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SHORT COMMUNICATION

Phytochemical investigation of *Gynura bicolor* leaves and cytotoxicity evaluation of the chemical constituents against HCT 116 cells

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1. Introduction

*Gynura bicolor* (Compositae) is a popular vegetable in Asia and believed to confer a wide range of benefits including anti-cancer. Our previous findings showed that the ethyl acetate extract of *G. bicolor* possessed cytotoxicity and induced apoptotic and necrotic cell death in human colon carcinoma cells (HCT 116). A combination of column chromatography had been used to purify chemical constituents from the ethyl acetate and water extract of *G. bicolor* leaves. Eight chemical constituents 5-p-trans-coumaroylquinic acid (I), 4-hydroxybenzoic acid (II), rutin (III), kampferol-3-O-rutinoside (IV), 3,5-dicaffeoylquinic acid (V), kampferol-3-O-glucoside (VI), guanosine (VII) and chlorogenic acid (VIII) were isolated from *G. bicolor* grown in Malaysia. To our best knowledge, all chemical constituents were isolated for the first time from *G. bicolor* leaves except rutin (III), 3,5-dicaffeoylquinic acid (V), guanosine (VII) and chlorogenic acid (VIII) demonstrated selective cytotoxicity (selective index > 3) against HCT 116 cancer cells compared to CCD-18Co human normal colon cells.

**Keywords:** *Gynura bicolor*; 3,5-dicaffeoylquinic acid; chlorogenic acid; guanosine; cytotoxicity; HCT 116 cells

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1. Introduction

*Gynura bicolor* (Compositae), which is a popular vegetable in Asia especially in Taiwan and Japan, is locally known as ‘Hong Feng Cai’ (Chinese) and ‘Sambung Nyawa Ungu’ (Malay). The leaves of *G. bicolor* show reddish purple colour on the abaxial side and green colour on the adaxial side. The aerial parts are often consumed and believed to confer a wide range of benefits such as anti-cancer, anti-hyperglycaemic, antioxidant and anti-inflammatory effects (Hayashi et al. 2002; Li et al. 2009; Lu et al. 2012; Wu et al. 2013). Traditionally, it had been used for
post-labour recovery, blood circulation improvement, treatment of dysmenorrhea and haemoptysis (Li 2006). Previous phytochemical analysis of *G. bicolor* leaves revealed the presence of sesquiterpenes, anthocyanins, flavonols and megastigmane-type norisoprenoids (Shimizu et al. 2009, 2010; Lu et al. 2010; Chen et al. 2012). Some people had used *G. bicolor* to treat colon cancer, and based on our previous findings (Teoh et al. 2013), the leaves of *G. bicolor* possessed cytotoxicity and induced apoptotic and necrotic cell death in human colon carcinoma cells (HCT 116). Hence, in this study, effort was put into phytochemical investigation on the leaves to explore potential chemical constituents that have cytotoxicity against HCT 116 cells.

2. Results and discussion
2.1. Identification of chemical constituents

$^1$H and $^{13}$C NMR assignments of all isolated constituents are provided in Supplementary material, available online. Chemical structures of all isolated constituents are shown in Figure 1. By comparison with NMR spectral data in the literature, we isolated 5-$p$-trans-coumaroylquinic acid (I) (Lu et al. 2000), 4-hydroxybenzoic acid (II) (Youn et al. 2010), rutin (III) (Kanada et al. 2012), kaempferol-3-$O$-rutinoside (IV) (Feng et al. 2007), 3,5-dicaffeoylquinic acid (V) (Lee et al. 2010), kaempferol-3-$O$-glucoside (VI) (Feng et al. 2007), guanosine (VII) (Kim et al.

![Figure 1. Structures of chemical constituents (I–VIII) isolated from the leaves of *G. bicolor*.](image-url)
Table 1. Cytotoxic effect of isolated chemical constituents against HCT 116 and CCD-18Co cells.

<table>
<thead>
<tr>
<th>Chemical constituents</th>
<th>IC$_{50}$a (µg/mL)</th>
<th>(SI)b</th>
<th>HCT 116</th>
<th>CCD-18Co</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-p-trans-coumaroylquinic acid (I)</td>
<td>&gt; 100</td>
<td>ND</td>
<td>&gt; 100</td>
<td>ND</td>
</tr>
<tr>
<td>4-Hydroxybenzoic acid (II)</td>
<td>&gt; 100</td>
<td>ND</td>
<td>&gt; 100</td>
<td>ND</td>
</tr>
<tr>
<td>Rutin (III)</td>
<td>&gt; 100</td>
<td>ND</td>
<td>&gt; 100</td>
<td>ND</td>
</tr>
<tr>
<td>Kampferol-3-O-rutinoside (IV)</td>
<td>&gt; 100</td>
<td>ND</td>
<td>&gt; 100</td>
<td>ND</td>
</tr>
<tr>
<td>3,5-Dicaffeoylquinic acid (V)</td>
<td>79.7 ± 4.5 (4.4)</td>
<td></td>
<td>350 ± 1.7</td>
<td></td>
</tr>
<tr>
<td>Kampferol-3-O-glucoside (VI)</td>
<td>&gt; 100</td>
<td>ND</td>
<td>&gt; 100</td>
<td>ND</td>
</tr>
<tr>
<td>Guanosine (VII)</td>
<td>81.0 ± 6.6 (&gt;6.2)</td>
<td></td>
<td>&gt; 500</td>
<td></td>
</tr>
<tr>
<td>Chlorogenic acid (VIII)</td>
<td>79.3 ± 3.1 (5.1)</td>
<td></td>
<td>403.3 ± 9.5</td>
<td></td>
</tr>
<tr>
<td>Cis-platinb</td>
<td>2.9 ± 0.1 (&gt;4.3)</td>
<td></td>
<td>&gt; 12.5</td>
<td></td>
</tr>
</tbody>
</table>

*a Data are presented as mean ± standard deviation from three independent experiments triplicate for each.

*b Selectivity index (SI).

c Positive reference standard; ND, not determined.

2003) and chlorogenic acid (VIII) (Lee et al. 2010) from G. bicolor leaves. The identities of all isolated constituents were confirmed by negative ionisation ESI-MS. To our best knowledge, all constituents were isolated for the first time from G. bicolor leaves except constituent III, rutin.

2.2. Cytotoxic effect of the isolated chemical constituents

As shown in Table 1, all isolated constituents did not show cytotoxic effect (with IC$_{50}$ values > 100 µg/mL) against HCT 116 cells at 72h incubation except 3,5-dicaffeoylquinic acid (V, IC$_{50}$ value of 79.7 µg/mL), guanosine (VII, IC$_{50}$ value of 81.0 µg/mL) and chlorogenic acid (VIII, IC$_{50}$ value of 79.3 µg/mL). Constituent with selectivity index (SI) more than 3 indicates high selectivity against cancerous cells compared to normal cells (Bézivin et al. 2003). The cytotoxic effect of constituents V, VII and VIII against CCD-18Co normal colon cells was comparatively low with IC$_{50}$ values of 350 ± 1.7, > 500 and 403.3 ± 9.5 µg/mL, respectively. Constituent VII appeared to be highly selective against HCT 116 cells with SI value of 6.2, followed by VIII with SI value of 5.1 and V with SI value of 4.4. To our knowledge, the SI of constituents V, VII and VIII on HCT 116 and CCD-18Co cells had not been reported. From SI point of view, constituents V, VII and VIII had showed cytotoxicity against colon cancer cells while exerting minimal cytotoxicity against normal colon cells. The selective property of these three chemical constituents of G. bicolor could be usefull against colon cancer.

3. Conclusion

In this work, seven chemical constituents were isolated from G. bicolor leaves for the first time. 3,5-Dicaffeoylquinic acid (V), guanosine (VII) and chlorogenic acid (VIII) from G. bicolor leaves showed selective cytotoxic effect on HCT 116 cells by in vitro MTT assay. By consuming G. bicolor, these chemical constituents may help in treatment of colon cancer. Further studies on the cell death mechanism of these constituents are under way in order to provide more convincing evidence.

Supplementary material

Experimental details relating to this article are available online together with Figure S1.
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