as **13**) showed pronounced activity
285 in the PMS/NADH-NBT assay (Table 2).
286
287 **NO Inhibitory Activity.** NO is a signal transmission molecule that act as a crucial
288 role in immune and inflammatory responses and neuronal transmission for brain. The
289 inhibitors of NO production had potential to turn into anti-inflammatory agents. The
290 isolates were tested on Abelson murine leukemia virus-induced tumor (RAW 264.7)
291 where NO production originated in the stimulation of LPS. And the Griess reaction
292 was applied to quantify the effect of evaluation. Indomethacin (IC_{50} 47.4 μM), a
293 typical and common non-steroidal anti-inflammatory agent, was selected as positive
294 control. Most of the tested compounds showed obvious inhibitory effects on NO
295 production compared to indomethacin, and the result were exhibited in Table 3.
296 Among them, Compounds **8**, **13**, and **16** showed significant inhibitory activity with
297 IC_{50} values below 10 μM, with the values of 3.56, 3.70 and 7.29 μM, respectively. It
298 was noteworthy that compound **8** possessed pronounced inhibitory activity, while
299 compound **7** was weak. It could infer that the coumarinoligan with an aromatic moiety
300 close to the C-8 position, showed more potent effects on NO inhibitory activity.

Meanwhile, the most of the tested compounds from durian had less IC_{50} values than positive control. The MTT assay was applied to evaluated cytotoxicity of the isolated phenolics to RAW 264.7 cells. The results showed that most of the tested isolates exhibited no cytotoxic effect at effective concentration.

In conclusion, twenty phenolics, including four novels, were purified and identified from durian. Some phenolics showed potent antioxidant and NO inhibitory activities. The results strongly demonstrated the phenolics isolated from durian may be partially responsible for its NO inhibitory activity.

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Notes

The authors declare no competing financial interest.

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Table 1. NMR data of compounds **1**–**4** (δ in ppm and J in Hz) [a, b]

	1		**2**		**3**		**4**	
no.	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1				124.2		128.0		
2		160.4	7.47 s	112.7	7.61 d (8.3)	129.6		160.2
3	6.34 d (9.5)	113.4		148.5	7.03 d (8.3)	116.4	6.27 d (9.5)	113.1
4	7.97 d (9.5)	144.2		150.0		158.7	7.91 d (9.5)	144.8
5	7.30 s	109.7	7.13 d (9.1)	114.3	7.03 d (8.3)	116.4	6.67 s	104.0
6		145.9	7.48 d (9.1)	122.5	7.61 d (8.3)	129.6		143.1
7		149.6		167.0	7.54 d (15.9)	143.4		136.6
8	7.14 s	103.0			6.40 d (15.9)	117.1		131.8
9		148.8				167.6		136.8
10		112.3						111.5
1′	5.18 d (7.3)	99.1	5.08 d (7.5)	99.2	5.00 d (7.4)	99.7		125.9
2′	3.30-3.36	76.4	3.20-3.32	76.5	3.20-3.40	76.2	6.76 s	105.6
3′	3.30-3.36	72.9	3.20-3.32	72.9	3.20-3.40	73.0		148.0
4′	3.16 m	70.1	3.16 m	70.0	3.16 m	70.0		136.1
5′	3.77 m	73.8	3.65 m	73.9	3.66 m	73.8		148.0
6′	4.03 dd (11.8, 8.0)		4.02 dd (11.7, 7.6)		4.04 dd (11.6, 7.3)			
	4.33 dd (11.8, 1.8)	63.6	4.31 dd (11.7, 1.8)	63.5	4.33 d (11.6)		6.76 s	105.6
7′							4.97 d (7.9)	76.4
8′							4.33 m	77.8
9′							3.65 m, 3.27 m	59.9
1″		175.6		175.4		175.4		
2″	2.35 m	40.1	2.32 m	40.2	2.35 m	40.1		
3″	1.32 m,1.49 m	26.1	1.34 m, 1.51 m	26.2	1.38 m, 1.52 m	26.1		
4″	0.68 t (7.4)	11.1	0.76 t (7.4)	11.3	0.77 t (7.4)	11.2		
5″	1.02 d (7.0)	16.1	1.03 d (7.0)	16.1	1.05 d (6.9)	16.1		
-OMe	3.78 s	56.0	3.80 s	55.6			3.77 s	56.1

[a] Measured in DMSO-d_6.

[b] Assignments were based on HSQC, HMBC, and ^{1}H-^{1}H COSY experiments

Table 2. Antioxidant activities of the phenolics from durian [a]

Compd.	IC_{50} [b] (μM)	
	DPPH	PMS/NADH-NBT
6	568.50±6.28	–
7	268.37±11.02	394.34±71.33
8	807.22±18.66	–
12	260.26±5.44	–
13	7.83±1.00	11.4±1.44
15	56.61±3.09	–
16	23.82±0.54	870.07±63.28
17	58.13±0.72	494.85±14.91
19	1201.88±30.76	–
20	90.97±2.10	–
70% Ethanol exact of durian	372.08±73.02 [c]	
Chloroform fraction	195.43±17.10 [c]	
Ethyl acetate fraction	230.38±15.13 [c]	
n-Butanol fraction	392.06±85.04 [c]	
Ascorbic acid	10.21±1.45	54.12±8.31
Gallic acid	19.10±2.23	83.29±14.22

[a] Data are represented as the mean value ± SD, n=3; [b] The test concentrations ranged from 0 to 1500 μM; [c] The concentration units are μg/ml.

Table 3. Inhibitory effects of the phenolics on NO production induced by LPS in RAW 264.7

Compd.	IC_{50} (μM)		Compd.	IC_{50} (μM)	
	NO Inhibitory Assay	MTT Assay		NO Inhibitory Assay	MTT Assay
1	36.32±1.39	>50.00	**14**	17.87±2.79	>50.00
2	38.07±2.40	>50.00	**15**	31.53±2.24	>50.00
3	41.26±0.44	>50.00	**16**	7.29±1.00	>50.00
4	35.23±1.79	>50.00	**17**	16.03±2.98	>50.00
5	>50.00	>50.00	**18**	>50.00	>50.00
6	21.70±2.35	>50.00	**19**	32.91±0.74	>50.00
7	28.88±2.08	>50.00	**20**	30.82±3.17	>50.00
8	3.56±0.49	>50.00	**70% Ethanol exact of durian**	21.29±2.54 [b]	>50.00 [b]
9	>50.00	>50.00	**Chloroform fraction**	32.98±2.85 [b]	>50.00 [b]
10	30.28±3.56	>50.00	**Ethyl acetate fraction**	28.70±1.35 [b]	>50.00 [b]
11	26.01±3.37	>50.00	**n-Butanol fraction**	33.06±1.12 [b]	>50.00 [b]
12	28.15±3.94	>50.00	Indo [a]	47.40±4.50	>50.0
13	3.70±1.75	>50.00			

[a] Indomethacin, positive control;

[b] The concentration units are μg/ml.

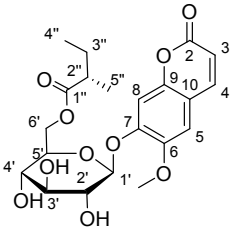
Figure Captions

Figure 1. Chemical structures of compounds **1-20** isolated from durian

Figure 2. The selected key ^{1}H-^{1}H COSY and HMBC correlations of compounds **1**–**4**

Figure 3. The key NOESY correlations of compound **4**

Figure 1



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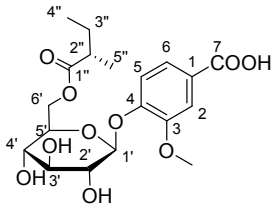

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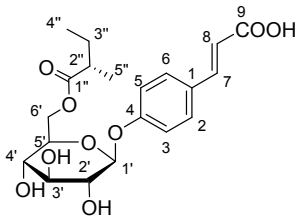

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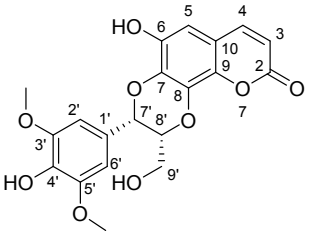

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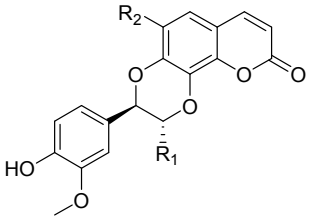

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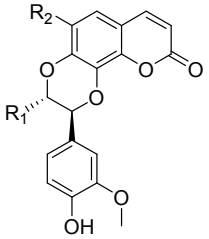
6 R_1=CH_2OH R_2=OH
7 R_1=CH_2OH R_2=OMe



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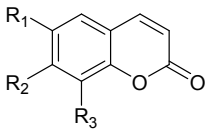
9 R_1=CH_3 R_2=OMe



10 R_1=H R_2=OH R_3=H
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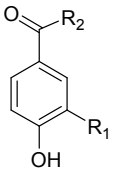
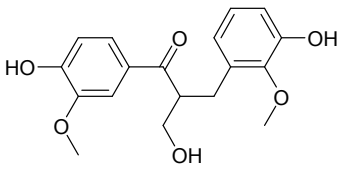
12 R_1=OMe R_2=OMe R_3=OH
13 R_1=OMe R_2=OH R_3=OH



14 R_1=OH R_2=OH
15 R_1=OMe R_2=OH
16 R_1=OH R_2=OC_2H_5
17 R_1=OH R_2=H



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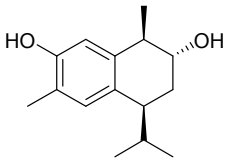

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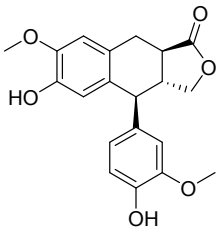


20

Figure 2

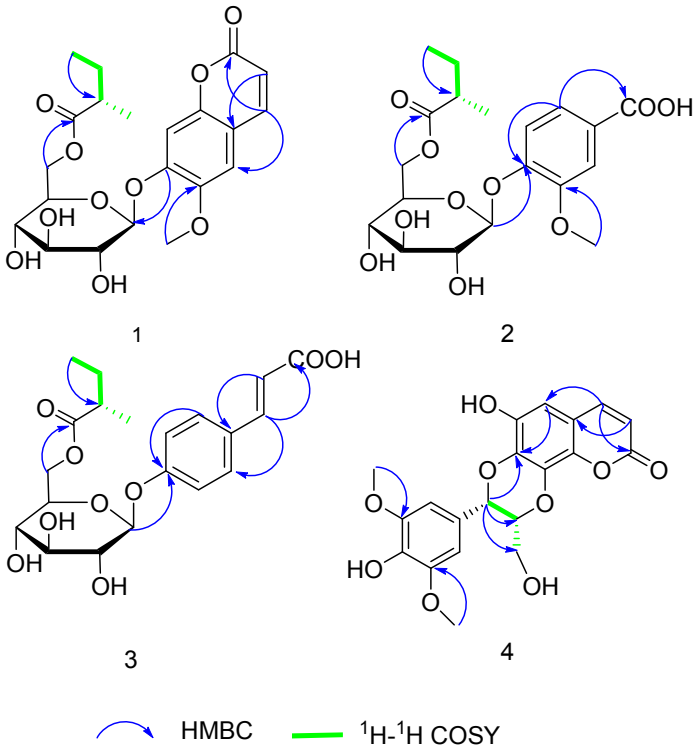
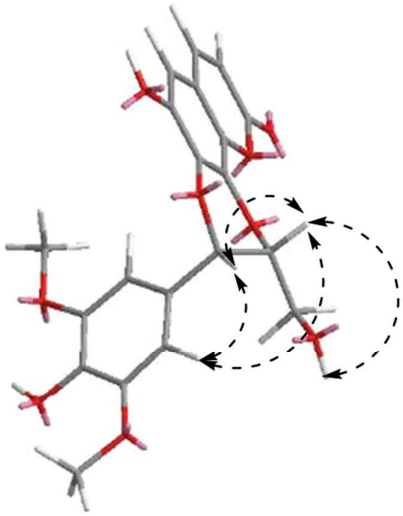


Figure 3



TOC Graphic