Antioxidant and Tyrosinase Inhibitory Constituents from a Desugared Sugar Cane Extract, a Byproduct of Sugar Production

Yu-Ming Chung, Hui-Chun Wang, Mohamed El-Shazly, Yann-Lii Leu, Ming-Ching Cheng, Chia-Lin Lee, Fang-Rong Chang, and Yang-Chang Wu

Graduate Institute of Natural Products, College of Pharmacy, Kaohsiung Medical University, Kaohsiung 807, Taiwan

Department of Pharmacognosy and Natural Products Chemistry, Faculty of Pharmacy, Ain-Shams University, Organization of African Unity Street 11566, Abassia, Cairo, Egypt

Graduate Institute of Natural Products, College of Medicine, Chang Gung University, Taoyuan 333, Taiwan

Department of Nutrition and Health Science, Chungchou Institute of Technology, Yuanlin, Changhua 51003, Taiwan

Natural Medicinal Products Research Center, China Medical University Hospital, Taichung 40402, Taiwan

Cancer Center, Kaohsiung Medical University Hospital, Kaohsiung 807, Taiwan

Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Kaohsiung 804, Taiwan

Graduate Institute of Integrated Medicine, College of Chinese Medicine, China Medical University, Taichung 40402, Taiwan

ABSTRACT: Recycling agricultural resources has become an important issue worldwide promoting the economical value of agricultural production processes. Desugared sugar cane extract (DSE) from Saccharum officinarum is a byproduct obtained during sugar production. Two new neolignan glucosides, saccharnan A (1) and saccharnan B (2), together with 10 known phenolics (3–12) were isolated from DSE, and their structures were elucidated on the basis of NMR spectral analysis. Compounds 3, 4, 8, and 9 showed good activity against DPPH radical (IC50 ≤ 51.20 μM) and compounds 3–8 and 12 exhibited strong ABTS⁺ free radical scavenging activity (IC50 ≤ 51.57 μM) compared to those of the positive controls, ascorbic acid and Trolox. Moreover, compounds 7 and 12 acted as potent tyrosinase inhibitors (IC50 ≤ 42.59 μM) compared to the positive control arbutin. Our results highlighted the economical value of recycling DSE for the future development of natural antioxidants and/or tyrosinase inhibitors.

KEYWORDS: desugared sugar cane extract, Saccharum officinarum, sugar cane byproduct recycling, neolignans, saccharnan A, saccharnan B, antioxidant, free radical scavengers, tyrosinase inhibitors

INTRODUCTION

Sugar cane (Saccharum officinarum), one of the major economic crops in the tropics and subtropics, is widely cultivated and consumed throughout the whole world. Taiwan used to be one of the most important sugar producing countries since World War II. In past decades, the research in the sugar industry almost focused on developing new harvesting technologies and improving the yield of current sugar production techniques. Less attention has been devoted to improving the economical and environmental value of the production processes. Industrial scale sugar production produces tons of wastes and byproducts which are difficult to handle and are considered as an additional burden to the environment. Limited resources and recent environmental legislations forced sugar companies to start active research aiming to develop greener procedures through recycling wastes and byproducts of sugar manufacturing processes.

Sugar cane products and isolated components have displayed a wide range of biological activities, including anti-inflammatory, antioxidant, antiatherosclerotic, and acetylcholine release modulatory activities. A number of polyphenolics, including flavonoids, benzenoids, lignans, and neolignans, were isolated from several sugar cane products such as brown sugar, juice, and molasses.

Higher levels of reactive oxygen species (ROS) and oxidative stress have been linked to a long list of life threatening diseases such as cancers, cardiovascular diseases, osteoporosis, and degenerative diseases suggesting the potential health benefits of antioxidants. Bioactive polyphenolics are able to protect living cells from the oxidative stress effects caused by large amounts of endogenous and exogenous of ROS exceeding the cells’ antioxidant defense capacity. Therefore, capturing harmful ROS by natural antioxidants is one of the effective ways to reduce oxidative stress damage. Because of the importance of antioxidants as food preservatives and the limited supplies of natural antioxidants, synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been widely used despite their proposed carcinogenic effects. However, several other studies claimed the beneficial effects of using such synthetic antioxidants and denied their relationship to cancer development. This debate and other health safety concerns are
the driving forces for the discovery of new inexpensive sources of natural antioxidants.

In recent years, tyrosinase inhibitors from natural plant extracts have been utilized in the cosmetic industry due to their skin whitening effect. In the food industry, the tyrosinase-mediated browning effect is directly linked to fruit injuries during harvesting; therefore, controlling this reaction with tyrosinase inhibitors is considered an essential step in the fruit pulp manufacturing process. While a number of natural compounds have been described to inhibit the activity of tyrosinase, phenolic compounds retain the ability to be the most effective tyrosinase inhibitors, due to their structural similarities to endogenous tyrosinase substrates.

Desugared sugar cane extract (DSE), a byproduct of the sugar refining process, is produced before sucrose crystallization from sugar cane juice. After squeezing sugar cane, resin is used as a matrix to absorb polyphenols and pigments from sugar cane juice. Most of the sucrose will pass through the resin and then is condensed into edible sucrose. The polyphenols and pigments sticking on the resin can be washed and desorbed using ethanolic solution, which is then evaporated to give DSE with a total yield of 0.035% of the raw sugar cane material. In other words, about 350 g of DSE can be produced from 1 ton of sugar cane. Of course with the global production of sugar cane, a considerable amount of DSE is produced every year. In the past, DSE was regarded as a waste byproduct and usually discarded. Recently, food production procedures are developing greener methods, and this new trend cannot be ignored any more. Agricultural models using eco-friendly food production are resulting in less pollution and efficient waste recycling techniques. The richness of sugar cane in polyphenolics and the lack of studies on the biological activities of sugar cane byproducts motivated us to investigate the presence of natural antioxidants and tyrosinase inhibitors in the DSE aiming to improve the economical and environmental value of sugar production processes.

**MATERIALS AND METHODS**

**General Procedures.** The 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), ascorbic acid, potassium persulfate, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), mushroom tyrosinase, tyrosine, 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox), and arbutin were purchased from the Sigma chemical Co. (St. Louis, MO, USA). Ultrapure water obtained using the Milli-Q system (Millipore, USA). Shimadzu LC-10AT system equipped with a Thermo ODS Hypersil column (10 × 250 mm, 5 μm).

**Extraction and Isolation.** DSE was donated by the Taiwan Sugar Corporation, Tainan, Taiwan, in August, 2008. In brief, DSE (228.1 g) was suspended in 2.5 L of water and was partitioned with the same volume of ethyl acetate, n-butanol, and water, successively, yielding ethyl acetate (DSEE, 11.2 g), n-butanol (DSEB, 49.2 g), and water (DSEW, 167.5 g) soluble layers. The DSEB layer exhibited a better tyrosinase inhibitory activity with 50.66% inhibition at 1 mg/mL compared to that of the other soluble layers (Figure 1). The DSEB layer was chromatographed over a Diaion HP20 column (5.0 cm × 30 cm) using a methanol and water mixture with gradual decrease in polarity resulting in the separation of seven fractions (A1–A7).

Fraction (Fr.) A3 (2.3 g) was passed through a Sephadex LH-20 column (2.5 cm × 30 cm) and eluted with methanol to obtain five fractions (A3-1–A3-5). Fraction A3-2 (120.5 mg) was purified further using silica gel (1.5 cm × 20 cm, 70-230 mesh, 100 g) eluted with a dichloromethane and methanol mixture (6:1 v/v) followed by preparative reversed-phase HPLC on a Thermo ODS Hypersil column (10 × 250 mm, 5 μm) using methanol and water (1.9 v/v; flow rate, 2 mL/min) as the eluent to yield compounds 5 (8.6 mg) and 6 (7.2 mg). Fraction A3-4 (520.5 mg) was chromatographed further on silica gel (2.0 × 20 cm, 230–400 mesh, 100 g) eluted with a dichloromethane and methanol mixture (6:1 v/v) to give four subfractions (A3-4-1–A3-4-4). Fraction A3-4-2 (150 mg) was purified further by preparative reversed-phase HPLC on a Thermo ODS Hypersil column (10 × 250 mm, 5 μm) with a methanol and water mixture (1:1 v/v, flow rate: 2 mL/min) as the eluent to afford compounds 9 (7.3 mg), 10 (11.6 mg), 11 (12.3 mg), and 12 (4.0 mg).

Fraction A6 (1.6 g) was subjected to a Sephadex LH-20 column and eluted with methanol to give five fractions (A6-1–A6-5). Compounds 1 (1.8 mg), 2 (3.7 mg), 3 (8.5 mg), 4 (5.5 mg), 7 (3.6 mg), and 8 (51.3 mg) were isolated from Fr. A6-4 (588.2 mg) using a silica gel column (2.0 cm × 20 cm, 230–400 mesh, 150 g). The column was eluted with a mixture of dichloromethane and methanol (9:1 v/v), and the compounds were further purified by preparative reversed-phase HPLC on a Thermo ODS Hypersil column (10 × 250 mm, 5 μm) and water (1:4 v/v; flow rate, 2 mL/min) as the eluent.

**Saccharman A (1).** Colorless gum; [α] D0 +52.5 (c 0.08, CH3OH); IR (Neat) νmax cm−1: 3367, 1734, 1637, 1601, 1509, 1169 cm−1. UV (CH3OH) λmax nm (log ε): 229 (3.20), 330 (3.32), 320 (3.35); 1H NMR (CD3OD, 600 MHz) and 13C NMR (CD3OD, 150 MHz) data are given in Table 1.
A solution of the test substance (1 mM) was mixed with the following substances: 40 μL of 10% aqueous DMSO, 80 μL of phosphate buffer solution (PBS, pH 6.8), and 40 μL of 150 U/mL mushroom tyrosinase. The mixture was incubated at 25 °C for 10 min and then followed by the addition of 40 μL of tyrosine (2.5 mM, PBS pH 6.8). The reaction mixture was incubated for 15 min. The inhibitory activity was determined by measuring the optical density (OD) at 475 nm using a Thermo Multiskan Ascent microplate reader. Arbutin was used as a positive control, and measurements were performed at least in triplicate. The percentage of tyrosinase inhibitory activity was calculated as follows:

\[
\%\text{inhibition} = \left\{ \frac{[(A - B) - (C - D)]}{(A - B)} \right\} \times 100
\]

where \(A\), OD at 475 nm without the test substance; \(B\), OD at 475 nm without the test substance and tyrosinase; \(C\), OD at 475 nm with the test substance; and \(D\), OD at 475 nm with the test substance, but without tyrosinase. The IC_{50} value represents the concentration of the tested sample needed to inhibit 50% of tyrosinase activity.

**Acid Hydrolysis of Compounds 1 and 2.** A solution of the tested compound (1 mg/50 μL TFA) was dissolved in 6 N HCl (150 μL), then refluxed for 4 h. The solution was diluted with water and extracted with ethyl acetate (1 mL × 3). The water layer was evaporated and then vacuum-dried to offer a monosaccharide residue. From the residue, gluco was proposed as the sugar part through comparing to an authentic sample.
glucose moiety, the glucose was confirmed as a D-glucose on the basis of the positive optical rotation.

**RESULTS AND DISCUSSION**

**General.** Desugared sugar cane extract (DSE) was suspended in water and was partitioned with ethyl acetate, n-butanol, and water, successively, yielding ethyl acetate (DSEE), n-butanol (DSEB), and water (DSEW)-soluble layers. The higher tyrosinase inhibitory activity exhibited by DSEB compared to that of other soluble layer encouraged us to further purify this layer utilizing bioassay-guided fractionation. The DSEB layer was fractioned on Diaion HP20, Sephadex LH-20, silica gel columns, and was finally purified using preparative reversed-phase HPLC. Twelve compounds (1–12) were isolated from the DSEB layer (Figure 2). Compounds 1 and 2 are new neolignan glucosides (1–2), and compounds 3–7, 9, 11, and 12 are identified for the first time from the genus *Saccharum*.

**Identification of Two New Neolignan Glucosides.** Saccharan A (1) was obtained as a colorless gum, and its molecular formula, C_{26}H_{30}O_{12}, was determined by the HRESI-MS at m/z 557.1638 [M + Na]^+. The IR spectrum of 1 indicated the presence of a hydroxyl group at 3367 cm\(^{-1}\) and an \(\alpha,\beta\)-unsaturated \(\gamma\)-lactone ring at 1637 and 1734 cm\(^{-1}\).\(^{16}\) In \(^1\)H NMR spectra, the aromatic region exhibited the presence of a typical AA’BB’ system at \(\delta_H 7.28\) (2H, d, \(J = 8.8\) Hz, H-2, 6) and \(\delta_H 6.66\) (2H, d, \(J = 8.8\) Hz, H-3, 5), which suggested the presence of a \(\beta\)-substituted benzoyl group. The 1,3,4,5-tetrasubstituted phenyl moiety was expected due to protons showing at \(\delta_H 6.57\) (2H, s, H-2, 6). Two methoxy groups at \(\delta_H 3.76\) (6H, s) and an anomeric proton at \(\delta_H 4.82\) (1H, \(J = 7.2\) Hz, H-1’) were also detected. Additionally, a \(^1\)H NMR signal at \(\delta_H 7.69\) (1H, \(br\) dd, \(J = 2.4\) Hz, H-7’) was assigned to an olefinic proton conjugated with a carbonyl group, which showed a long-range coupling with H-7. Twenty-six signals, including 8 quaternary, 14 methine, 2 methylene, and 2 methyl carbons, were observed in the \(^13\)C NMR and DEPT spectra. In the HSQC experiment, two additional proton signals \(\delta_H 4.54\) (1H, \(br\) dd, \(J = 3.6, 2.4\) Hz, H-7) and \(\delta_H 4.41\) (1H, dd, \(J = 7.6, 3.6\) Hz, H-8) were observed and correlated.
with the corresponding carbon signals at δC 47.5 (C-7) and δC 86.7 (C-8), respectively. The 1H−1H COSY and HMBC correlations of compound 1 are shown in Figure 3. The HMBC correlations of H-7/C-1, C-2, C-6, C-9; H-8/C-1, C-9; H-7′/C-2′, C-6′, C-8′, C-9′; and CH3O-/C-3, C-5 indicated that the 1,3,4,5-tetrasubstituted phenyl moiety is linked to C-7 and that the p-substituted benzoyl group is linked to C-7′ of olefinic carbon. Furthermore, two methoxy groups were located symmetrically at C-3 and C-5 in the 1,3,4,5-tetrasubstituted phenyl ring. Moreover, the HMBC correlation between the anomeric proton at δH 4.82 (1H, d, J = 7.2 Hz, H-1′) and C-4 was observed, and six sugar carbon signals at δC 105.3, 78.2, 77.8, 75.7, 71.2, and 62.5 indicated that the sugar is β-D-glucose. Furthermore, acid hydrolysis of 1 gave a glucosyl residue, and glucose was confirmed through comparison to an authentic sample using TLC and optical rotation. The stereochemistry was determined by a NOESY experiment shown in Figure 4, and a NOE effect was observed between H-7 and H-2′/H-6′ indicating that the geometry of the vinyl group is E configuration. Another NOE effect was observed between H-2 and H-8 revealing that H-7 and H-8 have a trans relationship. On the basis of the aforementioned analysis, compound 1 was confirmed as a new neolignan glucoside and was named saccharnan A.

Saccharnan B (2) was isolated as a colorless gum. The HRMS of 2 displayed a pseudomolecular ion peak at m/z 557.1637 [M + Na]+, corresponding to the formula of C32H30O12Na. The IR spectrum of 2 revealed the presence of a hydroxyl group at 3362 cm−1 and an α,β-unsaturated γ-lactone ring at 1644 and 1731 cm−1. Comparing the NMR data with those of 1 revealed that compounds 1 and 2 were analogues and that the major difference in NMR spectra was the shift in the anomeric carbon signal of compound 2 (δC 101.8) compared to the signal of the same carbon in compound 1 (δC 105.3). Six sugar carbon signals at δC 105.3, 78.2, 77.8, 75.7, 71.2, and 62.5 and the coupling constant (J = 7.2 Hz) of the anomeric proton suggested that the sugar is β-D-glucose as in compound 1. The 1H−1H COSY and HMBC correlations of compound 2 are shown in Figure 3. An obvious correlation between the anomeric proton at δH 4.91 (1H, d, J = 7.2 Hz) and C-4′ at δC 160.4 was observed in the HMBC experiment indicating that glucose is linked at C-4′ of p-hydroxybenzoyl moiety. In NOESY, the NOE correlations of H-7 to H-2′/H-6′ indicated that the vinyl group has an E configuration and that another NOE effect was detected between H-8 to H-2 suggesting a trans relationship between H-8 and H-7 (Figure 4). According to the reported literature data and NMR analysis, the structure of compound 2 was deduced as shown and was named saccharnan B.

The new compounds 1 and 2 possess a rare neolignan skeleton with C7, C8′-linkage, and a γ-lactone function. To the best of our knowledge, only one compound has this type of skeleton.16 The 10 known isolates were identified by comparing their physical and spectroscopic data with the corresponding authentic samples or literature values, and they are erthyo-secoisolariciresinol- O-β-D-glucoside (3),17 three-secoisolariciresinol-O-β-D-glucoside (4),18 erythro-1,2-bis(4-hydroxy-3-methoxyphenyl)-1,3-propanediol 4′-O-β-D-glucopyranoside (5),19 three-1,2-bis(4-hydroxy-3-methoxyphenyl)-1,3-propanediol 4′-O-β-D-glucopyranoside (6),19 lariresinol 4-O-β-D-glucoside (7),20 (7S,8R,7′R,8′S)-icaril A2-9-O-β-D-xylopyranoside (8),20 2′-(3-methoxy-4-hydroxyphenyl)-3-hydroxymethyl-5-trans-carboxylethylene-7-methoxy-2,3-dihydrobenzofuran (9),21 3,4-dimethoxyphenyl-β-D-glucopyranoside (10),22 3-hydroxy-4,5-dimethoxyphenyl-β-D-glucopyranoside (11),22 and three-guaiacylglycerol 7-O-β-D-glucopyranoside (12).23

Figure 3. HMBC correlations of compounds 1 and 2.

Figure 4. NOESY correlations of compounds 1 and 2.
Table 2. Antioxidative and Anti-Tyrosinase Activities of Compounds 1–12 Isolated from DSE

<table>
<thead>
<tr>
<th>Compounds</th>
<th>DPPH IC50 Value (μM)</th>
<th>ABTS IC50 Value (μM)</th>
<th>Tyrosinase Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>2</td>
<td>&gt;500</td>
<td>189.53 ± 1.88</td>
<td>287.95 ± 2.67</td>
</tr>
<tr>
<td>3</td>
<td>28.04 ± 0.85</td>
<td>22.20 ± 0.18</td>
<td>114.71 ± 3.03</td>
</tr>
<tr>
<td>4</td>
<td>23.48 ± 0.26</td>
<td>16.80 ± 0.01</td>
<td>153.07 ± 0.12</td>
</tr>
<tr>
<td>5</td>
<td>177.92 ± 1.15</td>
<td>47.57 ± 0.93</td>
<td>85.87 ± 5.86</td>
</tr>
<tr>
<td>6</td>
<td>91.39 ± 1.61</td>
<td>34.18 ± 0.23</td>
<td>95.81 ± 2.83</td>
</tr>
<tr>
<td>7</td>
<td>107.7 ± 0.30</td>
<td>43.78 ± 0.30</td>
<td>42.59 ± 3.46</td>
</tr>
<tr>
<td>8</td>
<td>30.11 ± 0.73</td>
<td>51.57 ± 0.45</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>9</td>
<td>51.20 ± 3.48</td>
<td>123.12 ± 1.30</td>
<td>798.02 ± 13.32</td>
</tr>
<tr>
<td>10</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>417.76 ± 10.12</td>
</tr>
<tr>
<td>11</td>
<td>284.61 ± 1.40</td>
<td>95.14 ± 1.05</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>12</td>
<td>&gt;500</td>
<td>48.25 ± 0.89</td>
<td>57.72 ± 5.13</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>95.08 ± 0.49</td>
<td>107.44 ± 0.40</td>
<td>N.D.</td>
</tr>
<tr>
<td>Trolox</td>
<td>70.07 ± 0.68</td>
<td>89.94 ± 2.12</td>
<td>N.D.</td>
</tr>
<tr>
<td>Arbutin</td>
<td>N.D.</td>
<td>N.D.</td>
<td>57.56 ± 2.06</td>
</tr>
</tbody>
</table>

*Concentration necessary for 50% inhibition (IC50). The data are expressed as the mean ± SD (n = 3), and the results are given in μM.

DPPH and ABTS* Radical Scavenging Activities of the Identified Compounds. Several studies have suggested the strong correlation between the phenolic composition of the sample and its free radical-scavenging activity.24 The DPPH radical-scavenging assay is usually used to evaluate the ability of certain compound or an extract to capture free radicals by producing the reduced form DPPH-H through hydrogen-donating action.25 ABTS* is another synthetic radical and is more versatile than DPPH as the ABTS* model can be used to assess the scavenging activity for both polar and nonpolar samples. The system also holds another advantage as the detected maximum absorption wavelength is far away from those of natural products, resulting in low experimental error.26 The results of the DPPH and ABTS* radical scavenging activities for compounds 2–12 isolated from DSE are summarized in Table 2. Unfortunately, due to the limited quantity of the isolated new compound 1 (1.8 mg), its biological activity was not tested. Among all tested compounds, 3, 4, 8, and 9 showed DPPH radical-scavenging activity with IC50 values of 28.04, 23.48, 30.11, and 51.20, respectively. In addition, compounds 3, 4, 5, 6, 7, 8, and 12 also displayed strong ABTS* radical-scavenging activity. It was found that the isomeric compounds 3 and 4 having two ortho-methoxy para-substituted phenols possessed the most potent DPPH/ABTS* radical-scavenging activity, with IC50 values of 28.04/22.20 and 23.48/16.80 μM, respectively. The IC50 values of compounds 3 and 4 were 2 to 5-fold more potent than the IC50 values of ascorbic acid and Trolox used as positive controls. Furthermore, compound 8 with two ortho-dimethoxy para-substituted phenols exhibited potent IC50 values of DPPH/ABTS* scavenging assays 30.11/51.57 μM. On the basis of the results, the number of hydroxyl groups on the aromatic rings of the tested compound can be correlated to its antioxidant activity, supporting reported results by Sroka and Cisowski.27 It can be stated that the greater the number of hydroxyl groups attached to the aromatic rings, the higher the antioxidative activity.28 Also, these results were in accordance with literature findings, which reported that the ortho- and para-substitution of phenols by electron donating groups reduced the bond dissociation energies and increased the transfer rate of the hydrogen atom to the peroxy radicals, improving compound antioxidant activity.29

Tyrosinase Inhibitory Activity of the Identified Compounds. Tyrosinase inhibitors play an important role in the cosmetic industry as they are incorporated in skin whitening creams. Tyrosinase enzyme is one of the key enzymes which takes part in the initial steps of pigment formation pathways, thereby indicating that tyrosinase can block pigment production.30 The tyrosinase inhibitory activity of the isolated compounds was similar to the antioxidant activity, which can also be correlated to the number of hydroxyl groups on the aromatic rings. These hydroxyl groups can form a hydrogen bond to the active site of the enzyme inhibiting tyrosinase activity. Tyrosinase inhibitory activity results of compounds 2–12 isolated from DSE are illustrated in Table 2. The isomeric compounds 5 and 6 displayed similar moderate tyrosinase inhibitory activity with IC50 values of 85.87 and 95.81 μM, respectively. The order of tyrosinase inhibitory activity was arbutin > 12 > 5 > 6 > 3 > 4 > 2 > 10 > 9. Compounds 7 and 12 showed the most potent tyrosinase inhibitory activity with IC50 values of 42.59 and 57.72 μM, respectively, and were equivalent to the positive control arbutin (57.56 μM). One characteristic structural feature of all compounds showing effective tyrosinase inhibitory activity is the presence of the free hydroxyl group at position 4 in the aromatic ring.31 It seems that this hydroxyl group is essential for forming a hydrogen bond with the active site of the tyrosinase enzyme. Interestingly, the structural similarity of compound 10 with the positive control arbutin did not lead to similar tyrosinase inhibitory activity, but the activity of compound 10 was lower. The absence of the free hydroxyl group in position 4 of the aromatic ring in compound 10 can be correlated to the lower activity of compound 10 compared to that of arbutin.32 Also, the activity of compounds 7 and 8 were markedly different with lower activity exhibited by compound 8. The lower activity of compound 8 may be attributed to the increase in the substituents bulkiness hindering the accessibility of compound 8 to the active site of the enzyme.33 Compound 9 exhibited the lowest tyrosinase inhibitory activity, which may be a direct result of the presence of the free carboxylic group capable of forming internal hydrogen bonding to the free hydroxyl group. The formation of the internal hydrogen bond prevents the hydroxyl group of compound 9 from forming a hydrogen bond with the active sites of the tyrosinase enzyme.

In summary, 2 new neolignan glucosides and 10 known compounds were isolated from desugared sugar cane extract (DSE). It is the second report of the rare neolignan skeleton of compounds 1 and 2 with C7, C8'-linkage and a γ-lactone function to be isolated from natural resources. In the antioxidative assays, compounds 3, 4, 8, and 9 showed good activity against DPPH (IC50 23.48–51.20 μM) and also compounds 3–8 and 12 exhibited strong ABTS* free radical scavenging activity (IC50 16.80–51.57 μM) even more potent than those of positive controls. Moreover, compounds 7 and 12 displayed potent inhibition of tyrosinase with IC50 values of 42.59 and 57.72 μM, respectively. These two compounds not only showed potent activity as tyrosinase inhibitors but also as antioxidants. Our research proved the economical value of recycling DSE as a potent source of inexpensive natural antioxidants and tyrosinase...
inhibitors which can be incorporated in different food and cosmetic products.

[1] AUTHOR INFORMATION

Corresponding Author
Tel: +886 4 220 57153. Fax: +886 4 220 60248. E-mail: aaronfrc@kmu.edu.tw (F.-R.C.); yachwu@mail.cmu.edu.tw (Y.-C.W.).

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[1] ABBREVIATIONS USED

DSE, desugared cane extract; DSEE, desugared cane extract ethyl acetate soluble layer; DSEB, desugared sugar cane extract n-butanol soluble layer; DSEW, desugared sugar cane extract water-soluble layer; ROS, reactive oxygen species; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; DPPH, 1,1-diphenyl-2-picrylhydrazyl radical; ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); OD, optical density; DEPT, distortion enhancement by polarization transfer; COSY, correlation spectroscopy; HSQC, heteronuclear single quantum coherence; HMBC, heteronuclear multiple bond coherence.

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