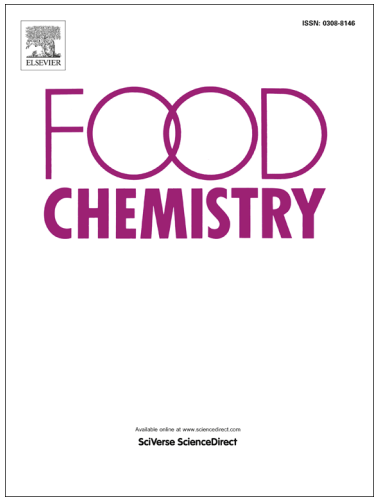

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Isolation, identification and antioxidant activity of bound phenolic compounds present in rice bran

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Abstract: The bound phenolic compounds in rice bran were released and extracted with ethyl acetate based on alkaline digestion. An investigation of the chemical constituents of EtOAc extract has led to the isolation of a new compound, *para*-hydroxy methyl benzoate glucoside (**8**), together with nine known compounds, cycloeucalenol *cis*-ferulate (**1**), cycloeucalenol *trans*-ferulate (**2**), *trans*-ferulic acid (**3**), *trans*-ferulic acid methyl ester (**4**), *cis*-ferulic acid (**5**), *cis*-ferulic acid methyl ester (**6**), methyl caffeate (**7**), vanillic aldehyde (**9**) and *para*-hydroxy benzaldehyde (**10**). The structures of these compounds were determined using a combination of spectroscopic methods and chemical analysis. Among the compounds isolated, compound **3, 5** and **7** exhibited strong DPPH and $ABTS^+$ radical scavenging activities, followed by compounds **4** and **6**. Compound **1** and **2** showed potent DPPH and $ABTS^+$ radical scavenging activities, compound **8** displayed moderate antioxidant activity against $ABTS^+$ radical, whereas compound **9** and **10** showed weak antioxidant activity.

Keywords: Rice bran; Alkaline hydrolysis; Bound penolic compounds; Structural identification; Antioxidant activities;

1. Introduction

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Rice bran, produced as the most abundant and valuable byproducts in the rice milling process, is of steadily growing interest in recent years due to its potential health benefits. It has been reported that rice bran contained high levels of phytochemicals, such as γ-oryzanol (Xu & Godber, 1999), phytic acid (Canan et al., 2011), tocopherols, tocotrienols (Xu, Hua & Godber, 2001), carotenoids (Stoggl, Huck, Wongyai, Scherz & Bonn, 2005), γ-aminobutyric acid (Parrado, Miramontes, Jover, Gutierrez, Collantes De Terán & Bautista, 2006), octacosanol (Chen et al., 2007), squalene (Sugihara, Kanda, Nakano, Nakamura, Igusa & Hara, 2010), unsaturated fatty acids (Xu & Godber, 1999), phytosterols and phenolic compounds (Liang et al., 2014). Among them, phenolics have been extensively investigated because they exhibited a diverse range of bioactivities such as antioxidative (Arab, Alemzadeh & Maghsoudi, 2011), antimicrobial (Kondo, Teongtip, Srichana & Itharat, 2011), antiviral (Ray et al., 2013), anti-inflammatory properties (Akihisa et al., 2000) and overall for the promotion of human health. These bioactive properties make these compounds play an important role in the prevention of certain major chronic diseases, such as diabetes (Lai, Chen, Chen, Chang & Cheng, 2012), chronic inflammation (Choi, Kim, Kang, Nam & Friedman, 2010), cardiovascular disease (Qureshi, Bradlow, Salser & Brace, 1997) and certain kinds of cancer (Verschoyle, Greaves, Cai, Edwards, Steward & Gescher, 2007), as strongly supported by animal studies and clinical trials.

Phenolics in rice bran are in soluble free, soluble conjugates and insoluble bound forms (Adom & Liu, 2002). Free forms present within the plant cell vacuoles (Pandey & Rizvi, 2009), whereas soluble esters or conjugates are esterified to sugars and other low molecular mass components, and insoluble bound forms are covalently linked to cell wall structural components, such as cellulose, hemicellulose, lignin, pectin and rod-shaped structural proteins (Arranz, Silvan & Saura-Calixto, 2010; McKee & Latner, 2000). The last is the major form in rice bran to enhance the mechanical strength of cell walls (Ryden, Sugimoto-Shirasu, Smith,

Findlay, Reiter & McCann, 2003) as well as providing both physical and chemical barriers, protection against pathogen invasion and in response to stress conditions such as infection, wounding and UV radiation, among others (Rice-Evans, Miller & Paganga, 1997). An increasing number of research groups turned their attention to these bound phenolics exist in fruits, vegetables and cereal grains, which have been given different names: nonextractable, unextractable, insoluble, or bound phenolics (Perez-Jimenez & Torres, 2011). About 74% of the total phenolics present in rice are in the insoluble bound forms, as also demonstrated by a comparison of the antioxidant capacity of bound phenolics significantly higher than that of free or soluble conjugated forms, with ferulic acid being the major phenolic compound present (Adom & Liu, 2002). In addition, some of these phenolics such as ferulic acid and diferulates are predominantly found in grains but are not present in significant quantities in fruits and vegetables (Adom & Liu, 2002).

Last decades, many studies have been reported the phenolic levels in rice bran using different combinations of water and organic solvents to extract soluble phenolics, however, most of these studies reported in the literature have largely ignored bound phenolics, hence underestimating the total content of phenolics present (Arranz, Silvan & Saura-Calixto, 2010). Although a few dozen papers have focused on the bound phenolics content and profiles, many of these studies were focused in the analysis of those known and particular types of phenolics. Limitations of these studies are that the individual phenolic composition in the sample usually must be known and the standards of some phenolics are commercially unavailable. Therefore, the precise composition of bound phenolics is crucial for evaluating their physicochemical properties, nutritional values, potential application, epidemiological and clinical studies addressing their potential health effects, as well as for the lipid composition analysis and quality control of rice bran oils. However, to date, there are no reports of the individual compositions of bound phenolics present in rice bran.

In general, bound phenolics can be released by alkali, acid, or enzymatic treatment of samples prior to extraction (Dai & Mumper, 2010). In most of cases, alkaline hydrolysis was the method mostly used for extracting esterified or bound phenolics (Su et al., 2014), for example, in our preliminary studies, the content of bound phenolics after alkaline hydrolysis of samples was significantly higher than after acid and enzymatic hydrolysis. It was for this reason that alkaline hydrolysis was selected for releasing bound phenolics and further chemical research.

In the context of our ongoing search for new bioactive components from fruits, vegetables, cereal grains and other natural products, a chemical investigation of the ethyl acetate (EtOAc) extract from an alkaline treatment sample of rice bran, led to the isolation of one new and nine known compounds and evaluation of their antioxidant activities. Herein, we describe the experimental details of the hydrolysis and isolation process as well as providing information pertaining to the elucidation of the structures of these compounds based on their spectroscopic properties and chemical reactivity.

2. Materials and methods

2.1. General methods

All of the ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 300, 400 and 500 spectrometer (Bruker BioSpin GmbH, Beijing, China), using tetramethylsilane (TMS) as an internal standard. The chemical shifts in the NMR spectra were recorded as δ values. Two-dimensional NMR spectra include COSY (Correlation Spectroscopy, COSY), HSQC (Heteronuclear Singular Quantum Correlation, HSQC) and HMBC (Heteronuclear Multiple Bond Correlation, HMBC). Electrospray ionization mass spectrometry (ESI-MS) analyses were measured on a Q-Tof Ultima Global GAA076 LC mass spectrometer (Waters Asia, Ltd, Singapore). TLC and column chromatography (CC) were performed on plates precoated with silica gel GF254

(10-40 μm), and over the silica gel (300-400 mesh, Qingdao Marine Chemical Factory, Qingdao, China), and reversed phase C18 (Octadecylsilyl, ODS) silica gel (Silicycle, 50 μm, Parc-Technologique Blvd, Canada) and Sephadex LH-20 (Sigma-Aldrich, St. Louis, MO, USA), respectively. Columns used for silica gel chromatography separation were 2.4cm in internal diameter and 30cm in length, and columns used for Sephadex LH-20 were 3.0cm in internal diameter and 100cm in length.

2.2. Materials and chemicals

Rice bran was purchased from WeiGang trade market (Nanjing, China). Di(phenyl)-(2,4,6-trinitrophenyl) iminoazanium (DPPH), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), L-Cysteine methyl ester hydrochloride, and N-trimethylsilylimidazole were purchased from Sigma Aldrich (St. Louis, MO, USA). Dimethyl sulfoxide-d_6 (DMSO-d_6) and $CDCl_3$ were obtained from Merck (Darmstadt, Germany). All of the other chemicals and solvents used in the current study were purchased as the analytical grade.

2.3. Alkaline hydrolysis of rice bran and extraction of bound phenolic compounds

The alkaline hydrolysis of rice bran was performed according to the method described by Adom et al. (2002) with minor modifications. Briefly, rice bran (1.2 kg) was grinded and homogenized in a Polytron homogenizer before being extracted three times with 12 L 80% acetone (1:10, w/v) at ambient temperature for 24 h. The supernatant was discarded after each extraction. The residues were then digested with 2 M 10L sodium hydroxide at ambient temperature for 2 h with shaking under nitrogen gas. The mixture was neutralized with an appropriate amount of hydrochloric acid and extracted with hexane to remove lipids. The final solution was extracted five times with EtOAc, and the combined EtOAc extracts were concentrated under vacuum at 40 °C to give the crude extract (8.2 g).

2.4. Isolation bound phenolic compounds

105 The crude extract (8.2 g) was separated into four fractions (Fr. 1-4) using normal-phase silica gel CC (20
106 g silica gel, 300-400 mesh) using a step-wise gradient elution of petroleum ether: acetone: MeOH
107 (100:0:0–0:100:0–0:0:100, v/v/v).

108 Fr. 1 (2.5 g) was separated into 2 sub-fractions Fr. 1-1 and Fr. 1-2 on a silica gel CC using cyclohexane
109 eluent. Fr. 1-1 was subsequently purified by CC over silica gel with a choloroform eluent to yield Fr. 1-1-1,
110 which was further purified over a silica gel CC using petroleum ether: acetone eluent (100:1, v/v) to yield **1**
111 (31.4 mg) and **2** (10.0 mg).

112 Fr. 2 (1.8 g) was purified by CC over silica gel with a petroleum ether: acetone eluent (30:1, v/v) to yield
113 Fr. 2-1, which was then passed through a Sephadex LH-20 column with a $CHCl_3$: MeOH eluent (1:1, v/v) to
114 give **3** (153 mg) and **4** (16.2 mg).

115 Fr. 3 (2.0 g) was also purified on a normal-phase silica gel CC using a cyclohexane: ethyl acetate eluent
116 (20:1, v/v) to yield Fr. 3-1, which was further purified by a reversed-phase C18 ODS CC using a step-wise
117 gradient elution of MeOH: H_2O (0:100-100:0) to give **5** (12.8 mg) and **6** (14.5 mg).

118 Fr. 4 (1.9 g) was subjected to CC over silica gel using a chloroform: acetone eluent (30:1, v/v) to give Fr.
119 4-1, Fr. 4-2 and Fr. 4-3. Fr. 4-1 was purified by CC over silica gel with a petroleum ether: acetone eluent (20:1,
120 v/v) to give **7** (6.8 mg). Fr. 4-2 was separated through a Sephadex LH-20 column with a $CHCl_3$: MeOH eluent
121 (1:1, v/v) to give **8** (4.6 mg). Fr. 4-3 was purified on a silica gel CC using cyclohexane: ethyl acetate eluent
122 (30:1, v/v) to yield **9** (11.7 mg) and **10** (9.8 mg). Fig. 1 showed the extraction and separation processes
123 associated with EtOAc extract of rice bran.

124 **2.5. Assay of DPPH and $ABTS^+$ radical-scavenging**

DPPH radical scavenging assay was performed according to a previously reported protocol (Rivero-Perez, Muniz & Gonzalez-Sanjose, 2007). Radical scavenging activity against $ABTS^+$ was performed as described in the literature (Zheleva-Dimitrova, Nedialkov & Kitanov, 2010). DPPH radical scavenging activity (%)=[1-($A_{517\ nm\ of\ sample}$/$A_{517\ nm\ of\ control}$)]×100, where $A_{517\ nm\ of\ sample}$ is the absorbance of DPPH radical solution mixed with the test compound/Vitamin E, $A_{517\ nm\ of\ control}$ is the absorbance of DPPH radical solution mixed with methanol solution. $ABTS^+$ radical scavenging activity (%)=[1-($A_{734\ nm\ of\ sample}$/$A_{734\ nm\ of\ control}$)]×100, where $A_{734\ nm\ of\ sample}$ is the absorbance of $ABTS^+$ radical solution mixed with the test compound/Vitamin E, $A_{734\ nm\ of\ control}$ is the absorbance of $ABTS^+$ radical solution mixed with methanol solution. The antioxidant activities of the test compounds were expressed as IC_{50}, with the IC_{50} being defined as the concentration of test compound required to inhibit the formation of radicals by 50%. Vitamin E was employed as a positive control.

2.6. Acid Hydrolysis of the Glucoside and Determination of the Absolute Configuration of the Monosaccharides

The assay used to determine the absolute configuration of the monosaccharide was performed as described in the literature (Liang, Hao, Zhang, Zhang, Chen & Yu, 2011).

2.7. NMR and ESI-MS Spectroscopic Data

***Cis*-ferulates cycloeucalenol (1)** and ***trans*-ferulates cycloeucalenol (2)**: White amorphous powder; ^{1}H NMR and ^{13}C NMR data, see Table 1; ESIMS *m*/*z* 601.1 $[M - H]^-$.

***Trans*-ferulic acid (3):** Colorless needle; ^{1}H NMR (300 MHz, DMSO-d_6, TMS): δ 7.50 (d, J = 15.87 Hz, 1H, H-7), 7.27 (d, J = 2.16 Hz, 1H, H-2), 7.08 (dd, J = 8.07, 1.89 Hz, 1H, H-6), 6.79 (d, J = 8.13 Hz, 1H, H-5), 6.38 (d, J = 15.87 Hz, 1H, H-8), 3.80 (s, 3H, 3-OCH_3); ^{13}C NMR: δ 126.5 (C-1), 110.3 (C-2), 149.1 (C-3),

147.8 (C-4), 115.1 (C-5), 123.0 (C-6), 145.2 (C-7), 114.9 (C-8), 167.7 (C-9), 55.4 (3-O**C**H$_3$); ESIMS *m/z* 193.0 [M - H]$^-$.

***Trans*-ferulic acid methyl ester (4):** Colorless needle; ^{1}H NMR (500 MHz, CDCl$_3$, TMS): δ 7.66 (d, *J* = 15.92 Hz, 1H, H-7), 7.10 (dd, *J* = 8.20, 1.84 Hz, 1H, H-6), 7.04 (d, *J* = 1.84 Hz, 1H, H-2), 6.95 (d, *J* = 8.16 Hz, 1H, H-5), 6.33 (d, *J* = 15.92 Hz, 1H, H-8), 5.98 (s, 1H, 4-OH), 3.94 (s, 3H, 3-OCH$_3$), 3.82 (s, 3H, 9-OCH$_3$); ^{13}C NMR: δ 126.9 (C-1), 109.3 (C-2), 148.0 (C-3), 146.8 (C-4), 115.1 (C-5), 123.0 (C-6), 145.0 (C-7), 114.7 (C-8), 167.8 (C-9), 55.9 (3-OCH$_3$), 51.6 (9-OCH$_3$); ESIMS *m/z* 207.0 [M - H]$^-$, 231.0 [M + Na]$^+$.

***Cis*-ferulic acid (5):** Colorless needle; ^{1}H NMR (300 MHz, DMSO-d_6, TMS): δ 7.66 (d, *J* = 1.83 Hz, 1H, H-2), 7.13 (dd, *J* = 8.25, 1.86 Hz, 1H, H-6), 6.75 (d, *J* = 8.19 Hz, 1H, H-5), 6.64 (d, *J* = 12.96 Hz, 1H, H-8), 5.75 (d, *J* = 12.96 Hz, 1H, H-7), 3.74 (s, 3H, 3-OCH$_3$), ESIMS *m/z* 193.0 [M - H]$^-$.

***Cis*-ferulic acid methyl ester (6):** ^{1}H NMR (500 MHz, CDCl$_3$, TMS): δ 7.82 (d, *J* = 2.15 Hz, 1H, H-2), 7.13 (dd, *J* = 8.25, 1.86 Hz, 1H, H-6), 6.91 (d, *J* = 8.35 Hz, 1H, H-5), 6.84 (d, *J* = 12.90 Hz, 1H, H-8), 5.82 (d, *J* = 12.90 Hz, 1H, H-7), 3.95 (s, 3H, 3-OCH$_3$), 3.75 (s, 3H, 9-OCH$_3$); ESIMS *m/z* 207.0 [M - H]$^-$.

Methyl caffeate (7): White amorphous powder; ^{1}H NMR (500 MHz, Acetone, TMS): δ (1H, d, *J* = 15.92 Hz, H-7), 7.17 (1H, d, *J* = 15.92 Hz, H-8), 7.06(1H, dd, *J* = 1.84, 8.16 Hz, H-6), 6.89 (1H, d, *J* = 8.20 Hz, H-8), 6.31(1H, d, *J* = 15.92 Hz, H-7), 3.72 (s, 3H, 9-OCH$_3$); ^{13}C NMR: δ 127.5 (C-1), 115.1 (C-2), 148.7 (C-3), 146.3 (C-4), 115.3 (C-5), 122.5 (C-6), 145.7 (C-7), 116.3 (C-8), 167.9 (C-9), 51.5 (9-O**C**H$_3$); ESIMS *m/z* 217.1 [M + Na]$^+$, 193.1 [M - H]$^-$.

***Para*-hydroxy methyl benzoate glucoside (8):** White amorphous powder; ^{1}H NMR and ^{13}C NMR data, see Table 1; HRESIMS [M + Na]$^+$ at *m/z* 337.0906 (calcd 337.0899).

Vanillic aldehyde (9): Colorless needle; ^{1}H NMR (400 MHz, CDCl$_3$, TMS): δ 9.86 (s, 1H, CHO), 7.47

(2H, d, J = 5.84 Hz, H-2, H-6), 7.09 (1H, d, J = 8.48 Hz, H-5), 4.00 (3H, s, 3-OCH_3); ^{13}C NMR: δ 127.6 (C-1), 129.8 (C-2), 147.2 (C-3), 151.8 (C-4), 114.4 (C-5), 108.7 (C-6), 191.0 (C-7), 56.1 (s, 3H, 3-OCH_3); ESIMS m/z 151.0 $[M - H]^-$.

***Para*-hydroxy benzaldehyde (10):** Colorless amorphous powder; ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 9.79 (s, 1H, H-7), 7.77 (2H, d, J = 8.48 Hz, H-2, H-6), 6.95 (2H, d, J = 8.48 Hz, H-3, H-5), ESIMS m/z 121.0 $[M - H]^-$.

2.8. Statistical analysis

All the data were analyzed according to Duncan's multiple comparison test ($p < 0.05$), using version 8.1 of the SAS software package. The resulting data were presented as mean ± SD.

3. Results and discussion

3.1. Structural elucidation of the isolated compounds

Compound **1** was isolated as a white amorphous powder. Its molecular formula was determined to be $C_{40}H_{58}O_4$ based on its ^{1}H and ^{13}C NMR, and ESI-MS data. ESI-MS analysis of the material revealed an m/z value of 601.1, corresponding to $[M - H]^-$. The ^{1}H and ^{13}C NMR spectra were typical of a feruloyl ester of triterpene alcohols. The 1D NMR spectrum showed the presence of one olefinic proton at δ 5.10 (t, J = 6.75 Hz, 1H, H-24), one oxygenated methine proton at δ 4.71 (s, 1H, H-3), seven methyl protons at δ 0.90 (s, 3H, H-18), 0.90 (s, 3H, H-21), 1.69 (s, 3H, H-26), 1.61 (s, 3H, H-27), 0.86 (s, 3H, H-28), 0.90 (s, 3H, H-29) and 0.90 (s, 3H, H-30), and two methylene protons of a cyclopropyl group at δ 0.35 (d, J = 3.95 Hz, 1H, H-19), 0.58 (d, J = 3.90 Hz, 1H, H-19), as well as two typical sp^2 olefinic carbon signals at δ 125.2 (C-24) and 130.9 (C-25). Taken together, these data were corresponding to a typical cycloartenol moiety. The ^{13}C NMR of **1** displayed the presence of 40 carbon signals, with 30 of these signals being assigned to the cycloartenol and 10

188 to the feruloyl ester moieties being connected to C-3 position of cycloartenol (Table. 1), which was confirmed
189 by the key correlation from H-3 to C-1′ from the HMBC experiment. The double bond of feruloyl ester
190 moieties was determined to be a *cis*-configuration by its $^{3}J_{\text{H-2'},\ \text{H-3'}}$ coupling constant of 12.60 Hz. Further
191 analysis of the 2D NMR data, including the COSY, NOESY, HSQC and HMBC spectra, allowed for the
192 complete assignment of the ^{1}H and ^{13}C NMR spectral data (Fig. 2A), and compound **1** was consequently
193 identified as *cis*-ferulates cycloeucalenol (Fig. 2). Although this compound has previously been isolated from
194 rice ran (Akihisa et al., 2000), the comprehensive NMR spectral data were not yet reported.

195 Compound **2** was isolated as a white amorphous powder. Its molecular formula was determined to be
196 $C_{40}H_{58}O_4$ on the basis of its ^{1}H and ^{13}C NMR, and ESI-MS spectra, with the latter of these analyses giving an
197 *m/z* value of 601.1 $[M - H]^-$. The ^{1}H and ^{13}C NMR spectra of **1** and **2** were very similar but not identical. A
198 direct comparison of the ^{1}H and ^{13}C NMR data of compound 2 with those of **1** revealed that they share the
199 same planar structure with the sole difference of the presence of a *trans*-configuration double bond at δ H 6.29
200 (d, J = 15.90 Hz, 1H) and 7.59 (d, J = 15.85 Hz, 1H) in **2** instead of the *cis*-configuration at δ H 5.84 (d, J =
201 12.60 Hz, 1H) and 6.79 (d, J = 12.85 Hz, 1H) in **1** (Table. 1). The structure of **2** was further confirmed by the
202 COSY and HMBC correlations and by comparing the NMR data with those of *trans*-ferulates cycloeucalenol
203 in the literature (Cho et al., 2012). Compound **2** was consequently identified as *trans*-ferulates cycloeucalenol
204 (Fig. 2).

205 Compound **3** was isolated as a colorless needle. The molecular formula of the material was determined to
206 be $C_{10}H_{10}O_4$ on the basis of its ^{1}H and ^{13}C NMR, and ESI-MS data. ESI-MS analysis of the material revealed
207 a peak at *m/z* 193.0, corresponding to $[M - H]^-$, indicating that the molecular weight of **3** was 194. The ^{1}H
208 NMR spectrum revealed the presence of two olefinic protons at δ 7.50 (d, J = 15.87 Hz, 1H, H-7) and 6.38 (d,

209 J = 15.87 Hz, 1H, H-8), and three aromatic ring protons at δ 7.27 (d, J = 2.16 Hz, 1H, H-2), 7.08 (dd, J = 8.07,
210 1.89 Hz, 1H, H-6) and 6.79 (d, J = 8.13 Hz, 1H, H-5), and one methoxyl at δ 3.80 (s, 3H, 3-OCH_3). The ^{13}C
211 NMR spectrum of **3** revealed two olefinic carbons at δ 145.2 (C-7), 114.9 (C-8), and six aromatic carbons at δ
212 126.5 (C-1), 110.3 (C-2), 149.1 (C-3), 147.8 (C-4), 115.1 (C-5) and 123.0 (C-6), and one methoxy carbon at δ
213 55.4, as well as a carboxyl carbon at δ 167.7 (C-9). The coupling constant of olefinic proton between C-7 and
214 C-8 was 15.87 Hz which allowed the determination of the double bond of **3** as a *trans*-configuration. Based on
215 a comparison of these data with data published in the literature (Yoshioka, Inokuchi, Fujioka & Kimura,
216 2004), compound **3** was identified as *trans*-ferulic acid (Fig. 2).

217 Compound **4** was isolated as a colorless needle, and its molecular formula was determined to be
218 $C_{11}H_{12}O_4$ on the basis of its ^1H and ^{13}C NMR and ESI-MS spectra. ESI-MS analysis of the material gave *m/z*
219 values of 207.0 and 231.0, corresponding to $[M - H]^-$ and $[M + Na]^+$, respectively. A careful comparison of the
220 ^1H and ^{13}C NMR spectra of **4** with those of **3** revealed the existence of a close structural relationship between
221 the two compounds. Compared to the spectra of **3**, an additional methoxyl signal at δ 3.94 (s, 3H, 9-OCH_3)
222 instead of the 9-OH signal at δ 9.78 (1H, br s) was observed in **4**. As expected, an additional methoxyl carbon
223 signal at δ 51.6 was observed in the ^{13}C NMR spectrum of **4**. By a comparison with data available in the
224 literature (Tanaka, Kato & Tsuchiya, 1971), compound **4** was determined to be *trans*-ferulic acid methyl ester
225 (Fig. 2).

226 Compound **5** and compound **3** share the same planar structure and the same molecular formula, $C_{10}H_{10}O_4$,
227 established on the basis of the ESIMS ions detected at *m/z* 193.0, corresponding to $[M - H]^-$. The ^1H NMR
228 spectrum revealed the presence of three aromatic ring protons at δ 7.66 (d, J = 1.83 Hz, 1H, H-2), 7.08 (dd, J
229 = 8.25, 1.86 Hz, 1H, H-6) and 6.75 (d, J = 8.19 Hz, 1H, H-5), and one methoxyl at δ 3.74 (s, 3H, 3-OCH_3), as

well as two olefinic protons at δ 5.75 (d, J = 12.87 Hz, 1H, H-7) and 6.38 (d, J = 12.96 Hz, 1H, H-8) with a lower coupling constant, corresponding to a *cis*-configuration double bond. Based on these data and a comparison with those reported in the literature (Akihisa et al., 2000), compound **5** was identified as *cis*-ferulic acid (Fig. 2).

Compound **6** and compound **4** share the same planar structure and the same molecular formula, $C_{11}H_{12}O_4$, determined on the basis of the ESIMS detected ions at *m/z* 207.0, corresponding to $[M - H]^-$. The ^1H NMR spectra for **6** and **4** showed very similar signals, with the exception that a pair of *trans*-configuration olefinic protons at δ 6.31 (d, J = 15.95 Hz, 1H, H-7) and 7.64 (d, J = 15.95 Hz, 1H, H-8) for **4** was replaced by a pair of *cis*-configuration olefinic protons at δ 5.85 (d, J = 12.90 Hz, 1H, H-7) and 6.84 (d, J = 12.90 Hz, 1H, H-8) for **6**. Based on the comparison of the NMR and MS spectral data with those reported in the literature (Tanaka, Kato & Tsuchiya, 1971), the structure of **6** was determined to be *cis*-ferulic acid methyl ester (Fig. 2).

Compound **7** was isolated as a white amorphous powder. The molecular formula was determined to be $C_{10}H_{10}O_4$ on the basis of its ^1H NMR, ^{13}C NMR and ESI-MS spectra. ESI-MS analysis gave *m/z* values of 217.1 and 193.1, corresponding to $[M + Na]^+$ and $[M - H]^-$, respectively. The ^1H and ^{13}C NMR spectra for **7** and **4** showed very similar signals, with the exception that the 3-OCH_3 singlet at δ H 3.92 for **4** was replaced by a new exchangeable proton at δ H 8.57 for **7**, and the absence of one carbon signal at δ C 55.9 corresponding to 3-OCH_3 carbon for **4**. These data indicated that the methoxy at C-3 in **4** was substituted by a hydroxy group in **7**. Based on a comparison of these data with information reported in the literature (Balachandran et al., 2012), compound **7** was identified as methyl caffeate (Fig. 2).

Compound **8** was isolated as a white amorphous powder. Its molecular formula was established as $C_{14}H_{18}O_8$ by High-resolution mass spectroscopy (HRMS) $[M + Na]^+$ at *m/z* 337.0906 (calcd 337.0899),

indicating six degrees of unsaturation. ^{1}H NMR and ^{13}C NMR spectrum of **8** revealed the presence of one *para*-disubstituted benzene ring, one sugar unit, one carbonyl and one methoxyl. Analysis of the ^{1}H-^{1}H COSY, HSQC and HMBC spectra of compound **8** allowed for the complete assignment of the ^{1}H and ^{13}C NMR spectral data (Table. 2). Key correlations from H-2 to C-1, C-4 and C-6, from H-3 to C-1, C-2, C-5 and C-7, from 7-OCH_3 to C-7 were observed in the HMBC experiments. The sugar unit, which was identified as a β-glucopyranosyl group on the basis of its $^{3}J_{\text{H-1′, H-2′}}$ coupling constant of 7.20 Hz, was placed at the C-1 position of the benzene ring because of the HMBC correlation between the anomeric proton at δ H 5.00 and the C-1 carbon resonance at δ C 161.5. The chemical shifts of all the individual protons and carbons of the sugar unit from C-1′ to C-6′ were assigned on the basis of ^{1}H-^{1}H COSY and HSQC spectra analysis (Fig. 2B). Acid hydrolysis of **8** with 1 N HCl liberated benzene acid and D-glucose, which were identified by gas chromatography-mass spectrometry (GC-MS) analysis of the corresponding trimethylsilyl L-cysteine derivative and a direct comparison with an authentic sample of the same material, prepared in the same manner. This result was further confirmed through a comparison of the retention time of this derivative with that of the authentic D-glucose derivative, with both samples providing the retention time of 7.92 min. Feng et al. (2008) isolated a new compound that had the same planar structure as that of compound **8** and named pseudolaroside C, however, the sugar moiety is not glucose but allose, therefore, they are a pair of isomers with the different sugar unit. Thus, the structure of **8** was unambiguously identified as *para*-hydroxy methyl benzoate glucoside, which is a new compound isolated and identified for the first time from a natural source (Fig. 2).

Compound **9** was obtained as a colorless needle. The molecular formula of the material was determined to be $C_8H_8O_3$ based on its ^{1}H and ^{13}C NMR, and ESI-MS data. ESI-MS analysis revealed an *m/z* value of

151.0, corresponding to $[M - H]^-$. Analysis of its ^1H NMR spectrum revealed the presence of a tri-substituted aromatic ring with signals at δ 7.47 (2H, d, J = 5.84 Hz, H-2, H-6) and 7.09 (1H, d, J = 8.48 Hz, H-5), as well as an aldehyde group signal at δ 9.86 (1H, br s, H-7), and three methoxy protons at δ 4.00 (3H, s, 3-OCH_3). Accordingly, the ^{13}C NMR spectrum of **9** showed six aromatic ring carbon signals at δ 151.8 (C-4), 147.2 (C-3), 129.8 (C-2), 127.6 (C-1), 114.4 (C-5) and 108.7 (C-6), one aldehyde group carbon signal at δ 191.0 (C-7), one methoxy carbon signal at δ 56.1 (s, 3H, 3-OCH_3). These data were the same as those reported for vanillic aldehyde in the literature (Wang, Zhang, Zhao, Wang, Liu & Xin, 2013), and compound **9** was consequently identified as vanillic aldehyde (Fig. 2).

Compound **10** was isolated as a colorless amorphous powder. The molecular formula of material was determined to be $C_7H_6O_2$ based on its ^1H NMR and ESI-MS data. ESI-MS analysis of the material gave an *m/z* peak of 121.0, corresponding to $[M - H]^-$. The ^1H NMR spectrum of **10** revealed the presence of an aldehyde group proton at δ 9.79 (s, H-7), and four aromatic ring protons at δ 7.77 (d, J = 8.48 Hz, H-2, H-6) and 6.95 (d, J = 8.32 Hz, H-3, H-5), corresponding to a *para*-disubstituted benzene ring. Based on these data and a comparison with data available in the literature (Shengan, Rong, Wenhan & Hongquan, 2012), compound **10** was determined to be *para*-hydroxy benzaldehyde (Fig. 2).

3.2. Antioxidant activities of the pure compounds isolated from rice bran

The antioxidant activities of the 10 pure compounds were tested according to their DPPH and $ABTS^+$ radical scavenging assay. The results of these experiments have been shown in Fig. 3 and Fig. 4. DPPH radical scavenging activity of compounds 1-10 (100μM) ranged from 15% to 97% (Figure 3A). Compound **3**, **4**, **5, 6** and **7** displayed a higher DPPH radical-scavenging activity than that of Vitamin E. Compound 3, 5 and 7 showed the highest antioxidant activity followed by compounds 4 and 6 and compounds 1, 2 and 8. Compound

293 **9** and **10** had the lowest DPPH radical-scavenging activity. There was no significant difference ($p > 0.05$) in
294 antioxidant activities among compound **3**, **5** and **7**, between **4** and **6**, **1** and **2**. Based on the data obtained,
295 compound **1-8** were selected to further determine whether their DPPH radical-scavenging capacity were
296 exhibited in a dose-dependent manner and the results were shown in Fig. 4A. The DPPH radical-scavenging
297 activities for compound **3**, **5** and **7** increased sharply with increasing concentration in a dose-dependent
298 manner at the range of 0-40 μM, and increased up to a maximal value of 91.68 % DPPH inhibitory effect at
299 the concentration of 40 μM, then keep constant, and other samples also showed a dose-dependent manner in
300 DPPH radical-scavenging assay. The IC_{50} of compound **1-8** and Vitamin E were 87.06, 84.98, 14.59, 61.16,
301 16.29, 63.16, 15.26, >100 and 71.17 μM, respectively. These results indicated that the DPPH
302 radical-scavenging activities of *trans*-ferulic acid, *cis*-ferulic acid and methyl caffeate were nearly the same
303 and stronger than that of Vitamin E, followed by *trans*-ferulic acid methyl ester and *cis*-ferulic acid methyl
304 ester. The antioxidant activities of cycloeucalenol *cis*-ferulate and cycloeucalenol *trans*-ferulate were almost
305 identical and comparable with Vitamin E. However, *para*-hydroxy methyl benzoate glucoside was less active
306 than Vitamin E since its IC_{50} value was higher. Similarly, as shown in Fig. 4B, all samples showed a
307 dose-dependent $ABTS^+$ scavenging-radical activity. IC_{50} values for compounds **1-8** and Vitamin E were 62.30,
308 60.60, 6.58, 35.35, 7.23, 37.63, 6.74, 65.52 and 57.91 μM, respectively.

309 It is well documented that ferulic acid and γ-oryzanol are the major antioxidants in rice bran, a
310 substantial of work has been carried out to investigate their antioxidant activity in *vitro* and *vivo* base on
311 different models. Hiramitsu et al. suggested that γ-oryzanol inhibited 61% reaction at a concentration of 10^{-4}
312 M in a lipid peroxidation system induced by porcine retinal homogenates using ferric iron or UV light, while
313 α-tocopherol only 14% in the same conditions (Hiramitsu & Armstrong, 1991). Xu & Godber demonstrated

314 that γ-oryzanol components (cycloartenyl ferulate, 24-methylenecycloartanyl ferulate, and campesteryl
315 ferulate) possessed significantly higher antioxidant activity than that of any of the four vitamin E components
316 (α-tocopherol, α-tocotrienol, γ-tocopherol, and γ-tocotrienol) in a cholesterol oxidation system accelerated by
317 2,2'-azobis(2-methylpropionamidine) dihydrochloride, of which 24-methylenecycloartanyl ferulate showed
318 the highest antioxidant activity (Xu, Hua, & Godber, 2001). The higher antioxidant activities of γ-oryzanol
319 components might because the structure of γ-oryzanol components is similar to that of cholesterol, an
320 important component in reducing oxidation stress and maintaining the functionality of cells, therefore, in
321 accordance with the structural relationship theory, the γ-oryzanol components may have greater ability to
322 associate with cholesterol in the small droplets of the emulsion and become more efficient in protecting
323 cholesterol against free radical attack. However, they also found that ferulic acid showed the highest
324 antioxidant activity, followed by vitamin E and γ-oryzanol at the three different ratios in a linoleic acid model
325 (Xu & Godber, 2001). The antioxidant properties of ferulic acid could be attributed to its aromatic phenolic
326 ring that stabilizes and delocalizes the unpaired electron within its aromatic ring, thereby acting as free-radical
327 scavengers (Srinivasan, Sudheer, & Menon, 2007). Whereas the triterpene portion of γ-oryzanol may affect its
328 antioxidant activity by lowering the mobility in the system due to its relatively larger molecular structure than
329 free ferulic acid (Xu & Godber, 2001). Wilson et al. reported that oryzanol has a greater effect on lowering
330 plasma lipid and lipoprotein cholesterol concentrations in hypercholesterolemic hamsters. However, they also
331 suggested that ferulic acid may have a greater antioxidant capacity via its ability to maintain serum vitamin E
332 levels compared to rice bran oil and oryzanol. Thus, both oryzanol and ferulic acid may exert similar
333 antiatherogenic properties, but through different mechanisms (Wilson, Nicolosi, Woolfrey, & Kritchevsky,
334 2007). To date there are various antioxidant activity assay models, all of them with strengths and limitations.

In some case, it may have some controversy when using different model. There is not one method that can provide unequivocal results and the best solution is to use various methods instead of a one-dimension approach (Carocho & Ferreira, 2013).

In the current study, alkaline hydrolysis was used to release phenolic compounds present in the insoluble bound form prior to extract with EtOAc. An investigation of the chemical constituents of EtOAc extract has led to the isolation of a new compound, *para*-hydroxy methyl benzoate glucoside (**8**), together with nine known compounds, cycloeucalenol *cis*-ferulate (**1**), cycloeucalenol *trans*-ferulate (**2**), *trans*-ferulic acid (**3**), *trans*-ferulic acid methyl ester (**4**), *cis*-ferulic acid (**5**), *cis*-ferulic acid methyl ester (**6**), methyl caffeate (**7**), vanillic aldehyde (**9**) and *para*-hydroxy benzaldehyde (**10**). The structures of these compounds were elucidated using a combination of spectroscopic methods and chemical analysis. Among the compounds isolated, compound **3, 5** and **7** exhibited strong DPPH hydroxy radical and $ABTS^+$ radical scavenging activities as compared with Vitamin E, followed by compounds **4** and **6**, compound **1** and **2** showed potent DPPH hydroxy radical and $ABTS^+$ radical scavenging activities, compound **8** displayed moderate antioxidant activity against $ABTS^+$ radical, whereas compound **9** and **10** showed weak activities, and compound **3** was the predominant antioxidant ingredient among the isolated bound phenolic compounds.

Compound **1** and **2** are a pair of isomers of γ-oryzanol naturally occurring in rice bran and rice germ. γ-oryzanol is a mixture of steryl ferulates, which are formed by esterification of the hydroxyl group of sterols (campesterol, stigmasterol, β-sitosterol) or triterpene alcohols (cycloartanol, cycloartenol, 2,4-methylene cycloartanol, cyclobranol) with the carboxylic group of ferulic acid (Goufo & Trindade, 2014). Since γ-oryzanol was first identified in rice bran oil in 1954 (Fang, Yu & Badger, 2003), at least 25 constituents of γ -oryzanol have been found to date, with five of them accounting for about 95% of the total γ-oryzanol content,

including 2,4-methylenecycloartanyl *trans*-ferulate (34-44%), cycloartenyl *trans*-ferulate (19-26%), campesteryl *trans*-ferulate (15-23%), *β*-sitosteryl *trans*-ferulate (7-17%), and stigmasteryl *trans*-ferulate (1-7%) (Goufo & Trindade, 2014). It has been reported that *γ*-oryzanol showed various important biological profile such as antioxidant activities, lowering serum cholesterol levels, treating inflammatory diseases, inhibiting tumor growth, decreasing platelet aggregation, promoting blood circulation, reducing blood pressure and also promoting growth and development in humans and animals (Goufo & Trindade, 2014; Cho et al., 2012). Besides the well-documented health benefits, *γ*-oryzanol also has been reported as a potential additive in various food products, pharmaceuticals and cosmetics (Goufo & Trindade, 2014). Akihisa et al. (2000) isolated and identified five pairs of *trans*- and *cis*-ferulate isomers include cycloeucalenol *cis*-ferulate and cycloeucalenol *trans*-ferulate from rice bran by column chromatography, TLC, HPLC and ^{1}H NMR. However, other studies suggest that these *cis*-ferulates were most likely formed during the manufacture of rice bran and the multistep separation process of individual *γ*-oryzanol components, because daylight and long-wavelength UV radiation can induce *cis-trans* isomerization of feruloyl esters (Goufo & Trindade, 2014; Fang, Yu & Badger, 2003). In the present study, it was found that the content of cycloeucalenol *cis*-ferulate was increasing continuously following the decreasing of cycloeucalenol *trans*-ferulate by TLC detection during purification, indicating that cycloeucalenol *trans*-ferulate was unstable and could convert to cycloeucalenol *cis*-ferulate. This result therefore implied that *trans*-ferulates were not really natural products, but artifact products.

Compound **3-6** are *cis-trans* isomers of ferulic acid and its methyl ester derivatives. Ferulic acid is widely distributed in fruits, vegetables and cereal grains. In rice, ferulic acid is esterified to cell wall components and thus form part of the dietary fiber and exist therefore in a insoluble bound form (Arranz,

Silvan & Saura-Calixto, 2010). Ferulic acid is considered as one of the most important phenolic acids, exhibiting a wide spectrum of therapeutic properties like anti-inflammatory, antiatherogenic, antidiabetic, antiageing, neuroprotective, radioprotective and hepatoprotective effects (Adom & Liu, 2002). It also protects against coronary disease, lowers cholesterol in serum and liver, and increases sperm viability (Adom, Sorrells & Liu, 2003). Many of these activities can be attributed to its potent antioxidant capacity because its phenolic nucleus and unsaturated side chain can readily form a resonance stabilized phenoxy radical delocalized across the entire molecule (Rice-Evans, Miller & Paganga, 1997). On account of these properties it is receiving increased attention with regard to applications in the food, health, cosmetic, and pharmaceutical industries. Four forms of ferulic acid and its derivates were isolated as the major components of bound phenolics from rice bran in this study, two *cis*-isomers are the converted products of the corresponding *trans*-isomers due to *cis-trans* isomerization of the side chain of olefinic protons. Antioxidant experiments revealed that *trans*-isomers possessed almost identical DPPH hydroxy radical and $ABTS^{+}$ radical scavenging activities to the corresponding *cis*-isomers and were the main antioxidant compounds among bound phenolics, and the antioxidant activity of the ferulic acid methyl ester was slightly lower than the corresponding ferulic acid due to a mehoxyl group instead of a hydroxyl group.

Compound **7**, methyl caffeate, is a methyl esterification product of caffeic acid which is the major representative of hydroxycinnamic acids widely present in almost every plant. Caffeic acid showed significant antioxidant activity and might inhibit the formation of mutagenic and carcinogenic nitrosamine compounds (Kuenzig et al., 1984). In vivo, when ingested with the diet, caffeic acid increase the plasma antioxidant capacity and inhibit oxidation of low-density lipoprotein (Gulcin, 2006). Methyl caffeate showed strong

397 antioxidant activities in DPPH and $ABTS^+$ assay, indicating that it might be one of the important contributors
398 for antioxidant activity of bound phenolics.

399 Compound **8**, *para*-hydroxy methyl benzoate glucoside, consists of a molecular of *para*-hydroxy benzoic
400 acid and glucose by esterification integrated to the aleurone, pericarp and embryo cell walls. Compound **8**
401 exhibited moderate antioxidant activity against $ABTS^+$ radical and might represent a new potential natural
402 antioxidant agent.

403 Compound **9**, vanillic aldehyde, was isolated from the seedpods of *Vanilla planifolia* originally. This
404 compound has been shown to have a wide range of bioactivities such as antioxidant, anti-mutagenic,
405 anti-inflammatory, analgesic, hypolipidemic, and hepatoprotective activities (Wang, Zhang, Zhao, Wang, Liu
406 & Xin, 2013). It has been demonstrated that vanillic aldehyde can be conversed from ferulic acid due to
407 microbial metabolism (Venturi, Zennaro, Degrassi, Okeke & Bruschi, 1998). In general, vanillic aldehyde is
408 used as flavoring agents in foods, beverages, cosmetics and pharmaceuticals.

409 Compound **10**, *para*-hydroxy benzaldehyde, is one of the three isomers of hydroxyl benzaldehyde. This
410 compound has been found in several plants such as the orchid *Gastrodia elata*, *Galeola faberi* and vanilla. It
411 has been reported that *para*-hydroxy benzaldehyde showed antioxidation and inhibition of
412 gamma-aminobutyric acid (GABA) transaminase associated with antiepileptic and anticonvulsive activity (Ha
413 et al., 2000).

414 It has been documented that cells in humans and other organisms are constantly exposed to a variety of
415 oxidizing agents, some of which are necessary for life (Wang, Zhang, Zhao, Wang, Liu & Xin, 2013).
416 Overproduction of oxidants can cause oxidative stress resulting in damage to DNA and proteins molecules, as
417 well as membrane lipid oxidation and an increased risk of degenerative diseases such as cancer,

418 atherosclerosis, ageing, cardiovascular and inflammatory diseases (Adom & Liu, 2002). Therefore, the
419 consumption of sufficient amounts of antioxidants has been recommended to prevent or slow the oxidative
420 stress induced by free radicals. Phytochemicals derived from natural resources, particularly from fruits,
421 vegetables and cereal grains containing high levels of bound phenolics, have played an important role
422 associated with a decreased risk of cardiovascular disease (Sun, Chu, Wu & Liu, 2002). Moreover, it has been
423 reported that most of bound phenolics, which are strongly covalently linked to cell wall components thus act
424 as part of the dietary fiber that are resistant to digestion and absorption in the human stomach and small
425 intestine, are entrapped by dietary fibre and reach the colon intact, where they are released under the
426 fermentation of colonic microflore, and exert beneficial effects by scavenging the free radicals and
427 counteracting the effects of dietary pro-oxidants (Adom & Liu, 2002). This may partly explain the mechanism
428 of grain consumption in the prevention of colon cancer and other digestive cancers, which is supported by
429 epidemiological studies (Adom, Sorrells & Liu, 2003). The high levels of bound phenolic compounds with
430 significant antioxidant activity present in rice bran indicated that it is a valuable potential resource and worthy
431 of further utilizing and developing on industry scale. It is worth mentioning, however, that the bound phenolic
432 compounds are only a minor component of rice bran. This research therefore suggests that the antioxidant
433 activities of rice bran are derived from the combination of phytochemicals and not from a single compound.
434 This result is therefore consistent with similar findings in whole fruits and vegetables, where the additive and
435 synergistic effects of phytochemicals in whole foods have been reported to be responsible for their potent
436 antioxidant activities, with the potential health benefits being attributed to the complex mixtures of
437 phytochemicals present in whole foods (He & Liu 2008).

In summary, this study has demonstrated that the individual bound phenolic compounds present in rice bran can be released by alkaline hydrolysis and obtained by chromatographic purification. The majority of the phenolic compound was ferulic acid with powerful antioxidant activity. *Para*-hydroxy methyl benzoate glucoside, in particular, has been identified as a new compound and showed moderate antioxidant activity against $ABTS^{+}$ radical. The current study represents a useful addition to understand the potential application of the bound phenolic compounds, lay the foundation for the clarification of mechanisms associated with combating human diseases, and enhance better understanding of the relationship between bound phenolics and human health.

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624 Figure Captions

625 Fig. 1 Extraction and separation process for the bound phenolics of rice bran

626 Fig. 2 Bound phenolic compounds isolated from rice bran and Key COSY and HMBC correlations for

627 compound **1** (A) and **8** (B)

628 Fig. 3 DPPH hydroxy radical-scavenging activity (A), ABTS radical-scavenging activity (B) of the isolated

629 compounds at the concentration of 100 μM. cycloeucalenol *cis*-ferulate (**1**), cycloeucalenol *trans*-ferulate (**2**),

630 *trans*-ferulic acid (**3**), *trans*-ferulic acid methyl ester (**4**), *cis*-ferulic acid (**5**), *cis*-ferulic acid methyl ester (**6**),

631 methyl caffeate (**7**), *para*-hydroxy methyl benzoate glucoside (**8**), vanillic aldehyde (**9**) and *para*-hydroxy

632 benzaldehyde (**10**). Each value has been presented as the mean ± SD of three experiments. Note: a-e, results

633 with a different letter differ significantly ($p < 0.05$).

634 Fig. 4 DPPH hydroxy radical-scavenging activity (A) and ABTS radical-scavenging activity (B) of

635 compounds **1-8** under different concentrations.

636 Table 1. NMR spectral data for compound **1** and **2** in $CDCl_3$ at 400 (^1H) and 100 MHz (^{13}C)

637 Table 2. NMR spectral data for compound **8** in DMSO-d_6 at 400 (^1H) and 100 MHz (^{13}C)

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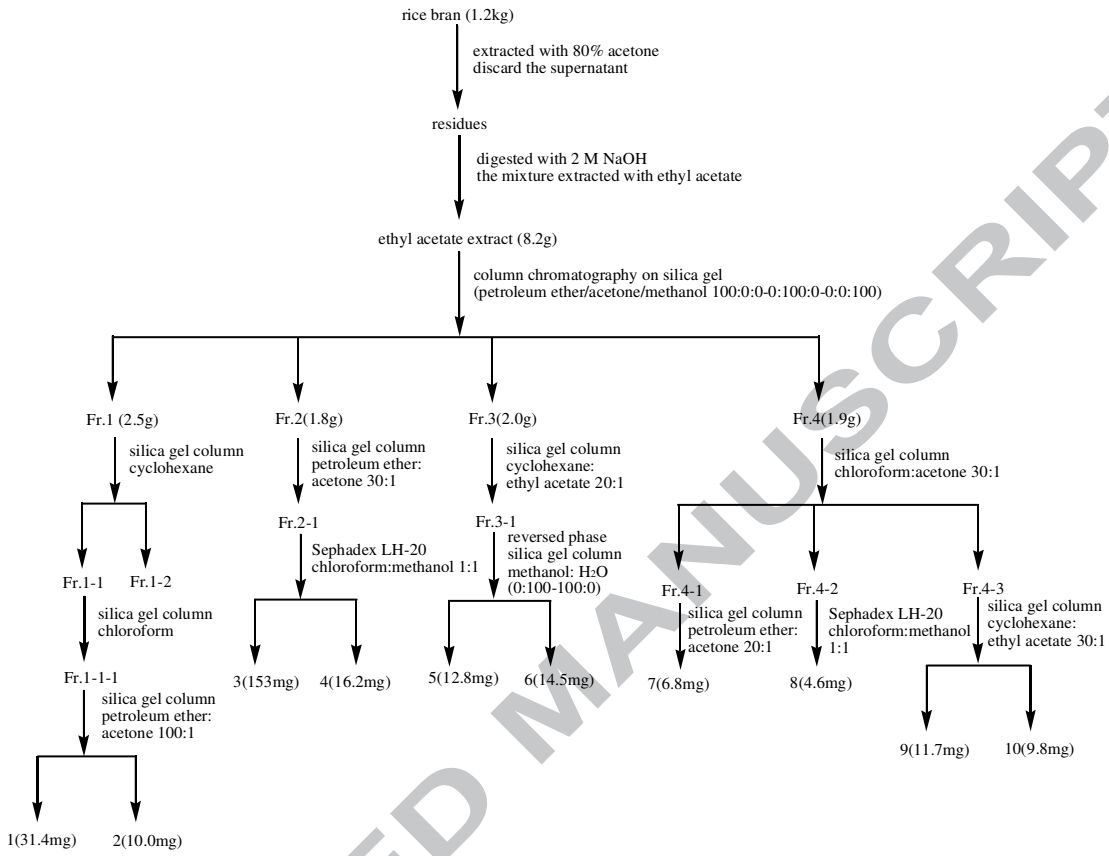
644 Figure. 1



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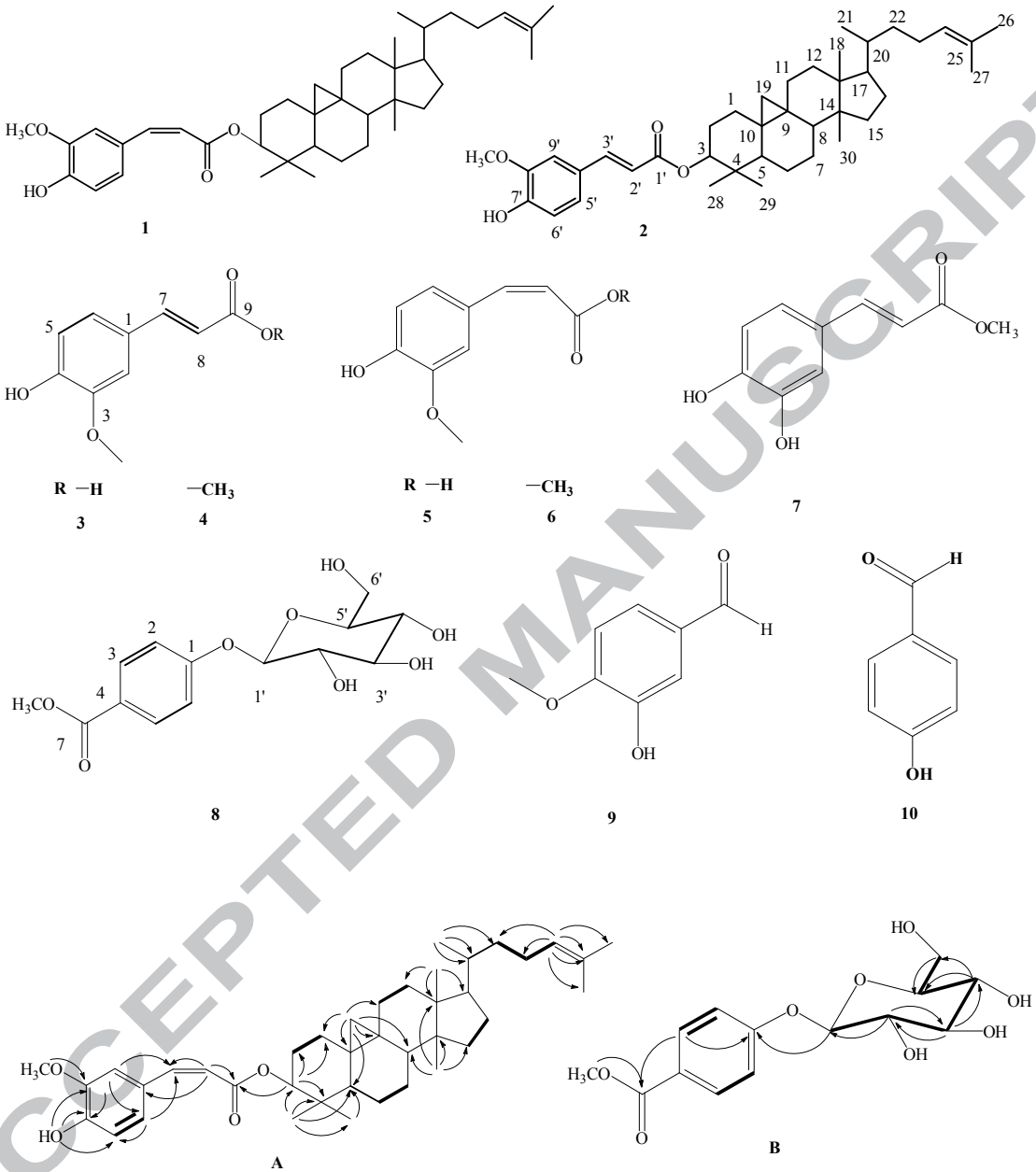
655 Figure. 2



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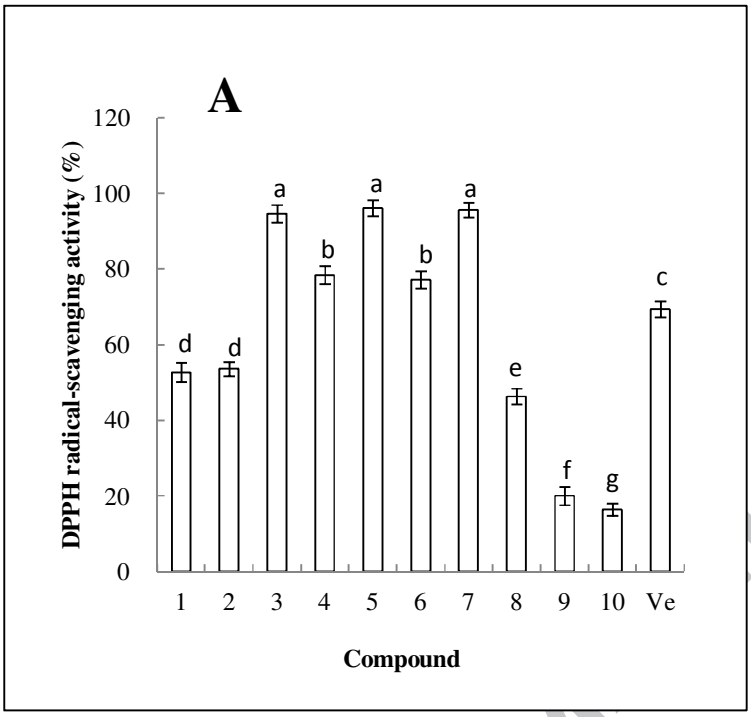
662 Figure. 3



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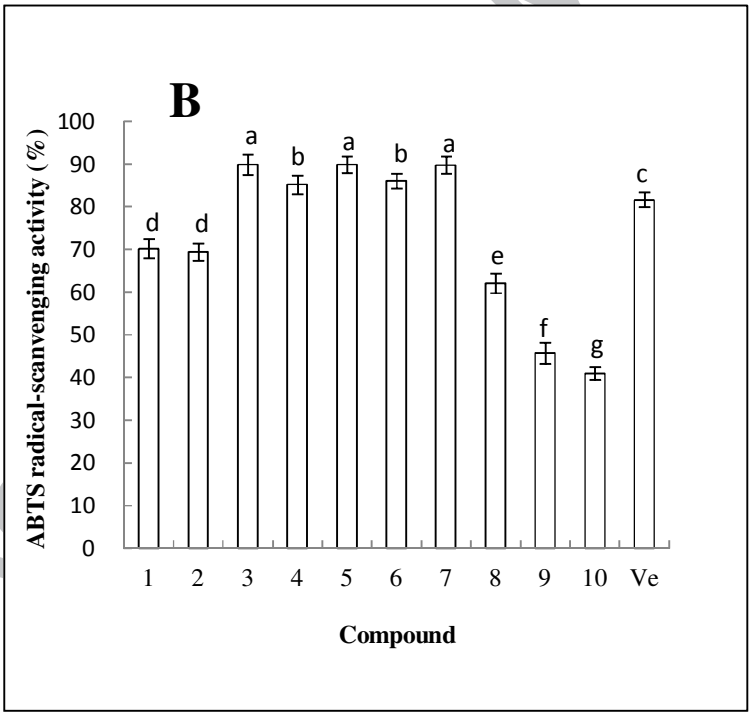

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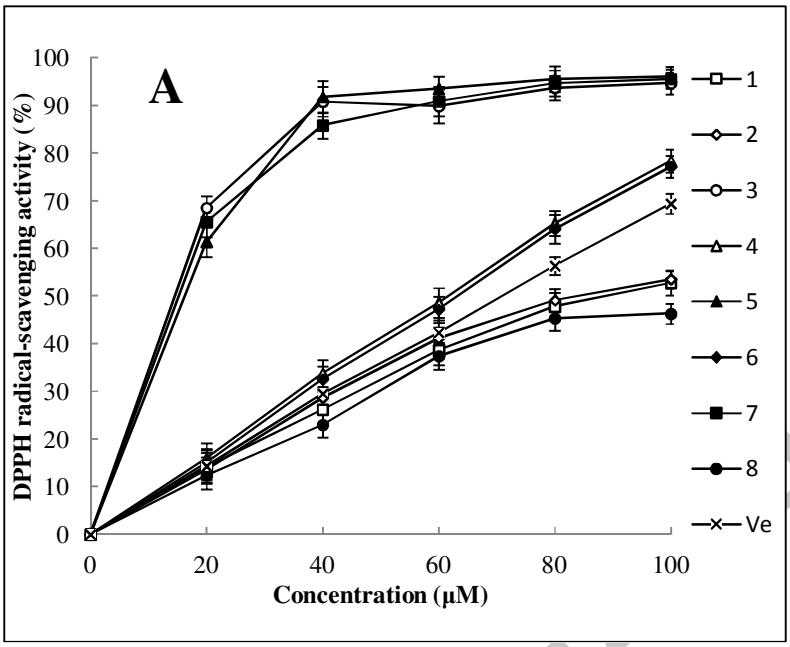
666 Figure. 4



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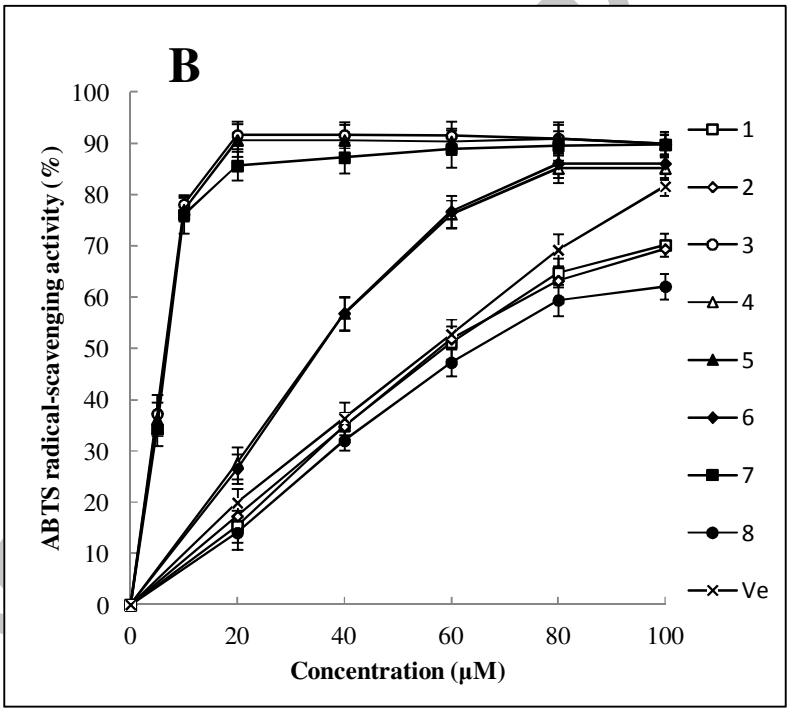

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670 **Table 1.** NMR spectral data for compound **1** and **2** in $CDCl_3$ at 400 (^1H) and 100 MHz (^{13}C)

	1		2	
Position	H (*J* in Hz)	C (*J* in Hz)	H (*J* in Hz)	C (*J* in Hz)
1	1.27 (m, 1H)	31.6	1.27 (m, 1H)	31.6
	1.65 (m, 1H)		1.65 (m, 1H)	
2	1.65 (m, 1H)	26.5	1.65 (m, 1H)	26.9
	1.83 (m, 1H)		1.84 (m, 1H)	
3	4.71 (s, 1H)	80.4	4.71 (m, 1H)	80.5
4		39.5		39.7
5	1.42 (d, *J* = 4.35, 12.40 Hz, 1H)	47.2	1.44 (d, *J* = 4.35, 12.40 Hz, 1H)	47.2
6	1.49 (d, *J* = 4.35, 12.40 Hz, 1H)	20.9	1.44 (d, *J* = 4.35, 12.40 Hz, 1H)	20.9
	1.58 (m, 1H)		1.59 (m, 1H)	
7	1.30 (m, 1H)	28.1	1.30 (m, 1H)	28.1
	1.90 (m, 1H)		1.93 (m, 1H)	
8	1.53 (dd, *J* = 3.70, 12.15 Hz, 1H)	47.8	1.53 (dd, *J* = 3.70, 12.15 Hz, 1H)	47.8
9		20.1		20.1
10		25.7		25.8
11	1.63 (m, 1H)	26.8	1.15 (m, 1H)	26.5
	2.00 (m, 1H)		2.00 (m, 1H)	
12	1.30 (m, 1H)	35.5	1.30 (m, 1H)	35.5
13		45.2		45.2
14		48.8		48.8
15	1.63 (m, 1H)	32.9	1.63 (m, 1H)	32.8
16	1.13 (m, 1H)	26.0	1.11 (m, 1H)	26.0
17	1.61 (m, 1H)	52.2	1.63 (m, 1H)	52.2
18	0.90 (s, 3H)	18.3	0.91 (s, 3H)	18.3
19	0.35 (d, *J* = 3.95 Hz, 1H)	29.7	0.36 (d, *J* = 3.95 Hz, 1H)	29.7
	0.58 (d, *J* = 3.90 Hz, 1H)		0.60 (d, *J* = 3.90 Hz, 1H)	
20	1.40 (m, 1H)	36.1	1.40 (m, 1H)	36.1
21	0.90 (s, 3H)	18.2	0.90 (s, 3H)	18.2
22	1.15 (m, 1H)	35.0	1.15 (m, 1H)	35.0
	1.58 (m, 1H)		1.58 (m, 1H)	
23	2.03 (m, 1H)	24.9	2.03 (m, 1H)	24.9
24	5.10 (t, *J* = 6.75 Hz, 1H)	125.2	5.10 (t, *J* = 7.20 Hz, 1H)	125.2
25		130.9		130.8
26	1.69 (s, 3H)	25.4	1.68 (s, 3H)	25.4
27	1.61 (s, 3H)	17.6	1.61 (s, 3H)	17.6
28	0.86 (s, 3H)	17.9	0.97 (s, 3H)	17.9
29	0.90 (s, 3H)	25.4	0.90 (s, 3H)	25.4
30	0.90 (s, 3H)	15.1	0.97 (s, 3H)	15.3
1′		166.4		167.0
2′	5.84 (d, *J* = 12.60 Hz, 1H)	117.7	6.29 (d, *J* = 15.90 Hz, 1H)	116.2
3′	6.79 (d, *J* = 12.85 Hz, 1H)	143.3	7.59 (d, *J* = 15.85 Hz, 1H)	144.3
4′		127.4		127.6
5′	7.14 (dd, *J* = 8.15, 2.10 Hz, 1H)	125.5	7.07 (dd, *J* = 8.15, 1.55 Hz, 1H)	122.9
6′	6.88 (d, *J* = 8.25Hz, 1H)	113.8	6.91 (d, *J* = 8.15 Hz, 1H)	114.8
7′		146.9		149.1
7′-OH	5.79 (s, 1H)		5.89 (s, 1H)	
8′		145.9		147.9
8′-OMe	3.92 (s, 3H)	56.0	3.92 (s, 3H)	55.9
9′	7.74 (d, *J* = 2.10 Hz, 1H)	112.8	7.03 (d, *J* = 1.50 Hz, 1H)	109.4

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675 **Table 2.** NMR spectral data for compound **8** in DMSO-d_6 at 400 (^{1}H) and 100 MHz (^{13}C)

Position	H (*J* in Hz)	C (*J* in Hz)	HMBC (H→C)	^{1}H-^{1}H COSY
1		161.5		
2	7.12 (dd, *J* = 8.60 Hz, 2H)	116.4	C-1, C-4, C-6	
3	7.91 (dd, *J* = 8.60 Hz, 2H)	131.5	C-1, C-2, C-5, C-7	H-2
4		123.3		
5	7.91 (dd, *J* = 8.60 Hz, 2H)	131.5	C-1, C-3, C-7	H-6
6	7.12 (dd, *J* = 8.60 Hz, 2H)	116.4	C-1, C-2, C-4	
7		166.2		
7-OCH_3	3.81 (s, 3H)	52.3	C-7	
1′	5.00 (dd, *J* = 7.20 Hz, 1H)	100.2	C-1	H-2′
2′	3.28 (m, 1H)	73.6	C-1′, C-3′	
3′	3.30 (m, 1H)	76.9	C-2′, C-4′	H-2′, H-4′
4′	3.19 (m, 1H)	69.9	C-5′, C-6′	
5′	3.39 (m, 1H)	77.5		H-4′
6′	3.48 (m, 1H), 3.69 (m, 1H)	61.0	C-5′	H-5′

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Highlights

1) The bound phenolic compounds present in rice bran were released by alkaline hydrolysis.
2) *Para*-hydroxy methyl benzoate glucoside was identified in the bound phenolic fraction.
3) *Trans*-ferulic acid was the major bound phenolic compound in rice bran with strong antioxidant activity.