SHORT COMMUNICATION

Development of beverage product from *Gynura bicolor* and evaluation of its antioxidant activity

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Abstract  *Gynura bicolor* (Roxb & Willd.) DC. is widely distributed in Asia and is very popular for vegetarian cuisine in Taiwan. This study used *G. bicolor* as the main ingredient to develop a new vegetable juice. Its chemical composition and antioxidant activity were analyzed, and consumer preferences for *G. bicolor* vegetable juice (GBVJ) evaluated. The results showed that the major compounds in GBVJ were rutin, \( \beta \)-carotene, anthocyanin, and chlorophyll. The antioxidant activity of GBVJ including 1,1-diphenyl-2-picrylhydrazyl free radical scavenging activity, 2,2ʹ-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), and superoxide anion were 85.1%, 95.9%, and 80.1%, respectively. The reducing power was about 60% of that of vitamin C (1 g/L). The consumer preferences for each investigated item were all above 5 points (total score 7 points). These results suggest that GBVJ may have positive effects on the antioxidant activity, and the high consumer acceptance may be used as the preliminary information to support that *G. bicolor* would be commercially viable in the food industry.

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Introduction

Epidemiological studies have shown that consumption of fruits and vegetables in the diet protects the human body from chronic illnesses. Because a number of phytonutrients have been identified to prevent diseases such as heart disease and cancer, consumers are being increasingly encouraged to eat more fruits and vegetables as a contribution to a balanced diet. Previous studies also show that fruits and vegetables contain antioxidant nutrients that significantly contribute to their total antioxidant capacity. Because plant-based foods are complex mixtures of bioactive compounds, information on the potential health effects of individual polyphenols is linked to information on the health effects of foods that contain those polyphenols.

Gynura bicolor (Roxb. & Willd.) DC. is widely distributed in Asia and is very popular for vegetarian cuisine in Taiwan. Leaves of G. bicolor distinctively show a reddish purple color on their abaxial sides, contrasting with the typical green color seen on the adaxial side. The leaves of this plant are often consumed in diet. Research shows that the contents of the leaves are nontoxic. The major constituents of pigment sources and physiological effects of G. bicolor are thought to be related to its rich flavonoids including anthocyanins, quercetin, kaempferol, quercitrin, isoquercitrin, and rutin. Gynura is usually used as a traditional medicine for the treatment of inflammation, herpes simplex virus, rashes, fever, rheumatism, kidney disease, migraines, constipation, diabetes mellitus, cancer, and hypertension. However, little effort has been made to explore the impacts of G. bicolor biological activities and applications of health products.

This study used G. bicolor as the main ingredient to develop a new vegetable juice and employed sensory evaluation to survey the consumer acceptance. We analyzed its chemical composition and determined the antioxidant activity including the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS+·), superoxide anion, and reducing power of G. bicolor vegetable juice. Simultaneously, the sensory attributes were also studied using descriptive sensory analysis.

Materials and methods

Preparation of the G. bicolor vegetables juices

G. bicolor was purchased from Yuanshan Village (Ilan, Taiwan). Leaves of G. bicolor were removed, cleaned, and blended in cold water, honey, and lemon juice (4°C, w/w: 1/1). The homogenates were filtered through Whatman No. 1 paper and disinfected at 100°C for 30 minutes. The G. bicolor vegetable juice (GBVJ) was kept at 4°C until used for chemical composition analysis and antioxidant activity assays.

Chemical composition analysis

Total flavonoid content was determined using the colorimetric method by Jia et al. The rutin content was determined using the high-performance liquid chromatography method of Krizman et al. The β-carotene content was analyzed as described by Xu et al. The chlorophyll content was determined using a procedure described by Whitham et al. Total phenol content was determined using the Folin–Ciocalteu procedure described by Singleton and Rossi with gallic acid as the standard and expressed (µg) as gallic acid equivalents (GAE)/g of extract. Anthocyanin content was determined according to the method of Padmavati et al. with quercetin as the standard and expressed (µg) as quercetin equivalent (QE)/g of extract. Ascorbic acid was determined by the direct colorimetric method using 2, 6-dichlorophenol-indophenol as decolorizing agent by ascorbic acid in GBVJ and in standard ascorbic acid solution.

Antioxidant activity assays

DPPH, superoxide anion radical scavenging activity, and reducing power of GBVJ different concentrations (10%, 25%, 50% and 100%) were determined with the method described by Shimada et al. The DPPH radical scavenging activity was 1.5 mL of sample solution with varying GBVJ different concentrations (10%, 25%, 50%, and 100%), 1.5 mL of 0.15 mM DPPH in 50% ethanol were added. The mixture was mixed vigorously and allowed to stand at room temperature in the dark for 30 minutes. The absorbance of the resulting solution was measured at 517 nm using a UV-2001 spectrophotometer (Hitachi, Japan). The superoxide radicals, generated by nonenzymatic phenazine methosulfate–nicotinamide adenine dinucleotide, reduce nitro blue tetrazolium to a purple formazan. A 1 mL aliquot of reaction mixture contained phosphate buffer (20 mM, pH 7.4), nicotinamide adenine dinucleotide (73 µM), nitro blue tetrazolium (50 µM), phenazine methosulfate (15 µM) and samples at various concentrations (0–20 g/L). After incubation for 2 minutes at room temperature (28–30°C), the absorbance at 562 nm was measured against a blank to determine the quantity of formazan generated. The reducing power was to 0.5 mL of sample solution mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 minutes. An aliquot (2.5 mL) of 10% trichloroacetic acid was added to the mixture, followed by centrifugation at 3000g for 10 minutes. The upper layer of solution (2.5 mL) was mixed with 2.5 mL of distilled water and 2.5 mL of 0.1% ferric chloride and the absorbance was read at 700 nm. The ABTS+ capacity of GBVJ was determined as described by Arnao et al. The capacity of a

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>GBVJ</th>
</tr>
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<tbody>
<tr>
<td>Rutin (µg/g)</td>
<td>7.55</td>
</tr>
<tr>
<td>β-Carotene (µg/g)</td>
<td>0.73</td>
</tr>
<tr>
<td>Ascorbic acid (µg/g)</td>
<td>7.55</td>
</tr>
<tr>
<td>Anthocyanin (µg/g)</td>
<td>100</td>
</tr>
<tr>
<td>Flavonoids (µg of QE/g)</td>
<td>120</td>
</tr>
<tr>
<td>Total phenolic (µg of GAE/g)</td>
<td>600</td>
</tr>
<tr>
<td>Chlorophyll (µg/g)</td>
<td>629</td>
</tr>
</tbody>
</table>
sample to scavenge the ABTS radical cation compared to a standard antioxidant (Trolox).

**Consumer preferences evaluation**

The sensations of the samples were evaluated by employing the acceptance test with 7-point hedonic scale (from 1 = disliked greatly to 7 = liked greatly). The panelists were untrained consumers older than 20 years, with 210 people in total. The project contains the color, aroma, sweetness, acidity, taste, flavor, and overall feel. Consumers rated the product according to personal preference.\(^{18}\)

**Statistical analysis**

Data are expressed as the mean ± standard deviation from at least three independent experiments. Differences among treatments were analyzed by a one-way analysis of variance and Duncan’s test using SPSS statistics 12.0 (SPSS Inc., Chicago, IL, USA). Statistically significant differences required that \( p < 0.05 \).

**Results**

The chemical composition of GBVJ includes rutin 7.55 µg/g, β-carotene 0.73 µg/g, ascorbic acid 7.55 µg/g, anthocyanin 100 µg/g, flavonoids 120 µg of QE/g, total phenolic 600 µg of GAE/g, and chlorophyll 629 µg/g (Table 1). The antioxidant activities of GBVJ are shown in Fig. 1. GBVJ was capable of quenching all three types of radicals, and the activity was dose-dependent. The DPPH free radical scavenging activity, ABTS\(^+\), and superoxide anion were 85.1%, 95.9%, and 80.1% in 100% GBVJ, respectively. The reducing power was about 60% of that of vitamin C (1 g/L; Fig. 2).

Of 210 fruit juice consumers who participated in this study, 50 were male and 160 female; the highest degree of consumer preference was in those older than 60 years (Table 2). The consumer preferences for each investigated item were all above 5 points (total score 7 points). There were significant differences in the aroma of the tasting results in 20–29 year and 30–39 year age groups, and significant differences in the color of the evaluation results in the 20–29 year and 40–49 year age groups (Table 2).

**Discussion**

Phytonutrients have been getting significant consideration, as more researchers uncover how important these nutrients are for health. Vegetables are important food crops, as

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**Figure 1** Scavenging activity of *Gynura bicolor* vegetable juice of (A) DPPH, (B) ABTS\(^+\), (C) superoxide anion. Values are mean ± standard deviations of three determinations. Values with different superscript letters are significantly different \((p < 0.05)\).

**Figure 2** Reducing power of *Gynura bicolor* vegetable juice. Values are mean ± standard deviations of three determinations. Values with different superscript letters are significantly different \((p < 0.05)\).
they supply ample amounts of flavonoids, phenolics, folic acid, ascorbic acid, carotene, riboflavin, and minerals such as iron, calcium, zinc, and phosphorous. The benefit of vegetable-rich diets has been partially attributed to the intake of variety of these phytochemicals. Eberhardt et al. suggested that the complex mixture of phytochemicals in fruits and vegetables provides protective health benefits mainly through a combination of additive and/or synergistic effects. The major compounds in GBVJ are rutin, β-carotene, anthocyanin, flavonoids, total phenolic, and chlorophyll. GBVJ contain complex mixtures of antioxidants, and are therefore responsible for many health benefits. Their advantage over single antioxidants is due to synergistic effects.

The neutralization of free radicals is one of the most important mechanisms by which peptides control oxidative reactions in foods. Because radicals produced by different sources have different relativities, multiple methods are generally recommended for assessing the impact of the specific oxygen radicals in food systems. In the present study, GBVJ has strong antioxidant properties due to its DPPH free radical scavenging activity, ABTS\(^+\), superoxide anion and reducing power. These results suggest that consumption of GBVJ may have health benefits.

Table 2

<table>
<thead>
<tr>
<th>Age (y)</th>
<th>N</th>
<th>Color</th>
<th>Aroma</th>
<th>Sweetness</th>
<th>Sourness</th>
<th>Taste</th>
<th>Flavor</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>20–29</td>
<td>46</td>
<td>6.17 ± 0.82 (^b)</td>
<td>4.98 ± 1.27 (^b)</td>
<td>5.54 ± 1.05 (^b)</td>
<td>5.24 ± 1.34</td>
<td>5.61 ± 1.00</td>
<td>5.35 ± 1.21</td>
<td>5.52 ± 1.05</td>
</tr>
<tr>
<td>30–39</td>
<td>60</td>
<td>6.33 ± 0.68 (^ab)</td>
<td>5.20 ± 1.29 (^b)</td>
<td>5.58 ± 1.05 (^b)</td>
<td>5.40 ± 1.50</td>
<td>5.52 ± 1.28</td>
<td>5.48 ± 1.31</td>
<td>5.63 ± 1.21</td>
</tr>
<tr>
<td>40–49</td>
<td>52</td>
<td>6.17 ± 0.73 (^b)</td>
<td>5.52 ± 1.19 (^ab)</td>
<td>5.85 ± 1.07 (^b)</td>
<td>5.56 ± 1.16</td>
<td>5.79 ± 1.09</td>
<td>5.56 ± 1.21</td>
<td>5.67 ± 1.08</td>
</tr>
<tr>
<td>50–59</td>
<td>32</td>
<td>6.38 ± 0.61 (^ab)</td>
<td>5.41 ± 1.32 (^ab)</td>
<td>5.53 ± 1.04 (^b)</td>
<td>5.38 ± 1.41</td>
<td>5.84 ± 0.92</td>
<td>5.63 ± 1.07</td>
<td>5.88 ± 0.87</td>
</tr>
<tr>
<td>≥60</td>
<td>20</td>
<td>6.55 ± 0.51 (^a)</td>
<td>5.85 ± 1.42 (^a)</td>
<td>6.35 ± 0.93 (^a)</td>
<td>5.75 ± 1.52</td>
<td>5.90 ± 1.33</td>
<td>5.90 ± 1.07</td>
<td>5.95 ± 1.19</td>
</tr>
<tr>
<td>Total</td>
<td>210</td>
<td>6.29 ± 0.71 (^ab)</td>
<td>5.32 ± 1.29 (^ab)</td>
<td>5.70 ± 1.06 (^b)</td>
<td>5.43 ± 0.94</td>
<td>5.69 ± 1.13</td>
<td>5.53 ± 1.21</td>
<td>5.69 ± 1.09</td>
</tr>
</tbody>
</table>

Different letters (a and b) indicate that differences between the same column were significant (p < 0.05) for Gynura bicolor vegetable juice. N = numbers of consumers. Data are mean ± standard deviation scores (1 = dislike greatly, 4 = neither like nor dislike, 7 = like greatly) of consumers.

Acknowledgments

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References