

The Development of Plant-Based Jelly Candy for *Chrysanthemum indicum* L. Flower Extract and Evaluation of The Antioxidant Activity

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ABSTRACT

Increasing awareness of wellness in society increased the demand for health supplements. Free radicals negatively impact vital organs resulting in the progression of neurodegenerative and cardiovascular disease. Herbal medicine such as (*Chrysanthemum indicum* L.) flowers has been reported to have strong antioxidant effects. However, the available products for *Chrysanthemum* is tea and capsules which is considered old-fashioned for many consumers. To improve consumer acceptance, *Chrysanthemum* can be formulated as jelly candy. However, applying heat during jelly candy production potentially reduced its antioxidant activities. This research is aimed to develop a *C. Indicum* L. jelly candy that can retain its antioxidant activity. *C. Indicum* L. flower extract is formulated into a jelly candy using plant-based gelling agents namely glucomannan and kappa carrageenan. Eight formulas were designed according to Design Expert software. Jelly candy's physical characteristics such as organoleptic, weight uniformity, elasticity, and moisture content were assessed. An antioxidant assay using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method was performed on *C. Indicum* L. flower extract before and after jelly candy formulation. The optimum formula identified from this study has a composition of kappa carrageenan 1.327% and glucomannan 0.673%. Assessment of antioxidant activity suggested that the jelly candy can retain its antioxidant activity compared to crude extract. The optimum formula exhibited strong antioxidant activity with an IC50 value of 72.91 ± 3.36 $\mu\text{g/mL}$. Through a minimal heating process, this study suggested that retaining antioxidant activity post-manufacturing process is feasible.

Keywords: *Chrysanthemum indicum*, Antioxidant, Jelly candy, Glucomannan, Kappa-Carrageenan

INTRODUCTION

Chrysanthemum indicum L., commonly known as East Asian traditional herbal medicine, is widely utilized for medicinal purposes in East Asia. This herb has a long-standing history of use in traditional medical practices across the region (Kim *et al.*, 2017). *Chrysanthemum* species have

been identified to have an extensive array of flavonoids, phenols, and phenolic acids (Oh *et al.*, 2011). *Chrysanthemum* isolates were reported to contain more than 190 chemical compounds, some of which are quercitrin, myricetin, linarin, acacetin, luteolin, kaempferol, and quercetin. These compounds have a broad biological spectrum

based on in vivo and in vitro tests (Shao *et al.*, 2020). Scientific investigations on nutrition have revealed that the consistent consumption of polyphenolic phytochemicals has beneficial outcomes in addressing and preventing various conditions caused by oxidative stress. Persistent oxidative stress is associated with the development of cancerous conditions, cardiovascular, and neurodegenerative disease (Ma & Wako, 2017). Chrysanthemum often time serve as a spice in culinary applications and can be integrated into various food items and pharmaceutical preparations. It offers natural preservative properties with its antioxidant potential (Youssef *et al.*, 2020).

Studies have reported chrysanthemum flower applications for health (Dong *et al.*, 2017). The potential content of chrysanthemum flowers has great prospects for being processed into snacks such as candy, chips, and instant drinks (Rahmasari *et al.*, 2024). One example of a chrysanthemum flower product that is growing in the market is chrysanthemum herbal tea. However, this herbal tea produces a taste that tends to be astringent and bitter. In addition, the use of tea is also considered less practical (Shao *et al.*, 2020).

Jelly candy is one alternative to formulate Chrysanthemum-based nutraceuticals. Jelly candy is a candy made from water or fruit juice with a gelling agent that has a transparent appearance and a texture with a certain elasticity (Godhwani *et al.*, 2012). Jelly candy has a soft and easy-to-swallow texture and a sweet taste with an attractive appearance. With these characteristics, making the consumption of jelly candy is considered practical and fun. However, pharmaceutical manufacturing to herbal formulations tends to include water and heat. This process potentially reduces the antioxidant activity of an extract. Typically, thermal processing is employed to prolong the shelf life of food items. Nevertheless, it is widely recognized that the bioactives in the extract can undergo substantial degradation during heating, drying, and boiling due to the vulnerability of the compound to heat (Choi *et al.*, 2006). Subjecting flavonoids to a temperature of 100 °C for 6 hours resulted in a 15% decline in their antioxidant efficacy, accompanied by reductions of 22% and 16% in their respective quantities (Ioannou *et al.*, 2020).

In addition to jelly candy, gummy candy is an alternate formulation for Chrysanthemum-based nutraceuticals. Tarahi has prepared watermelon-

gummy candy which produces a high moisture content in the range of 23.3 to 29.4%. In gummy candy preparation, a cooling process is required at 4°C for 4 hours and then left at 25°C for 24 h (Tarahi *et al.*, 2023). Meanwhile, the manufacture of jelly candy does not require a cooling process, so it was assumed it will minimize jelly candy-moisture content (Utomo *et al.*, 2014).

The characteristics of jelly candy are influenced by the gelling agent used. The most commonly used gelling agent is gelatine. Gelatine is obtained from the partial hydrolysis of collagen which comes from the skin, connective tissue and bones of animals. Most of the gelatine obtained comes from imported products and their halal status is doubtful, so the exploration on several plant-based polymers is necessary (Yusof *et al.*, 2019). Therefore, this study is aimed design a plant-based jelly candy in order to retain the antioxidant activity of *Chrysanthemum indicum* L. flower extract. The studies include the formulation of *C. indicum* L. in plant-based jelly candy with evaluation of its antioxidant activity before and after manufacturing.

MATERIALS AND METHODS

The materials of study were chrysanthemum flower (PT. AV Jaya Store Herbal, Bandung Indonesia), 70% ethanol (PT. Progo Mulyo, Yogyakarta, Indonesia), glucomannan (isolated by Faculty of Agricultural Technology, Gadjah Mada University), kappa carrageenan (Indo Food Chem Ltd.), multiflora honey (PT. Natura Alamindo Utama, Tangerang, Indonesia), sugar (PT. Gulaku Sugar Group Company, Lampung Tengah, Indonesia.), citric acid (PT. Multiverse Anugerah Chemindo, Tangerang, Indonesia), lemon essence (PT. Gunacipta Multirasa, Tangerang Indonesia), yellow dye (PT. Gunacipta Multirasa, Tangerang Indonesia), sodium benzoate (PT. Gunacipta Multirasa, Tangerang Indonesia), aquadest (PT. Progo Mulyo, Yogyakarta Indonesia), vitamin C (Sigma Aldrich, MO, USA), DPPH (PT. Smart Lab Indonesia.), ethanol p.a. (Merck Ltd, Darmstadt, Germany), and jelly candy reference product Relaxa Jelly Candy (PT. Natural Food Success, Bekasi, Indonesia).

Plant Determination

Plant determination is required as an initial step in the research process and has been used to determine the plant species.

Table I. Formulation of jelly candy

Ingredients	Formula							
	1	2	3	4	5	6	7	8
CI extract (g)	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Kappa carrageenan (g/100 mL)	1.25	0.5	1	1.5	1.5	1	0.75	0.5
Glucomannan (g/100 mL)	0.75	1.5	1	0.5	0.5	1	1.25	1.5
Honey (g)	20	20	20	20	20	20	20	20
Sugar (g)	30	30	30	30	30	30	30	30
Citric acid (g)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Sodium benzoate (g)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Essence (g)	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Colorant (g)	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Aquades (mL)	ad 150	ad 150	ad 150	ad 150	ad 150	ad 150	ad 150	ad 150

Plant determination was conciseness at the Pharmacognosy-Phytochemical Laboratory, Department of Pharmaceutical Biology, Gadjah Mada University, Indonesia (Baiti, 2023).

Preparation of *C. indicum* L. Extract

About 1000 g of the dried of *C. indicum* L. flowers were macerated with 7000 mL of 70% ethanol at room temperature for 24 hours. Subsequently, re-maceration was performed using 3000mL of 70% ethanol. The mixture was then filtered using a sieve cloth, and the solvent was evaporated at 40°C using a water bath until the concentrated extract was achieved. Finally, the ethanolic extract was kept in a 4°C refrigerator until used (Debnath *et al.*, 2013). The percentage of yields (w/w) were calculated (Yield [%] = [total extracted sample mass ÷ total dry sample mass] x 100).

Extract Characterization Phytochemical Analysis

One milliliter of the extract solution was put into a 10 ml test tube. Using pasteur pipette, 2-4 drops of concentrated HCL and Mg were added. The mixture was vortexed until Mg dissolved. The formation of an orange color indicates the presence of flavonoids of the flavonol and flavanol groups (Farmakope Herbal Indonesia 3rd Ed).

Thin Layer Chromatography (TLC)

Thin-layer chromatography (TLC) was performed to identify the presence of the compound myricetin in the concentrated extract of *C. Indicum* L. flowers. Absorbent silica gel 60 F₂₅₄ was coated on aluminum foil. A small spot of the solution obtaining the sample was applied on the plate 1 cm from the bottom marked by a line ruled.

Multiple spotted plates are used to prevent cross-contamination and interference, with each spot applied 1 cm apart. The sample is allowed to dry on the plate before placing it into the pre-saturated chromatographic chamber. The chamber cap was closed immediately to keep the chamber saturated with the mobile phase. The progress of the reaction is then monitored as the solvent moves up the plate, eluting the sample. The mobile phase consists of a solvent ratio of 19:11:2 of toluene, acetone, and acetic acid, respectively. Once the solvent reaches the 1 cm for the top border, it is carefully removed and dried. The plate was sprayed with citroborate reagent heated at 100°C for 5-10 min. Flavonoid spot was visualized under UV₃₆₅ light (Indonesian Ministry of Health, 2017).

Jelly Candy Formulation

Different formula was prepared to produce *C. Indicum* L. jelly candy (Table I). The procedure of jelly candy production was described as follows: sugar, glucomannan, kappa carrageenan, citric acid, sodium benzoate, aqua destilata, and honey was poured into a pot and mixed until homogenous. Subsequently, the extract was added to the mixture and heated until two minutes after boiling point was reached. Subsequently, the mixture was poured into a jelly candy molder and left for 3 h in the 4°C before being removed from the molder. The jelly candies were dried in an oven at 55°C for 24 h.

Physical Properties of Jelly Candies

The organoleptic (shapes, color, aroma, and texture) of the jelly candies under study were verified according to the previous method (Godhwani *et al.*, 2012). The whole jelly candies were tested for weight uniformity in accordance with United States Pharmacopeia (USP) Chewable Gels Monograph. Individually weigh units from

each formula in equal quantities to achieve a total of at least 20 individual weights. Then, calculating the average weight. The criteria will be satisfied if the individual weights do not deviate from the average weight by more than 7.5%. In case one unit exceeds these limits, perform the procedure again with an additional batch of at least 20 chewable gels. The requirements will be met if none of the tested units exhibit a difference from the average weight exceeding 10% (Davydova, 2018).

Evaluation of elasticity is performed by placing a plate on the jelly candy, then was given a pendulum weighing 50 grams. The weight were placed in the plate for 5 min. Changes in jelly candy thickness before and after treatment were measured using a caliper. Elasticity test data was obtained through the average percentage change in jelly candy thickness. The percent elasticity of jelly candy was calculated as follow:

$$\% \text{ Elasticity} = \frac{A - B}{A} \times 100\%$$

Where *A* is the thickness of jelly candy before treatment and *B* is the thickness of jelly candy after treatment.

Determination of water content is carried out using a Moisture Balance (OHAUS MB35) instrument. It shows the percentage of water content contained in the preparation. The test was performed by setting the instrument temperature at 105°C to warm up.

DPPH Radical Scavenging Activity

The DPPH scavenging activity was evaluated according to Kikuzaki (2002) method with slight modification. 0.4 mM DPPH solution was prepared in ethanol pro analysis. The extract solution was made by weighing 500 mg of chrysanthemum flower extract and dissolving them in 10 mL of ethanol pro analysis. Then extract solution was diluted 10x. The jelly candy was weighed an amount equivalent to 500 mg of chrysanthemum flower extract, then was suspended with 20 mL of 70% ethanol and homogenized using ultrasonic. After that, it was centrifuged at 10,000g for 20 minutes. The solutions with various concentration ranges for both extracts and jelly candy were prepared (20, 40, 60, 80, and 100 ppm). Vitamin C was used as a positive control. A hundred milligrams of vitamin C were weighed and dissolved in 100 ml of ethanol p.a. Some series of concentration series was made into 2, 3, 4, 5, and 6 ppm. One milliliter of each sample/standard

solution was mixed with 1 mL DPPH-ethanol solution, then was added ethanol pro analysis up to 5 mL. After that, the tubes were kept in complete darkness for 30 min. The absorbance of the samples were determined at 517 nm. The percent rate of DPPH free radicals was calculated as follow:

$$\% \text{ DPPH scavenging rate} = 1 - \frac{A_{\text{sample}}}{A_{\text{blank}}} \times 100\%$$

Where *A_{blank}* is the absorbance of the blank and *A_{sample}* is the absorbance of the sample (Kikuzaki *et al.*, 2002).

Hedonic Test

Samples were evaluated by 30 untrained panelists aged 18-25 among the students from Gadjah Mada University. Panelists were given two samples consisting of a product comparator and an optimum jelly candy formula. The comparator product was a jelly candy that used gelatin and pectin as a gelling agent instead of kappa carrageenan-glucomannan that were used in this study. Each sample was given a three-digit code and randomly served to the panelists. Panelists also received a glass of water and plain crackers for cleansing and neutralizing the palate between each evaluation and formulation. The evaluated sensory attributes were color, aroma, taste, and texture. A seven-point hedonic scale was conducted to identify significant differences between each sample (7 rating: extremely like; 1 rating: extremely dislike).

Statistical Analysis

The optimum formula was obtained from the response analysis for each physical characteristic (weight uniformity, elasticity, and moisture content) using the simple lattice design (SLD) method with the Design Expert tool using one sample t-test with 95% confidence. Determination of antioxidant activity was performed by the DPPH radical scavenging method, which then obtained the absorbance profile to calculate the percent inhibition and IC50 value. Antioxidant activity in the form of the resulting IC50 value was then analyzed using the IBM SPSS Statistics tool with one way ANOVA (*P*<0.05) and continued with the Post Hoc Tukey test.

RESULTS AND DISCUSSION

Extraction and characterization of Chrysanthemum

The identification of the sample species were conducted at the Pharmaceutical Biology

Laboratory at Gadjah Mada University. It was confirmed that the flower belongs to the Asteraceae family and specifically identified it as *C. indicum* L (Document # 19.5.1/51/UN1/FFA.2/BF/PT/2003)

The crude extract obtained from the maceration process had a yield of 31.36%. This result met the requirements stated in Farmakope Herbal Indonesia 3rd Edition, which specifies a minimum yield of 22.7% for *C. indicum* L. The extract from chrysanthemum flowers was yellowish-brown in colour and possessed a distinctive aroma. The results of the extract phytochemical test by adding citroborate reagent to the *C. indicum* L extract solution resulted on color changes from light yellow to brick red (Kementrian Kesehatan RI, 2017). This specific color formation upon addition of citroborate reagent suggested that the sample contains flavonoid compounds.

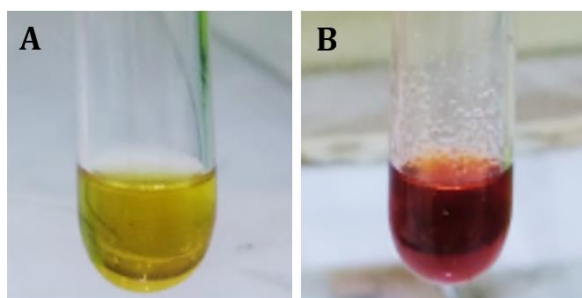


Figure 1. Formation of dark red color of the the Chrysanthemum extract solution upon addition of citroborate reagent.

Studies suggest that flavonoids in *Chrysanthemum morifolium* extract exert antioxidant activities (Han *et al.* 2019). To confirm the presence of flavonoids in *C. indicum* L. extract, thin-layer chromatography (TLC) qualitative test was performed to confirm the presence of flavonoid compounds on the extract. The principle of the TLC test is the separation of chemical components based on the adsorption and partition principles determined by the stationary phase (adsorbent) and the eluent (Alen *et al.*, 2017). The flavonoid reference used in this study was quercetin since it has similar structure and polarity to myricetin, a flavonol compounds that was abundant in *C. indicum* L. flower. Spots in TLC containing flavonoids will fluorescence yellow after being sprayed with citroborate reagent while observed under UV light 365 (Harwoko & Warsinah, 2020).

TLC separation resulted spots that appear on the chrysanthemum extract sample (M) had an Rf value of 0.57 while the quercetin (Q) as a standard had an Rf value of 0.62 (Figure 2). The spots on the chrysanthemum flower extract samples (Figure 2) are suspected to be spots of myricetin compounds. Myricetin is an identity compound in chrysanthemum flowers. The results of this study were in agreement with Farmakope Herbal Indonesia (2017) which mentioned that myricetin exhibits Rf value of 0.57 when being separated with Thin Layer Chromatography with the mobile phase that is similar to this current study. Indeed, data on logP for myricetin and quercetin support the result. logP value has been widely used to determine the polarity of a compound. Lower the logP value suggesting the polarity of the substance. Myricetin has a logP value of 1.42 (*Myricetin* | C15H10O8 | CID 5281672 - PubChem, n.d.). Meanwhile, quercetin has a logP value of 1.48 (*Quercetin* | C15H10O7 | CID 5280343 - PubChem, n.d.). Thus the Rf of myricetin is smaller than quercetin.

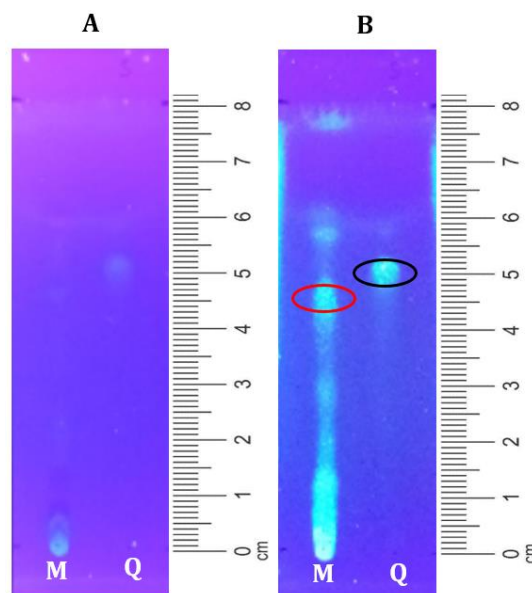


Figure 2. Chrysanthemum indicum flower extract upon separation using thin layer chromatography. Stationary phase was silica gel 60 F254 result while the mobile phase was toluene, acetone, and acetic acid (19:11:2). Plate A: before spraying with citroborate reagent. Plate B: after been sprayed with citroborate reagent. (M) The line of chrysanthemum flower extract (Q) The line of quercetin as standard.

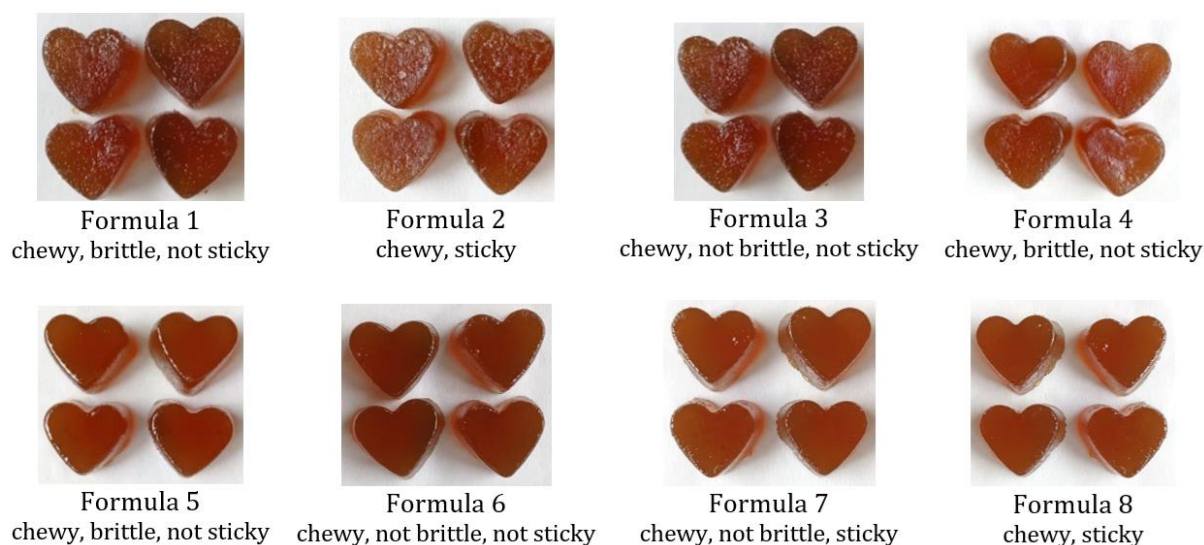


Figure 3. The organoleptic of jelly candy with various concentrations of glucomannan and kappa carrageenan as gelling agent used.

In earlier studies, the mobile phase utilized in HPTLC to isolate myricetin underwent optimization by experimenting with various solvents of different polarities. After testing, the mobile phase is ultimately chosen, a combination of toluene, ethyl acetate, formic acid, and methanol in proportions of 3:3:0.6:0.4 (v/v), yielded a distinct band for myricetin at Rf 0.57. This separation was clear, distinguishing myricetin from neighboring bands within the plant extract. The myricetin standard displayed a single peak (Rf 0.57) in the HPTLC chromatogram (Patel *et al.*, 2010).

Formulation Chrysanthemum Jelly Candy

The jelly candy in this study was formulated using a plant-based gelling agent. The use of a plant-based gelling agent instead of gelatin was chosen to improve consumer acceptability. For example, vegetarian and Moslem populations are concerned with the pork gelatin in the jelly candy. The optimal proportions of glucomannan and kappa carrageenan were identified by utilizing SLD and Design Expert software.

A total of eight experimental formulas were conducted, each using different concentrations of the gelling agents. The total weight of the extract used is 7.5 g (5%) per run with a total ingredient requirement of 150 g. Each run produces 60 jelly candies, each containing 125 mg of chrysanthemum flower extract.

The physical properties of the prepared samples were evaluated through organoleptic testing, weight uniformity test, elasticity test, and moisture content test. The results of the organoleptic testing revealed that the *C. indicum L.* jelly candy were heart-shaped in appearance with a yellow-brown in colour. It had a distinct aroma of chrysanthemum flower extract and a chewy texture (Figure 3).

The weight uniformity test was performed to evaluate the uniformity of the preparations and ensure that each preparation has similar amount of the extract. The test was initiated by weighing 20 jelly candy samples one by one at each formula and then calculating the average weight so that the CV value could be obtained. The lowest CV value is generated in formula 5 which is 1.16% (Figure 4a). This means that the weight uniformity produced by formula 5 has the most uniform weight compared to the other formulas. This may be caused by the role of kappa carrageenan which was able to produce more solid jellies compared to other formulas. The solid texture of the jellies provide an easiness in removing jelly candy from the molder and to minimize physical damage of preparations due to scratches. The highest CV value is generated in formula 2 which is 1.40%. The jelly candy obtained in formula 2 was stickier than the other formulas so it affected the process of removing jelly candy from the molder which had an impact on the CV value of the resulting weight uniformity.

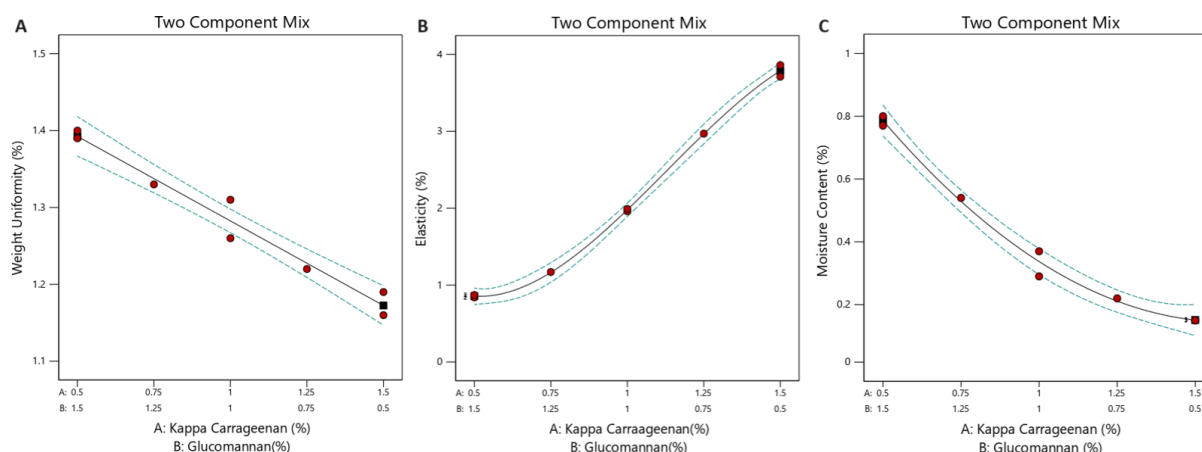


Figure 4. Profile of physical properties of jelly candy based on expert design. (A) Weigh uniformity. (B) Elasticity. (C) Moisture content

Table II. Comparison of Simple Lattice Design (SLD) Predicted Value with Optimum Formula Test Results

Response	Predicted Value	Experiment Value	Sig.
Weigh Uniformity	1.21%	1.20%	0.922
Elasticity	3.25%	3.52%	0.235
Moisture Content	0.19%	0.22%	0.535

These studies suggested that the composition of the gelling agent affects the texture as well as the easiness to be removed from the mold.

The average range of percent elasticity in the jelly candy test samples for the entire formula was 0.84% -3.86%. The commercial product (Relaxa Play Gummy Jelly Candy) had an elasticity value of 3.25%. The highest elastic jelly candy was formula 2 with an elasticity value of 0.84% (Figure 4b). This is produced due to the dominating presence of glucomannan. The stickiness of a preparation is greatly influenced by the ability of the gelling agent to hold water molecules. In this context, the ability of glucomannan to retain water is compared with kappa carrageenan. When glucomannan is included to increase the stickiness of a dosage form, this is correlated with a reduced influence of kappa carrageenan, which normally retains water through the presence of anhydrogalactose groups, resulting in a decrease in the amount of sulfate esters in its structure. As a result, kappa carrageenan becomes better able to retain water through its hydroxyl functional group (R-OH) (Alvita *et al.*, 2021).

Then, increasing the composition of glucomannan in the formulation resulted in a weak

bond zone being formed and causing the deformation of kappa carrageenan intermolecular interaction. This event leads to a reduction in jelly candy hardness and an increase in jelly candy elasticity. The lowest elasticity is at formula 5 due to the ability of kappa carrageenan to form a strong double helix structure and resulted in a harder, brittle, and less elastic jelly candy (Utomo, *et al.*, 2014).

Another evaluation of the jelly candy is the moisture content. The lowest moisture content resulted from formula 5 with only 0.15% while the highest moisture content were observed in formula 2 with a value of 0.80% (Figure 4c). Previous studies had described the structure of kappa carrageenan and its properties. Kappa carrageenan had the ability to retain water due to the presence of anhydro-galactose groups thereby reducing the number of sulphate esters in its structure, kappa carrageenan was more capable to retain water through its hydroxyl functional group (Utomo *et al.*, 2014). The addition of glucomannan caused an increase in water content as glucomannan had the highest hydrophilic properties compared to other hydrocolloids. The difference between glucomannan and other hydrocolloids is related to

their ability to absorb 100 grams of water per gram of sample. The greater the amount of glucomannan used, the greater the water absorbed (Karo *et al.*, 2021). In this study, kappa carrageenan was more dominant in increasing the moisture content than glucomannan.

Verification of Optimum Formula

To validate the optimal formula for jelly candy containing chrysanthemum flower extract, the physical properties of the eight experimental formulas were analyzed using Design Expert software version 13. The physical properties examined included weight uniformity, elasticity, and moisture content. The software calculated a desirability value of 0.971, indicating a highly favorable outcome. The ideal formula, according to analysis using the software, consisted of 1.327% kappa carrageenan and 0.673% glucomannan for the 150 g batch size. Table 2 shows the predicted values for each response provided by the software.

Evaluation on the Antioxidant Activity of *C. indicum L.* extract jelly candies

Numerous techniques in the field of food technology are employed to preserve nutritional properties and improve sensory characteristics, shelf life, and safety of food products. Various manufacturing processes have an impact on the antioxidants, leading primarily to a reduction in antioxidant activity. These losses can occur dramatically during heating (Poljsak *et al.*, 2021). Due to findings from multiple studies indicating a declining activity of some active ingredients such as natural antioxidants due to inadequate manufacturing processes, the food industry is challenged to determine which food processing methods can minimize the impact on pharmacological activity. Consequently, the additional aim of this study is to assess the antioxidant activity after various manufacturing processes.

DPPH radical scavenging assay is widely known as a simple method to determine antioxidant activity. DPPH is a stable free radical because of the delocalization of its spare electron over the whole molecule. In the delocalization condition, it shows a deep violet color with the lambda max around at 517 nm. When a substrate having hydrogen atom-donating capacity meets DPPH, the color turns into yellow (Debnath *et al.*, 2013). In this study, vitamin C was used as a positive control because it is a secondary antioxidant that has a free hydroxy group as an

electron donor to capture radicals resulting to its high antioxidant activity.

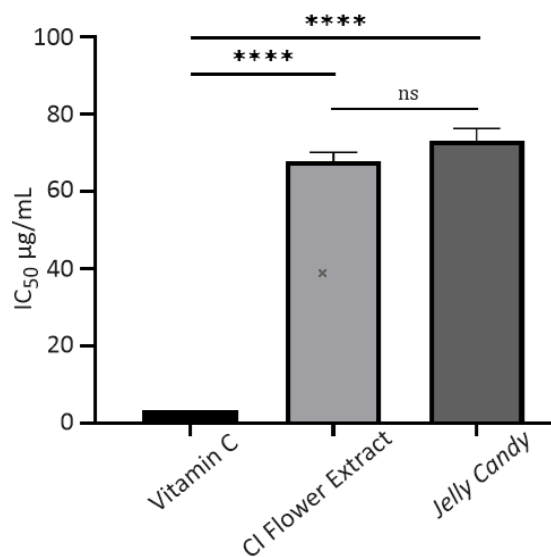


Figure 5. Antioxidant activity of *Chrysanthemum indicum* jelly candy compared to *Chrysanthemum indicum* crude extract (CI) and vitamin C as a positive control. The analysis of antioxidant activity was performed using One Way ANOVA followed by Post Hoc Test. Each bar represents mean ± SD, n = 3; **** = p-value <0.0001; ns = p-value 0.0851, CI (*C. indicum L.*).

The IC₅₀ value of vitamin C as a positive control was 3.60 ± 0.01 µg/mL. The IC₅₀ of antioxidant activity of *C. indicum L.* extract was 67.80 ± 2.37 µg/mL and while the IC₅₀ of the jelly candy containing similar amount of extract was 72.99 ± 3.24 µg/mL (Figure 5). Both the *C. indicum L.* flower extract and *C. indicum L.* jelly candy showed strong antioxidant activity based on Molyneux (2004). In this study, there is no significant difference between the IC₅₀ of *C. indicum L.* crude extract compared to those that been formulated in the form of jelly candy (P>0.05). The antioxidant activity of vitamin C was categorized very strong antioxidant, while *C. indicum L.* extracts and jelly candy are included in the strong category. The antioxidant activity of *C. indicum L.* extract is lower than vitamin C. This is in line with research conducted by Agustiarini (2022) on the antioxidant activity test of the ethanol extract of rosella flowers at 43 µg/mL lower than vitamin C at 2,058 µg/mL. Free radical scavenging by vitamin C was stronger than the test sample because the tested extract was a mixture containing various compounds including compounds that did

not have antioxidant activity while vitamin C was a pure compound.

In another study conducted by Rahmasari who also examined the antioxidant activity of chrysanthemum flower extract but in the form of gummy candy. It was found that the water content was higher, while the antioxidant activity of gummy candy was lower and significantly different from the extract. This difference may be due to the longer heating process used in their study, namely 5 minutes of heating at 80°C, compared to the 2 minutes heating to boiling process used in the jelly candy production in this study (Rahmasari, 2023).

Lower processing temperatures play a crucial role in maintaining nutritional value and functional properties. By utilizing gentle heat treatments during processing, thermosensitive nutrients, and functional components are better preserved. High temperatures can lead to the degradation or loss of antioxidants, which are known for their health-promoting benefits. Therefore, employing low processing temperatures ensures to retain their nutritional integrity and bioactive compounds, enhancing the overall quality and potential health benefits of the final products (Avelar *et al.*, 2020).

In order to retain the antioxidant activity in jelly candy, careful consideration should be given to the concentration of the sweetener utilized and the packaging of the product. Additionally, the pH stability of the product is also a crucial factor to consider. Comparatively, goji fruit jelly experiences a greater reduction in antioxidant capacity when compared to goji jam. This disparity can be attributed to the higher sugar content and lower pH level of the jam, which effectively shield the bioactive compounds from degradation (Cedeño-Pinos *et al.*, 2020).

Compared to the previously published studies, the current research provides information on the involvement of glucomannan in affecting the physico-characteristic of jelly candy. The report on utilizing glucomannan as a gelling agent in jelly candy is limited compared to gelatin or pectin. Glucomannan as a plant-based polymer has an advantage related to its halal status as a biological ingredient. In addition, glucomannan has some health benefit. It can lower cholesterol levels, reduce the risk of constipation, a therapeutic agent in type 2 diabetes mellitus, and as a dietary supplement for weight loss (Sharma & Wadhwa, 2022).

This study utilizes honey as a sweetener at a concentration of 20% for each formulation. The use

of honey is intended to replace the use of high fructose syrup (HFS) as a sweetener which has been commonly used for the last 40 years. Consumption of HFS can increase the risk of diabetes and various other side effects when compared to honey (Moeller *et al.*, 2009). Honey is a natural sweetener that has therapeutic potential for disease with its phytochemical content as anti-inflammatory, antimicrobial, and antioxidant. Honey is highly recommended to be used as a sweetener or even to play a role in medicine (Samarghandian *et al.*, 2017).

The physical characteristics test of the chrysanthemum flower extract jelly candy produced in this study requires a better method for collecting elasticity data. The elasticity test method should be done by Texture Profile Analysis (TPA) on testing the physical and sensory characteristics of jelly candy so that more precise test data is produced because this tool has the advantage of being more sensitive and accurate (Yusof *et al.*, 2019).

The current research provides information on the antioxidant status of *C. indicum* L. extract post-formulation. Tests for antioxidant activity in previous studies were only carried out on extracts. Research conducted by Kang *et al.* (2021) that testing the antioxidant activity of chrysanthemum flower extract. The resulting IC50 value is 14.95 µg/mL. This research resulted in the antioxidant activity of chrysanthemum flower extract with an IC50 value of 68.0 ± 2.37 µg/mL. The difference in the magnitude of the IC50 value was obtained due to the difference in the DPPH concentration used, which was 0.15 mM, while in this study the DPPH was used with a concentration of 0.4 mM (Kang *et al.*, 2021).

A formulation of a pharmaceutical dosage form requires an equivalent dose of the active substance so that it can provide health effects as expected as an antioxidant agent. Recommended Dietary Allowance (RDA) is 90 mg/day for adult men and 75 mg/day for adult women is set based on the vitamin C intake to provide antioxidant protection (Institute of Medicine (US) Panel on Dietary Antioxidants and Related Compounds, 2000). Then, the safe upper limit of vitamin C is 2000 mg a day. In this research, the antioxidant activity of vitamin C was 20.25x stronger than jelly candy extract based on IC50. Therefore, the daily dose of extract required is 20.25×90 mg = 1823 mg which, if converted, requires 15 jelly candies per day. This is not a problem because both men and women are acceptable based on the dose limits as

explained previously. So, people can eat 5 jelly candies in the morning, afternoon, and evening to get a total of 15 jelly candies per day. Each package will be designed to contain 3 small packages, each containing 5 jelly candies to make it easier for consumers and increase compliance. The calculation of the daily dose of chrysanthemum extract jelly candy of 1823 mg has also been adjusted to be equal to the recommended safe dose, namely 18 mg - 2.25 grams of extract (Harima, 1996).

Hedonic Test

A sensory evaluation was conducted to investigate the consumer acceptance of the gummy candy prepared from Chrysanthemum extract, honey, and natural polymer kappa-carrageenan and glucomanan.

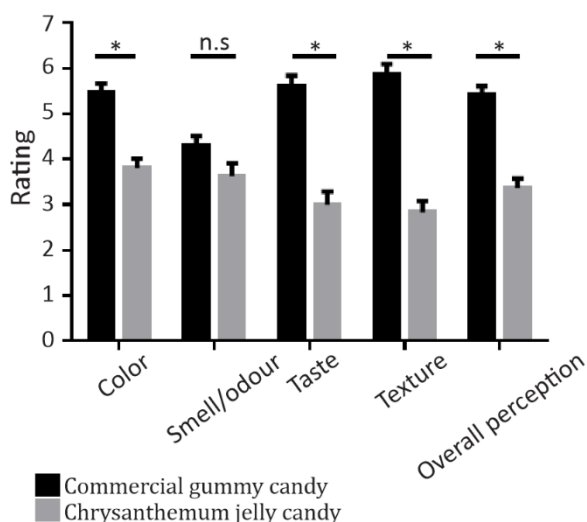


Figure 6. Hedonic test comparator product vs *C. indicum L.* jelly candy. Thirty respondents were recruited to assess the color, odor, taste, texture, and overall rating of the two products. Rating 7 represents the highest preference while rating 1 represents the lowest preference. Data were analyzed using Two Way ANOVA followed by Sidak tests. * represent significant differences.

Based on Figure 6, panelists prefer to market products to gummy candy on all parameters except or smell/odor properties. Significant differences were found in taste, color, and texture parameters. In terms of taste parameters, jelly candy has a distinctive taste that comes from the active substance of the chrysanthemum extract used. This is acceptable because the panelists are not familiar

with this taste. In texture parameters, the differences are caused by differences in the gelling agent used. In the comparator product, the gelling agent gelatin-pectin was used, while in the gummy candy, the gelling agent kappa-carrageenan was used. The differences in the gelling agent and the addition of honey instead of sucrose might contribute to the differences in the texture and response acceptability. This hedonic test suggests that although kappa carrageenan and glucomannan are plant-based polymers, they potentially influence the texture and response acceptability. The gelatin-pectin combination, although it is an animal-based polymer and can raise an issue among Moslem consumers, it provides robust and acceptable consistency. Meanwhile, the kappa-carrageenan combination tends to be softer because it is made from seaweed so the texture is more like jelly.

The hedonic test implies that gelatin is still the gold standard for jelly candy preparation. This gelling agent compared to plant-based polymers provides a more robust acceptable texture compared to the current formulation. Thus studies that develop a Moslem-friendly gelatin will be beneficial to further develop this *C. indicum L.* jelly candy.

CONCLUSION

In conclusion, the optimal formula for jelly candy containing *C. indicum L.* flower extract was determined to be 1.327% of kappa carrageenan and 0.673 % of glucomannan. The resulting formula exhibited weight uniformity, elasticity, and moisture content that closely matched the predicted values and experimental observations. Furthermore, both the *C. indicum L.* flower extract and the jelly candy displayed similarly strong antioxidant activity, with no significant difference between the before and after formulation of jelly candy. The product has no significant differences in odor properties but has significant differences in texture.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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