The Development of Antioxidant Nutraceuticals containing
Chrysanthemum indicum L. Gummy Candy

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ABSTRACT

Chrysanthemum flowers (Chrysanthemum indicum L.) have been shown to contain flavonoid compounds and exert antioxidant activity. This brings Chrysanthemum indicum potential to be developed as a nutraceutical product. This study aims to evaluate the antioxidant activity of Chrysanthemum flowers before and after the formulation of Chrysanthemum indicum flower extract as a gummy candy. Gummy candy formulas were developed using variations of gelatin and pectin as gelling agents. In this study, Chrysanthemum flower extract was formulated into gummy candy. The physical characteristics evaluated include organoleptic tests, weight uniformity, elasticity, and moisture content. Optimization was performed using the simplex lattice design (SLD) method with the aid of the Design Expert software Ver. 13. The antioxidant activity of the chrysanthemum flower extract and gummy candy extract was evaluated using the DPPH radical scavenging method. Ascorbic acid was used as a positive control. The optimum formula for preparing the gummy candy was 11.51% of gelatin and 1.24% of pectin. The evaluation of weight uniformity, elasticity, and moisture content suggested that there is no significant difference between the optimum formula and the predicted value. Both the chrysanthemum flower extract and chrysanthemum flower gummy candy had strong antioxidant activity. The IC50 value of the extract was 67.80 ± 2.37 mg/mL while the gummy candy IC50 value was 82.93 ± 2.55 mg/mL. The antioxidant activity of Chrysanthemum indicum was slightly decreased after being formulated into gummy candy. These studies suggested that scientists are expected to anticipate the decrease of Chrysanthemum antioxidant activity in the gummy manufacturing process.

Keywords: antioxidant activity; Chrysanthemum indicum; gelatine; gummy candy; pectin

INTRODUCTION

Degenerative diseases such as stroke, ischemic heart disease, COPD; cancer of the trachea, bronchi, and lungs; Alzheimer’s and other dementia diseases; diabetes; and kidney disease are the leading causes of death in the world (WHO, 2020). In Indonesia in 2017, there was an increase in the burden of non-communicable diseases with stroke and ischemic heart disease being the largest burden of disease (Ministry of Health RI, 2019). Degenerative diseases are non-communicable diseases that occur due to decreased organ function due to the aging process (Nandi et al., 2019). Oxidative stress is closely related to the aging process and the pathophysiology of degenerative diseases. Factors that cause oxidative stress are exposure to radiation, smoking, alcohol consumption, consumption of foods high in fat and sugar, pollution, and exposure to pesticides or chemicals (Vatner et al., 2020). In addition, oxidative stress can occur because endogenous antioxidants are not sufficient to counteract reactive oxygen species (ROS) production, thereby triggering oxidative stress and cell damage. Under certain limits, there is a shortage of endogenous antioxidants which require consumption of exogenous antioxidants. Several epidemiological studies have shown that the consumption of foods containing antioxidants plays a role in preventing various diseases such as...
cancer, hypertension, neurodegenerative diseases, and stroke (Oppedissano et al., 2020).

Chrysanthemum indicum is a plant that has been used in traditional Chinese medicine (TCM). Chrysanthemums have anti-inflammatory, antioxidant, anticancer, antimicrobial, hepatoprotective, and neuroprotective activities (Shao et al., 2020). Compared to other parts i.e. leaves, seeds, and stems, Chrysanthemum indicum flowers exhibited the highest antioxidant activity (Cao et al., 2020). Active ingredients in Chrysanthemum indicum that pose antioxidant activity include flavonoids (myricetin, acacetin, linarin, luteolin, kaempferol), dihydroflavonoids (eriodictyol), phenylpropanoids, and phenolic acids (cafeic acid, chlorogenic acid, vanillic acid), as well as other compounds such as camphor, thymol, and α-terpineol (Shao et al., 2020). Commercial products from chrysanthemum flowers available on the market are herbal teas. However, these products are less practical to use and leave a bitter and astringent aftertaste (Hartanto et al., 2021). Therefore, it is necessary to innovate processed chrysanthemum flowers into products that are more practical, easy to use, attractive, and have a long shelf life.

Gummy candy is a gel-based preparation with an attractive shape and delicious taste so it can be used widely because it can be accepted by all ages (Matulyte et al., 2021). Gummy candy can be an alternative to processed chrysanthemums as a nutraceutical product that contains antioxidants. In addition, the manufacture of gummy candy aims to mask the bad taste, easy to use, and increase consumer acceptance. The gel in gummy candy is formed from hydrocolloids such as gelatin, pectin, and starch. Each hydrocolloid has different properties so that it can produce different characteristics of gummy candy (Hartel et al., 2018). The development of gummy candies from chrysanthemum flower extract has not been reported. In addition, the antioxidant activity of chrysanthemum flower preparations after it was formulated into nutraceutical preparation has not been examined in previous studies. This study aimed to develop Chrysanthemum indicum flower extract gummy candy and to evaluate the antioxidant activity of Chrysanthemum indicum before and after gummy candy formulation.

MATERIALS AND METHODS

Materials

Chrysanthemum indicum extract, gelatin (Gelita NZ LTD), pectin (Adimitra Kaformulaiia Inc, pharmaceutical grade), distilled water (pharmaceutical grade), DPPH (2,2-Diphenyl-1-Picrylhydrazyl) (PT Smart Lab Indonesia), ascorbic acid pro analysis (Merck, Darmstadt, Germany), ethanol absolute pro analysis (Merck, Darmstadt, Germany), distilled water (PT Progo Mulyo), 70% technical ethanol (pharmaceutical grade, PT Progo Mulyo), citric acid (PT Sirsate Inc), sucrose (PT Point), multiflora pure honey (PT Natura Alamindo Utama), lemon essence (PT Gunacipta Multiras), yellow dye (PT Gunacipta Multiras), and sodium benzoate (PT Gunacipta Multiras).

Plant Determination

The dried Chrysanthemum indicum flower, which was obtained from PT AV Jaya Herbal, Jakarta, Indonesia, was determined at the Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada (Rahmasari, 2023).

Preparation of Chrysanthemum indicum Extract

Chrysanthemum indicum extract was prepared using the maceration technique. Before the extraction, the chrysanthemum flowers were re-dried again in the oven at 50°C for 2 hours and then micronized using a grinding machine. The amount of 980 g of simplicia powder was obtained and was then macerated using 70% ethanol as much as 6,860 L for 24 hours. The extract was filtered using a Buchner funnel and vacuum pump, followed by a re-maceration with 2,940 L of fresh ethanol 70%. The extract was then concentrated using a water bath and stored in the refrigerator at 4°C.

As the crude extract was obtained, physical evaluation of the extract such as moisture content and extract spreadability was performed. The moisture content was determined using moisture balance (Ohaus MB120). For the extract spreadability test, as many as 0.5 g of extract were placed in the middle of a 15 cm diameter round glass. Subsequently, another glass was placed on top of that round glass. After being loaded with a glass lid for 1 minute, the diameter of the extract spread was measured. An additional 150 g of load was placed on the top of the glass lid for one minute. Usually, the extract will spread bigger. The diameter of the spread was calculated at three different diameters and the average number was calculated.

Quercetin analysis using Reverse Phase Ultra-High-Performance Liquid Chromatography (RP-UHPLC)

The qualitative and quantitative analysis of quercetin were conducted using an RP-UHPLC (Thermo Scientific) following Ang, et al (2020).
with modification. The Thermo Hypersil Gold column C-18 (250 x 4.6 mm, particle size 5µ) was used to analyze the sample. The separation process was performed using isocratic elution with a flow rate of 0.7 mL/min, a mobile phase of MeOH, and water (90:10) containing 1% acetic acid. Quercetin was monitored using a UV-Vis detector at 370 nm. The injection volume was 10 µL and the total run time was 20 min. The raw chromatogram data was processed with Chromeleon 7, Version 7.2.10.24543 (Thermo Fisher Scientific). The quercetin stock solution was diluted in MS grade MeOH (1 mg/mL) and then diluted to various concentrations of solutions. These solutions were then used as a calibration standard for the quantitative determination of Chrysanthemum sp. extracts. The extract of this flower was dissolved into MS grade MeOH (1 mg/mL) and then diluted to 0.25 mg/mL for injection.

**Evaluation of Chrysanthemum indicum L. Extract and the Gummy Candy Antioxidant Activity**

Evaluation of the antioxidant activity of chrysanthemum flowers was performed using the DPPH radical scavenging method. The sample solutions of gummy candy were prepared by grinding and dissolving it in ethanol p.a. Sample solutions were diluted into five concentration series then reacted with 1 mL of 0.4 mM DPPH and added absolute ethanol to 5 mL. The mixture was gently vortexed and then placed in a dark cabinet and protected from light for 30 minutes. The absorbance of the solution was measured with a UV-Vis spectrophotometer at a wavelength of 516.5 nm. In this study, ascorbic acid was used as a positive control. The percentage of inhibition was calculated based on Equation 1. The value of antioxidant activity is expressed by IC\(_{50}\) which is calculated by the linear regression equation of \( y = bx + a \), where \( x \) is the concentration of the sample while \( y \) is the % inhibition.

\[
\% \text{ inhibition} = \frac{\text{DPPH absorbance} - \text{sample absorbance}}{\text{DPPH absorbance}} \times 100\%
\]

**Formula Optimization of Chrysanthemum Flower Gummy Candy**

Exploration of the gummy candy optimum formula was performed by preparing some formulas with various compositions of gelatin and pectin using the aid of Design Expert software (version 13). Eight formulas were produced with variations of gelatin from 8.5 to 9.5 g and pectin from 0.5 to 1.5 g for the batch size of 78.4 g.

**Physical Characteristic Evaluation**

Each formula was evaluated for its physical characteristics, including organoleptic, weight uniformity, elasticity, and moisture content. Color, shape, smell, and texture were also examined. In terms of the weight uniformity test, twenty pieces of gummy candy were sampled from each formula, and the weight of each gummy candy was recorded. Following that, the weight average, standard deviation (SD), and coefficient of variation (CV) are calculated. The elasticity test was carried out by sampling 4 gummy candies from each formula. The height and thickness of each gummy was measured. The 4 gummy candies were placed on a flat surface and covered with a flat-light metal lid. The weight of 200 g was placed on the top of the metal lid for 5 minutes. The thickness of the gummy candy was measured after being loaded with 200 g of weight. The difference in thickness was calculated. The elasticity value is indicated by the percentage of elasticity which is the percentage difference in the thickness of the gummy candy before and after being loaded (Eq.2).

\[
\% \text{ elasticity} = \frac{(\text{initial thickness} - \text{final thickness})}{\text{initial thickness}} \times 100\%
\]

**Statistical Analysis**

The response for each formula including weight uniformity, % elasticity, and moisture content were analyzed using Design Expert software (version 13). The pH values are not analyzed using Design Expert ver 13, as pH measurements were in-process control testing, not an optimization parameter of the gummy candy’s physical characteristics. The response for physical characteristics of the optimum formula was compared to the predicted value produced by the software using one sample t-test (SPSS software version 25). The antioxidant data (IC\(_{50}\)) were analyzed using One-way ANOVA followed by Tukey tests.

**RESULTS**

**Extraction of Chrysanthemum Flower**

Based on the results of the determination from the Department of Pharmaceutical Biology at Universitas Gadjah Mada, the flower samples used...
were identified as Chrysanthemum indicum L. from the Asteraceae family. The yield of extract obtained from the extraction process was 31.3%. These results meet the requirements of the Indonesian herbal pharmacopoeia, which states that chrysanthemum flower extract should not be less than 22.7% (Indonesian Herbal Pharmacopoeia, 2017).

Physical analysis of the extract which been characterized including moisture content and spreadability. In this study, the Chrysanthemum indicum flower extract exhibited 0.48±10% moisture content and 33.68±0.45 mm for the spreadability test. The spreadability test is commonly used as a surrogate approach to assess extract viscosity.

Quercetin analysis in Chrysanthemum indicum L. Extract

Phenolic and flavonoid compounds are commonly analyzed using RP-HPLC with a C-18 column as a stationary phase (Khodami et al., 2013; Benincasa et al., 2022). This method is suitable for qualitative and quantitative identification of those metabolites in the extract. One of the kinds the flavonoid compounds is quercetin. This study showed that Chrysanthemum indicum flower extract contains quercetin with a retention time of 4.517 min (Figure 1). Quantitative analysis of the sample suggested quercetin concentration in this extract was 35.08 mg/mL. Quercetin has been shown by several studies that it has an activity to combat free radicals (Xu et al., 2019; Lesjak et al., 2018). As an antioxidant, quercetin donates its hydrogen to scavenge Reactive Oxygen Species (ROS), and affect the enzyme activity that is associated with oxidative properties (Qi et al., 2022). Quercetin also has been reported to inhibit the damage of neurons under concurrent administration with ascorbic acid. Studies also reported neuroprotective activity of quercetin by suppressing neuroinflammation and enhancing memory (Victor et al., 2016). The confirmation of quercetin in the extract along with the antioxidant data from previous studies suggested the potential development of Chrysanthemum extract as an antioxidant supplement.

Table 1. Chrysanthemum flower gummy candy formula

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Chrysanthemum flower extract (g)</td>
<td>8</td>
</tr>
<tr>
<td>Gelatin (g)</td>
<td>9</td>
</tr>
<tr>
<td>Pectin (g)</td>
<td>1</td>
</tr>
<tr>
<td>Sucrose (g)</td>
<td>18</td>
</tr>
<tr>
<td>Honey (g)</td>
<td>21</td>
</tr>
<tr>
<td>Citric acid (g)</td>
<td>0.3</td>
</tr>
<tr>
<td>Sodium benzoate (g)</td>
<td>0.1</td>
</tr>
<tr>
<td>Lemon essence (g)</td>
<td>q.s.</td>
</tr>
<tr>
<td>Colorant (g)</td>
<td>q.s.</td>
</tr>
<tr>
<td>Aquadest (mL)</td>
<td>20</td>
</tr>
</tbody>
</table>

The development of Chrysanthemum indicum flower extract as an antioxidant supplement was done by formulating it as a gummy candy. The formula development was performed using the simplex lattice design (SLD) method using Design Expert software Optimization. To form a gummy structure, gelatin, and pectin were used as gelling agents. In the production of gummy candy, in the control process testing is carried out by measuring the pH of liquid gummy candy mass. The pH value of all formulas is 4. Each formula was evaluated for its physical characteristics, including organoleptic tests, weight uniformity, elasticity, and moisture content. The gummy candy obtained exhibits a brownish-yellow in color with a distinctive odor of extract, heart shape, and chewy texture.

The weight uniformity test was carried out to evaluate whether the gummy candy in each formula had the same weight. The requirement to meet the weight uniformity test is that no gummy candy has a weight difference of more than 7.5% from the average weight. Whenever there is 1 sample that exceeds the limit, the above-mentioned sampling procedure is repeated (weighing another 20 pieces of gummy candy one by one). The condition for weight uniformity is that no gummy candy differs by more than 10% from the average weight (Davydova, 2018). All the current formulas showed a coefficient of variation.
of 0.7-1.8% which suggested that the gummy candy produced met the weight uniformity requirement (Figure 2a). There was no single gummy candy whose weight was bigger than 7.5% of the average weight.

An elasticity test was performed to evaluate the elasticity of gummy candy. The percentage of elasticity is obtained from the difference in thickness of the gummy candy before and after being loaded. The smaller the percentage, the chewier the gummy candy. The gummy candy is elastic when post-weight burden, it can return to its original shape and size. Based on the current elasticity test, the addition of pectin to the formula reduced the elasticity of the gummy candy. The results of this study are in line with the results reported by Renaldi et al. (2022), which found that the addition of pectin to gelatin-based gummy candy would reduce the chewiness/elasticity of gummy candy. In this study, the percentage elasticity of gummy candy is compared to a commercial product that is already available in the market (Pome apple vinegar®). The elasticity percentage of commercial gummy candy, which was 1.743%, is used as the target for the optimum gummy candy formula elasticity value. The 8 formulas produce the range elasticity value of 1.5-7.5% (Figure 2b). This suggested that some formulas meet the % elasticity value which is almost similar to the reference product.

Measurement of the moisture content of gummy candy was performed using a moisture analyzer (Ohaus MB120). Moisture content is very important in determining the stability of the gummy candy. As the moisture content increases in the product, there is a greater chance for microbial to grow. According to the National Standardization Institute, the maximum moisture content of gummy candy is 20% (National Standardization Agency of Indonesia, 2008). The current study revealed that formulas have a moisture content ranging from 3.0 to 6.0% (Figure 2b). This value falls within the range set by the National Standardization Agency of Indonesia for gummy candy moisture content.

The correlation between the composition of the gelling agent to the response is shown below (Table II). Pectin contributes to the stickiness which makes gummy candy hard to remove from the mold. Both gelatin and pectin synergistically improve elasticity compared to gelatin and pectin alone. Gelatin contributed to the moisture content. The more gelatin added to the formula, the more water is trapped in the gummy candy. Due to this contradictive contribution of each gelling agent, SLD is used to identify the optimum proportion of gelatin and pectin.

Preparation of the Optimum Formula Chrysanthemum Flower Gummy Candy

The determination of optimum gummy candy formula for chrysanthemum flowers was determined by the SLD method using software design expert version 13. The parameters used to determine the optimum formula are those that produced a significant difference upon changes in gelling agent composition. In addition,
the parameter should produce an insignificant lack of fit. Weight uniformity, % elasticity, and moisture content parameters produce a significant model and insignificant lack of fit. Thus suggested that they can be used as parameters to determine the optimum formula.

The optimum formula is expected to have good weight uniformity so that the expected target CV value is as small as possible (minimize) with importance 5 (+++++). The elasticity parameter has a target of 1.743% and importance 4 (++++) because the optimum formula is expected to produce a percentage of elasticity that is close to that of the comparison product. The optimum formula is expected to have the smallest possible moisture content. Based on the above data, the optimum desirability formula is 0.627 with a combination of 11.51% gelatin and 1.24% pectin with a batch weight of 78.4 g. The predicted values for the optimum formula characteristics of the software are weight uniformity (CV) of 1.306%, elasticity of 1.743%, and moisture content of 4.747%. The results of the statistical analysis show that the optimum gummy candy formula has physical characteristics that are not significantly different from the target determined by Design Expert software (Table III). This suggested that the gummy candy produced exhibited similar physical characteristics that had been predicted/designed.

**Table II. correlation between the proportion of gelatin and pectin to the physical characteristics of Chrysanthemum gummy candy**

<table>
<thead>
<tr>
<th>Response</th>
<th>Equation</th>
<th>Target value (%)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight uniformity</td>
<td>$Y_1 = 0.9002A + 1.75 B$</td>
<td>&lt;7.5</td>
<td>Greater pectin contributed to greater weight uniformity</td>
</tr>
<tr>
<td>% elasticity</td>
<td>$Y_2 = 1.75A + 7.49B - 10.97AB$</td>
<td>± 1.74</td>
<td>Both pectin and gelatin reduced the gummy elasticity</td>
</tr>
<tr>
<td>Moisture content</td>
<td>$Y_3 = 6.01A + 3.36B$</td>
<td>&lt;20</td>
<td>Gelatin increases moisture content</td>
</tr>
</tbody>
</table>

A: gelatin proportion; B: pectin proportion, AB: gelatin and pectin proportion

**Determination of Chrysanthemum Flower Antioxidants Before and After Formulation**

Evaluation of antioxidant activity was performed to compare the antioxidant activity of chrysanthemum flowers before and after they were formulated into gummy candy. In this study, ascorbic acid was used as a positive control. In the current study, the IC$_{50}$ value of ascorbic acid was 3.60 μg/mL which is categorized as a very strong antioxidant category. Chrysanthemum flower extracts antioxidant activity showed an IC$_{50}$ value of 67.80 μg/mL which suggested that it is a strong antioxidant. Meanwhile, gummy candy chrysanthemum flower extract has an IC$_{50}$ value of 82.93 μg/mL which is still been categorized as a strong antioxidant (Figure 3). Studies on the gummy candy without extract showed that the percentage inhibition of the DPPH reaction could not reach 50%. This study suggested that the antioxidant activity of the excipient is very low or negligible.
The Development of Antioxidant Nutraceuticals containing Chrysanthemum Indicum L.

Table III. One sample T-Test Results for Each Optimized Parameter of the Gummy Candy optimum formula

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Predicted target</th>
<th>Test results</th>
<th>p-value</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight uniformity (CV)</td>
<td>1,306%</td>
<td>1,310%</td>
<td>0.636</td>
<td>not significantly different</td>
</tr>
<tr>
<td>Elasticity</td>
<td>1,743%</td>
<td>1,769%</td>
<td>0.509</td>
<td>not significantly different</td>
</tr>
<tr>
<td>Moisture content</td>
<td>4,747%</td>
<td>3,13%</td>
<td>0.07</td>
<td>not significantly different</td>
</tr>
</tbody>
</table>

**DISCUSSION**

This study is the first published report on the development of Chrysanthemum indicum as a gummy candy. Although several studies have reported the formulation of gummy candy, the studies on Chrysanthemum gummy candy formulation are not available. The optimum formula obtained (11.15% gelatin and 1.24% pectin) has met the target product profile (TPP in terms of weight uniformity, elasticity, and moisture content). This study still has limitations in the measurement of the ethanol content of the product. Therefore, hedonic tests were not performed because this study still required further research to determine the ethanol content of gummy candy.

In addition to the optimum formulation, this study also evaluated the antioxidant activity of the extract and the finished product. This study will be useful to increase insight into how much the antioxidant activity is reduced during the process. Studies on Garcinia atroviridis gummy candy reported the antioxidant activity in the finished product but did not compare or evaluate how far the antioxidant activity will be decreased during the process (Renaldi et al., 2022). The current data can be used to increase understanding and explore a mitigation effort to prevent the loss of antioxidant activity during the manufacturing process.

This study also contributed to the availability of data on the use of honey as a sweetener in gummy candy. Sucrose and high fructose corn syrup are commonly used as a sweetener in gummy candy. Honey contains various types of sugars such as fructose, glucose, maltose, and sucrose. In addition to carbohydrates, proteins, amino acids, and some volatile components, honey contains minor components such as phenolic compounds, minerals, organic acids, and vitamins (Wu et al., 2017). Honey has several benefits, such as antibacterial, antiproliferative, and antioxidant properties. Although honey possesses antioxidant properties due to its phenolic compound (Alvarez-Suarez et al., 2014), the incorporation of honey into the gummy formula did not contribute significantly to the antioxidant activity of Chrysanthemum gummy candy. This is based on the examination of antioxidant activity on the gummy candy which did not contain the extract. It did not reach 50% inhibition of DPPH which suggests a very low/negligible antioxidant activity. This discrepancy could be due to the low

Figure 3. The antioxidant activity of Chrysanthemum indicum flower extract, Chrysanthemum indicum gummy candy, and ascorbic acid. a. ascorbic acid; b. Chrysanthemum flower extract; c. Chrysanthemum flower extract gummy candy; The antioxidant IC\textsubscript{50} (DPPH), triplicated. The value represents mean±SD. The data were analyzed using One-way ANOVA followed by the Tukey post-comparison test. * represent p<0.05; ** represent p<0.01; *** represent p<0.001; **** represent p<0.0001.
proportion of honey compared to the whole formula. Honey incorporation as a sweetener is expected to improve the taste and acceptance of gummy candy.

The obtained gummy candy meets the requirement from the National Standardization Agency of Indonesia (2018), which states that the maximum moisture content of gummy candy is 20%. The antioxidant activity of the chrysanthemum flower is decreased after it is formulated into gummy candy. The results of the gummy candy antioxidant activity test are expected to be taken into consideration in the development of nutraceutical products containing antioxidants.

CONCLUSION
The optimum gummy candy formula for chrysanthemum flower extract is produced by a combination of 11.51% gelatin and 1.24% pectin. The optimum formula obtained produces physical characteristics that are not significantly different from the predicted value obtained from the SLD. Chrysanthemum indicum flower extract gummy candy was confirmed to contain quercetin and exhibited antioxidant activity which is slightly reduced from the crude extract.

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