Formulation and Antioxidant Activity of Gotu Kola Jelly Candy with Plant-based Polymers as a Gelling Agent

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ABSTRACT

Centella asiatica or gotu kola has a long history as a brain supplement. Gotu kola supplements are sold as liquid and dried extract which is less attractive for a younger generation. Jelly candy is an alternative dosage form with better acceptability across ages. However, the use of animal-derived polymers such as pork gelatine in the candy restricts those who practice vegetarian and halal lifestyles from consuming the products. This study aims to explore plant-based polymers glucomannan and kappa-carrageenan as gelling agents in the preparation of gotu kola jelly candy. Preparation of the jelly candy formula was designed based on Simplex Lattice Design. Evaluation of physical characteristics of jelly candy includes organoleptic, weight uniformity, moisture content, pH, and elasticity. The antioxidant activity of gotu kola before and after the manufacturing process was evaluated. The results showed that a combination of kappa-carrageenan 1.33% and glucomannan 0.67% is the optimum formula. Adding more proportion of kappa-carrageenan reduced jelly elasticity and moisture content. While adding glucomannan improved its elasticity responses but increased moisture content. Evaluation of the antioxidant activity of gotu kola in jelly candy suggested that gotu kola experienced a significant reduction in antioxidant activity following the production process. The IC50 of the crude extract initially was129.23 ppm while post jelly candy manufacturing, the IC50 increased to 197.49 ppm. This study suggested that improvement in extraction and production processes is necessary to maintain gotu kola antioxidant activity.

Keywords: antioxidant; glucomannan; Centella asiatica; jelly candy; kappa carrageenan

INTRODUCTION

Brain oxidative stress triggered by free radicals is the underlying mechanism of brain inflammation. Free radicals are produced endogenously and exogenously by an imbalance of antioxidants and pro-oxidants in the body. Unbalanced antioxidant defense mechanisms, excessive production, and incorporation of free environment radicals from the lead to neurodegeneration which impairs cognitive function and brain memory (Uttara et al., 2009).

Gotu kola (*Centella asiatica* (L.) Urban) is one of the plants that is believed by many societies to improve memory. It strengthens nerve function and improves focus and memory (Hannan et al., 2021; Rao et al., 2005). *Studies showed that gotu kola contains* bioactive compounds that exhibit antioxidants and neuroprotective activity (Yuliani & Linar, 2019). Antioxidants in gotu kola have the

*Corresponding author : Marlyn Dian Laksitorini Email : marlyn_fa@mail.ugm.ac.id potential to slow brain aging and neurodegenerative diseases (Umka Welbat et al., 2016; Wong et al., 2021; Xu et al., 2012). Antioxidant activities of *C. asiatica* correlated with its total phenolic and total flavonoid compounds. Another major constituent of *C. asiatica* is triterpenoid such as asiaticoside and asiatic acid (Long et al., 2012).

Gotu kola has usually been consumed as a fresh vegetable or extract (Gomez, 2021). However, this consumption option is considered less practical. Jelly candy is an alternative solution for the younger generation that prefers attractive shapes, various flavors, and practical dosage forms (Sunil et al., 2020). Hydrocolloids in the jelly candy promote the formation of a chewy and attractive product. Most of the chewable gel preparations use gelatin as a gelling agent. However, the use of gelatin especially those originating from pork is prohibited in Moslem and vegetarian consumers (Zin et al., 2021).

In this study, a combination of glucomannan and kappa-carrageenan hydrocolloid was used as a gelling agent for chewable jelly candy. The kappa carrageenan and glucomannan were chosen because of their synergistic effect which can produce stronger plastic gel products. Kappacarrageenan forms a rigid gel with a high degree of syneresis. Meanwhile, glucomannan forms an elastic gel. Formula optimization was conducted to obtain a good jelly candy texture. Evaluation of antioxidant activity in extracts and jelly candy preparations of Centella asiatica was carried out using the DPPH reduction method to validate the potential antioxidant power as a brain supplement (Subathra et al., 2005). The DPPH assay is considered a reliable, precise, straightforward, and cost-effective approach for assessing the antioxidant properties of compounds by measuring their ability to scavenge radicals (De Torre et al., 2019). In addition, this method has been well established and has been adopted by several research groups which allows researchers to assess the antioxidant data obtained to be compared with the published paper.

MATERIALS AND METHODS Materials

The materials used in this study included extracts of gotu kola (Centella asiatica) from Omah Djamoe Arroyan, Karanganyar Regency, Central Java Province, Indonesia, kappa-carrageenan (Food grade, Indo Food Chem, Ltd.), glucomannan (isolated by Faculty of Food Technology, Gadjah Mada University), sucrose (Sugar Group Company, Ltd.), citric acid (Multiverse Anugerah Chemindo, Ltd.), tutti-fruity flavor (Gunacipta Multirasa, Ltd.), purple coloring (Gunacipta Multirasa, Ltd.), aquadest (Progo Mulya, Ltd), 70% ethanol (Progo Mulya, Ltd.), jelly candy reference product (Natural Food Success, Ltd.), DPPH (Smartlab, Ltd.), ascorbic acid (Sigma Aldrich), multiflora honey (Natura Alamindo Utama, Ltd.), and methanol p.a. (Merck, Ltd.).

Methods

Centella asiatica Extraction

The confirmation of species identity was done at the Department of Biological Pharmacy, Faculty of Pharmacy, Gadjah Mada University. Before extraction, the herb was ground into a fine powder using a disk mill. Nine hundred grams of *Centella asiatica* powder was macerated using 6.3 L of ethanol 70% for 24 hours at room temperature. This amount is equal to a sample-tosolvent ratio of 1:7. After the maceration process, the solution was filtered using a Buchner funnel. The sludge was re-macerated and soaked into 2.7 L of 70% ethanol with a ratio of sample to solvent (1:3) for another 24 hours. The filtrate obtained from the first and second maceration was combined and concentrated using a temperature-controlled water bath.

Qualitative analysis of Total Phenolic Contents (*TPC*)

The qualitative analysis of the TPC of *C. asiatica* extract was evaluated according to (Jaradat et al., 2015) with slight modification. Briefly, a solution of FeCl₃ was added to the crude extract. The presence of phenolic compounds in the crude extract was indicated by the development of black or blue-green color.

Quantitative analysis of Total Flavonoid Content (*TFC*)

The TFC of the extract was determined according to (Aryal et al., 2021) slight modification. Briefly, the extract (0.5 mL) was subjected to 1.5 mL of methanol and 100 μ L of 10% AlCl₃. The mixture was added with 100 μ L of 1 M CH₃CO₂Na. The volume of the mixture was completed to 5 mL using distilled water. The mixture was gently mixed and then incubated at room temperature for 30 min. The absorbance of the sample was measured using Spectrophotometer UV-Vis (Agilent Cary 60) at 420 nm. Similar treatments were done for the standard compound (quercetin). The concentration of flavonoid in the C. asiatica was expressed as quercetin equivalent (mg QE/g sample).

Confirmation of Asiaticoside in Thin Layer Chromatography Profile

Asiaticoside is one of several compounds in *Centella asiatica* that is responsible for its neuroprotective activity. To confirm the presence of asiaticoside in gotu kola extract, thin-layer chromatography was used. The concentration of the extract used was 20% w/v. The stationary phase used was Silica Gel 60 F254 plates with a size of 10 cm x 10 cm and 5 mL of ethyl acetate: methanol: water (8:2:1) as the mobile phase. After the elution process, the plates were sprayed with an anisaldehyde sulfuric acid reagent before being heated for 10 minutes at 110°C. Next, the plates were visualized in 366 nm UV light.

Evaluation of Antioxidant Activity Using DPPH Radical Scavenging Assay

The antioxidant activity was measured on the gotu kola extract before and after being formulated into jelly candy. Ascorbic acid and jelly candy without extract were used as a positive and negative control. Briefly, to prepare 0.4 M DPPH solution, 15.77 mg DPPH powder was weighed and dissolved in 100 ml of ethanol p.a. The solution was stored at refrigerator temperature and protected from light (Susiloningrum & Mugita Sari, 2021).

To prepare gotu kola solution for DPPH assay, as much as 500 milligrams of gotu kola extract was dissolved in 10 ml of ethanol p.a. This stock solution was then diluted into various concentrations of 50, 75, 100, 125, and 150 ppm. To prepare gotu kola jelly candy for antioxidant assay, a total of five gotu kola jelly candies were homogenized using a mortar and stamper. The homogenate which is equivalent to 500 g of gotu kola extract was weighed. This corresponds to 5.17 g of the jelly candy homogenate. The mass was dissolved in 10 mL of ethanol p.a. The solution was vortexed and centrifuged to obtain the supernatant. This solution was diluted in concentrations 42, 83, 125, 167, and 208 ppm. To prepare the blank solution, ethanol was used (Pindan et al., 2021).

To prepare a sample for measuring the antioxidant activity of blank jelly candy, a similar protocol as gotu kola jelly candy was used. To prepare the control solution, 1 mL of DPPH solution (50 ppm) was mixed with 4 mL of ethanol p.a and vortexed to homogenize with the vortex. The optimum wavelength was identified by scanning the solution using spectrophotometry at λ 400-600 nm. Ascorbic acid is used as a positive control. To measure ascorbic acid antioxidant activity, 1 g of ascorbic acid p.a was dissolved in 100 ml of ethanol p.a. Then, the solution was homogenized using an ultrasonic sonicator. After reaching homogenous, some dilutions were prepared to achieve ascorbic acid 2, 3, 4, 5, and 6 ppm.

To determine the antioxidant activity of the sample, 1 ml of DPPH solution 1 mL was added to the 1 ml of the sample solution. In this study, there are four samples to be measured namely, ascorbic acid, extract, gotu kola jelly candy, and blank jelly candy. The mixture (2 ml) solution was homogenized and incubated for 30 minutes. The color change of the solution was observed and the absorbance was read with a UV spectrophotometer at the optimum wavelength. The determination of IC50 of each sample was done in triplicate.

Preparation of Gotu Kola Jelly Candy

The development of Gotu Kola jelly candy was performed through a simplex lattice design approach as previously described (Hidayah et al., 2023). The formula for preparing gotu kola jelly candy is shown in Table I. Jelly candy was prepared by dissolving the gotu kola extract in 10 mL of distilled water. In a separated beaker, sugar, kappa-carrageenan, and glucomannan were mixed until homogeneous and added to 25 mL of distilled water gradually. Subsequently, the gotu kola extract solution and honey were added to the solution. To the mixture, 29.5 mL of distilled water, citric acid, and sodium benzoate were added to the solution and stirred until completely dissolved. The mixture was cooked using low heat for 2 minutes to boil (90°C) with occasional stirring. Subsequently, the temperature was reduced to 80°C and the essence and food coloring were added. As the heating stopped, the mixture was set aside until all the bubbles disappeared. The mixture was immediately printed and baked at 50°C for 23 hours (Devi, 2023).

Evaluation of Jelly Candy Physical Characteristics

Weight Uniformity

The test was carried out by weighing 20 jelly candies for each formula and then calculating the average weight. According to USP Chewable Gels, the requirement is met if no jelly candy deviates by more than 7.5% of the average weight. If there is one preparation that deviates from the requirements, then the test is carried out again with an additional 20 pieces of jelly candy. Requirements are met if there is not a single unit whose weight deviates from the average by more than 10%.

Elasticity

The elasticity test was carried out by sampling 4 gotu kola jelly candies from each formula. A metal plate was then placed on top of the jelly candy layer and 200 g weight was placed on the plate for 5 minutes for each jelly candy. The percent elasticity of jelly candy for each formula was calculated based on the formula:

% elasticity =
$$\frac{initial \ height - final \ height}{initial \ height} x100\%$$

Loss on Drying

An aluminum plate was placed on the moisture analyzer scales and set for tare (zeroing). One jelly candy from one formula was added before the lid of the moisture balancer was closed. The weight before heating was recorded. To measure the moisture content, a temperature of 105°C was used. After several minutes, the scale reaches a constant weight and the LOD value appears in the form of a percentage. The experiment was done in triplicate for each formula.

Statistical Analysis

To identify the optimum proportion of plant-based gelling agents, Design Expert 13 software was used. Observational data on

evaluation parameters which included tests for moisture content, weight uniformity, and elasticity were entered as response parameters to determine the optimum jelly candy formula. Verification of the optimum formula using SPSS 25 software by performing tests for normality, homogeneity, and one sample t-test with a 95% confidence level on physical characteristics by comparing the predicted values from Design Expert 13. Antioxidant test data were analyzed using linear regression to determine antioxidant activity. Oneway ANOVA followed by LSD Fisher's test was used to compare the antioxidant activity of the positive control, gotu kola extracts, and gotu kola jelly candies.

RESULTS

Gotu Kola Herb Extract

In this study, extraction using the maceration method was chosen to extract triterpenoid glycoside from gotu kola. The maceration was followed by one re-maceration to improve yield. Important triterpenoid glycosides in gotu kola include asiatic acid, asiaticoside, madecasic acid, and madecassoside (Jamil et al., 2007). Asiaticoside has a polar group due to the glycosidic bond of a sugar molecule and a benzene group. 70% ethanol was reported to extract more asiaticoside and obtained a high level of flavonoids compared to 30% and 50% ethanol (Pramono & Ajiastuti, 2004). Organoleptically, gotu kola extract was dark green in color, viscous, and had a characteristic odor of Centella asiatica. The current extraction method resulted in a 22.73% yield.

The qualitative analysis of the phenolic compound of the crude extracts was determined using $FeCl_3$ solution. The result showed that the ethanolic extract of *C. asiatica* contains phenolic compounds, which form a blue-green color after dropping 1% FeCl_3 solution (Figure 1).

The quantitative of TFC extract was also determined in this study. The result revealed that the crude extract of C. asiatica contains flavonoid compounds with a value of 66.01±0.62 mg QE/g extract. This result was in agreement with the previous study (Gray et al., 2018; Mohammad Azmin & Mat Nor, 2020). The study reported that the ethanolic extract of C. asiatica contains flavonoid acids such as gallic acid, rutin, kaempferol, catechin, quercetin, and luteolin by HPLC analysis. Those compounds have been reported to have antioxidant activity (Pittella et al., 2009). In this study, the standard asiaticoside solution was not used, so the TLC test comparator used the results of other studies with the same eluent to strengthen the results of identifying asiaticoside compounds in *Centella asiatica* herb extracts.

A phytochemical screening using the Thin Layer Chromatography (TLC) was performed to confirm the presence of asiaticoside in the crude profile extract. The TLC with ethvl acetate:methanol: water (8:2:1 v/v) and anisaldehyde sulfuric acid spray produced purple stains in the presence of terpenoid compounds. The results of this TLC showed 3 spots of the active compound at Rf 0.38; 0.47; and 0.61. The results of this study have similarities to the Rf value in the study (Zainol et al., 2008), namely 0.35 for asiaticoside (bright orange), 0.45 for madecasside acid (dark orange), and 0.59 for asiatic acid (bluish green) (Figure 2a). These results are close to the value of retention time with previous research even though there are differences in the origin of the plants used. Differences in Rf values are also possible because the eluent is not maximally saturated.

The Rf value from the TLC results with the solvent chloroform:methanol: water (65:25:4) with Liebermann-Burchard spray, produced was only detectable for asiaticoside compounds of 0.26 as shown in Figure 2b. Research conducted by Harwoko *et al.* (2014) for Rf asiaticoside of 0.24. Additionally, (Sathiyanarayanan et al., 2010) also reported that Rf 0.26 is asiaticoside. It can be concluded that the herb extract of gotu kola contains asiaticoside compounds.

Physical Characteristic Test of Jelly Candy

As the TLC studies suggested that the extract has an asiaticoside compound, the studies continued with the preparation of gotu kola jelly candy. Jelly candy is made by mixing and dissolving all the ingredients, then cooking for 2 minutes at 90°C. Immediately pour the mixture into the mold and let it rest for 1 hour at room temperature. Remove the jelly candy from the mold and bake for 23 hours at 50° C. The difference between jelly candy and gummy candy lies in the gelling agent used and the gelling temperature. Optimization of the glucomannan and kappa-carrageenan gelling agents aims to evaluate the interaction of the two hydrocolloids. Glucomannan solution is unable to form gels but is capable of forming gels when used with other hydrocolloids (Lin et al., 2024). Meanwhile, the gel formed by kappa-carrageenan is strong, stiff, and has high syneresis (Fateha et al., 2021). The combination of glucomannan and kappa-carrageenan creates а synergistic interaction that can form a more elastic gel with low syneresis (Tunieva et al., 2021). After that, an evaluation of the physical characteristics of the jelly candy was carried out.

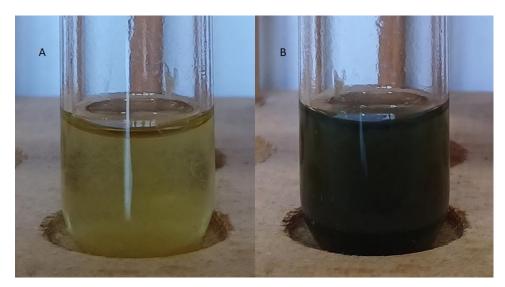


Figure 1. Qualitative analysis of the phenolic compound in the *C. asiatica extract* was performed by adding 1% FeCl₃ solution to the extract. The extract solution before (a) and after (b) FeCl₃ addition

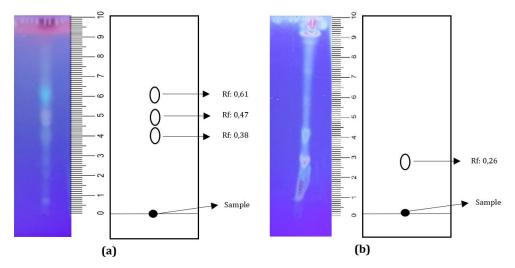


Figure 2. Separation of Centella asiatica ethanol extract using Thin Layer Chromatography. Ethyl acetate:methanol: water (8:2:1 v/v) was employed as a mobile phase. Visualization of the spot was performed by spraying anisaldehyde sulfuric acid (panel a) and Liebermann-Burchard (panel b). Rf of the separation was calculated and compared to the published paper regarding the asiaticoside's Rf with similar separation conditions.

The results of weight uniformity of gotu kola jelly candy from each run are presented in Table II. Jelly candy weight uniformity ranged from 1.27-1.37 g with a range of %CV ranging from 1.551-1.99. The results of the Design Expert 13 software analysis provide a quadratic model with the simplex lattice design (SLD) equation as follows: Y₁ = 1.56A + 1.98B - 0.2731AB. Glucomannan (B) has a greater coefficient compared to kappacarrageenan (A) which suggests that glucomannan has a more dominant influence on weight uniformity (%CV).

Based on the graph of the relationship between the proportions of kappa-carrageenan

and glucomannan to weight uniformity (Figure 3a), an increase in the concentration of glucomannan and less kappa-carrageenan as a gelling agent causes an increase in %CV weight uniformity and vice versa.

Elasticity

Elasticity is the rate or how well the ability of a product that has been deformed to return to its full size and shape. The more elastic the jelly candy, the chewy the texture will be and the easier it will return to its original size and shape. The percent elasticity in this study shows that the greater the elasticity, the less elastic the products.

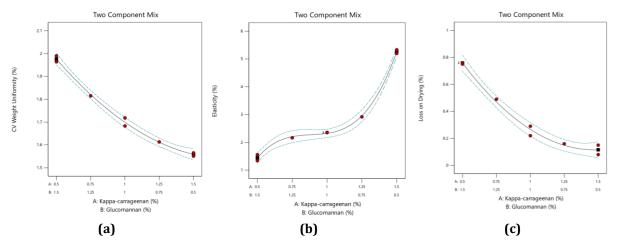


Figure 3. Relationship between kappa carragenan and glucomannan toward some physicochemical characteristics of gotu kola jelly candy in terms of (a) weight uniformity chart; (b) elasticity chart; (c) loss on drying chart. Each data point represents the mean of three replications. Data were analyzed using a simple lattice design.

Table II. Parameter H	Response of Each F	Formula (Run)
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Parameters (%)	R1	R2	R3	R4	R5	R6	R7	R8
Weight uniformity	1.565	1.613	1.683	1.815	1.990	1.551	1.718	1.964
Elasticity	5.199	2.916	2.350	2.163	1.553	5.327	2.354	1.339
Moisture content	0.08	0.16	0.22	0.49	0.76	0.15	0.29	0.75

*R is the running experiment

The results of measuring the elasticity of jelly candy extract of gotu kola herb are presented in Table II. The results of the analysis of the Design Expert 13 program provide a Cubic model for the elastic response with the simplex lattice design (SLD) equation as follows: Y2 = 5.26A + 1.44B -4.11AB - 6.16AB(A-B). Based on the equation obtained, it can be seen that the coefficient of kappa-carrageenan is greater than that of glucomannan so the greater the composition of the kappa-carrageenan it reduced the chewiness. This study is in agreement with (Rusli et al., 2017) who reported that a higher concentration of carrageenan produces a stronger gel matrix and reduced product elasticity.

Loss on Drying (LOD)

LOD data generated in the 8 run formulas produces data with varying percentages and the significance test with ANOVA shows that there is a significant difference between the run formulas in the Design Expert model. The results of the LOD measurement of jelly candy from gotu kola herb extract from each formula are presented in Table II. The LOD of jelly candy ranged from 0.08-0.76%. The results of the Design Expert 13 software analysis provide a quadratic model with the simplex lattice design (SLD) equation as follows: Y3 = 0.1158A + 0.7581B - 0.6808AB. Glucomannan (B) shows a greater coefficient when compared to kappa-carrageenan (A), this indicates that glucomannan has a more dominant influence on the LOD response. Based on Figure 3c, a higher concentration of glucomannan causes an increase in LOD. This is because carrageenan binds water more strongly so the amount of free water decreases (Thommes et al., 2009).

Optimum Formulation

Formula optimization was carried out using Design Expert 13 software. Data from each observational response was processed using the Simplex Lattice Design method. This method is a numerical optimization approach and produces an optimal formula with a composition of kappacarrageenan (1.33%) and glucomannan (0.67%) with a desirability value of 0.943. The desirability value is a value that indicates the achievement of a model used against the desired target.

Optimal Formula Verification

The LOD value is not significantly different from the predicted value, this is shown through a one-sample t-test with significancies more than 0.05 (Table III). The LOD value of jelly candy products meets the requirements, namely

Response	Predictive Value	Result Value	Significance
Weight uniformity	1.591	1.587	Not significant
Loss on Drying	0.136	0.18	Not significant
Elasticity	3.450	3.495	Not significant

Table III. Test Response Value and Predictive Value

<10%.LOD indicates the content of water and volatile matter in the product. The higher the LOD, the faster the shelf life of the product.

The advantage of jelly candy in this study is the texture which melts more easily in the mouth compared to gummy candy derived from animal hydrocolloids. The use of honey in this study aims to replace the high fructose syrup sweetener commonly used in making jelly candy. HFS can trigger excess weight. The elasticity of gotu kola extract jelly candy was not significantly different from commercial products. This can be seen from the one sample t-test with sig. 0.812.

Evaluation of the Antioxidant Activity

From the optimum formula, the antioxidant activity of Centella asiatica jelly candy was compared to Centella asiatica extract. As shown in Figure 4, Centella asiatica has moderate antioxidant activity with IC50 fo $129,32 \pm 2,08$ ppm. This activity is significantly different from ascorbic acid which showed IC50 of $3,60 \pm 0.01$ ppm. Based on the research of Yahya and Nurrosyidah (2020) stated that the ethanol extract of Centella asiatica herb has an IC50 of 78.20 ppm. Meanwhile, Djoko et al. research. (2022) stated that the IC50 of the ethanol extract of Centella asiatica herb was 76.66 ppm. The difference in the results of the IC50 value with this research is possible due to differences in the drying process of dried Simplicia, harvesting, and the heating temperature used.

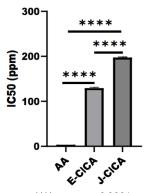
Evaluation of the gotu kola jelly candy antioxidant activities showed that it is known that there was a decrease in the IC50 value to $197,47 \pm$ 1,24 ppm. Evaluation on the blank jelly candy which does not contain Centella asiatica exhibited IC50 of 3.960,25 ± 17,35ppm. This value suggested that the excipient does not exhibit antioxidant activity and the antioxidant activity in the gotu kola gummy candy solely came from the Centella asiatica extract. The decrease in the IC50 value might be due to the extract evaporation process and heating process. Heat and the presence of water have been reported to accelerate the oxidation of antioxidant activity. The decrease in the IC50 value is affected by the heating temperature which causes the degradation of antioxidants thereby accelerating the oxidation of antioxidant activity (Ameliya et al., 2018). The process of oxidation of antioxidants can be accelerated due to the heat of the oxidizing agent and additional catalysts (iron). Oxidized antioxidant compounds will undergo structural changes.

DISCUSSIONS

In this study, a plant-based jelly candy containing C.asiatica was developed as an alternate option for gelatin as a gelling agent for jelly candy. The main findings in this study are: 1) The optimal composition of plant-based gelling agents tested are kappa-carrageenan (1.33%) and glucomannan (0.67%) 2) The antioxidant activity of the C.asiatica is reduced after the gummy preparation process. 3) The more kappa carrageenan added, the smaller the chewiness of the jelly candy. 4) Adding more glucomannan will increase the moisture content of the gummy.

Researchers have been interested in vegan gummy candy because of the increasing number of people who are claiming to be vegan (Sexton et al., 2022). Veganism has grown in developed countries where people also adhere to healthy lifestyles. Evidence has shown that vegetarian diets may improve cardiovascular performance and lipid profile. In addition, a vegan diet also normalizes body mass index and reduces the risk of diabetes and cardiovascular diseases (Selinger et al., 2023)In these countries, taking supplements is relatively common.. This phenomenon creates a new sector in pharmaceutical industries to develop a vegan-friendly health supplement. Jelly candy is one of the examples of supplement formulations to maintain health (Habilla et al., 2011; Utomo et al., 2014).

A common gelling component in jelly candies is gelatin, a byproduct of collagen obtained from a variety of sources, such as pigskin, goatskin, and cow skins (Alipal et al., 2019). Gelatin has several beneficial qualities, such as its consistency, and gel-forming ability, however, it is become less popular due to the growing demand for kosher, vegan, and halal food products. Consequently, studies on gummy and jelly candy have explored gelatin substitutes and nonanimal hydrocolloids, including exocarp of jaban watermelon, pectin, agar, starch, carrageenan, and Arabic gum (Ghiraldi et al., 2021; Rashmi & Mona, 2023; Formulation and Antioxidant Activity of Gotu Kola Jelly Candy



Antioxidant Activity

Figure 4. Evaluation of the antioxidant activity of the ascorbic acid (AA) as a positive control, a *Centella asiatica* extract (E-CICA), and a jelly candy of *Centella asiatica*. Antioxidant activity was evaluated using DPPH assay. Each data represents the mean of three replications. Data were analyzed using one-way ANOVA followed by the LSD Fisher test.

Tarahi et al., 2023) In this study we add information on the alternate on vegan-friendly gelling agent that can be used for jelly candy particularly combination of kappa carrageenan and glucomanan. The current studies suggested that a combination of 1.33% kappa-carrageenan and 0.67% glucomannan produces jelly candy with acceptable physical characteristics. However, the jelly candy exhibits a slight reduction in antioxidant activity compared to the crude extract.

Regarding to the decline in antioxidant activity, there are several approaches to maintaining the antioxidant activity of the herbal product among others by regulating the water content of the product as well as minimizing the heat contact during the manufacturing process. This is supported by the research (Suzery et al., 2020) that increasing temperature and heating time causes the degradation of antioxidant compounds so that their antioxidant activity decreases. Heating is also able to increase the reduction of power (Jeong et al., 2004). However, Ioannou et al., (2020) stated that the antioxidant activity associated with heat and light treatment could remain constant, decrease, or increase. This is due to the presence of degradation products that can support or reduce antioxidant activity. Therefore, the heating process in this study can be minimized at the jelly candy cooking stage.

CONCLUSION

In summary, the combination of 1.33% kappa-carrageenan and 0.67% glucomannan gave the optimum response for gotu kola jelly candy. Plant-based polymers as gelling agents in gotu kola jelly candy exhibited comparable elasticity compared to references products gotu kola extract

has medium antioxidant activity. Formulation of gotu kola to jelly candy with the current protocol, increased the IC50 of antioxidant activity by 34%. These studies suggested that exploration of the manufacturing process is imperative to maintain the antioxidant activity of gotu kola jelly candy.

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REFERENCES

- Ameliya, R., . N., & Handito, D. (2018). The effect of boiling time on vitamin C: Antioxidant activity and sensory properties of Singapore cherry (*Muntingia calabura* L.) syrup. *Pro Food*, 4(1), 289–297. https://doi.org/10.29303/profood.v4i1.77
- Aryal, B., Adhikari, B., Aryal, N., Bhattarai, B. R., Khadayat, K., & Parajuli, N. (2021). LC-HRMS profiling and antidiabetic, antioxidant, and antibacterial activities of *Acacia catechu* (L.f.) Willd. *BioMed Research International*, 2021, 7588711. https://doi.org/10.1155/2021/7588711
- De Torre, M. P., Cavero, R. Y., Calvo, M. I., & Vizmanos, J. L. (2019). A Simple and reliable method to quantify antioxidant activity in vivo. *Antioxidants, 8*(5), 142. https://doi.org/10.3390/antiox8050142
- Davydova, N. (2018) USP Chewable gels monographs. USP Dietary Supplements Stakeholder Forum, May 15, 2018. pp. 1-20.

^{****} means p<0.0001

- Fateha, Ulya, N., Asmanah, & Agusman. (2021). Comparison of gel preparation methods on gel strength measurement of carrageenan. *IOP Conference Series: Earth and Environmental Science*, 715(1), 012055. https://doi.org/10.1088/1755-1315/715/1/012055
- Gomez, L. A. (2021). Growth evaluation and proximate analysis of gotu kola (Centella asiatica L.) in response to organic growing media combinations. Asian Journal of Fundamental and Applied Sciences, 2(2).
- Gray, N. E., Alcazar Magana, A., Lak, P., Wright, K. M., Quinn, J., Stevens, J. F., Maier, C. S., & Soumyanath, A. (2018). Centella asiatica: Phytochemistry and mechanisms of neuroprotection and cognitive enhancement. *Phytochemistry Reviews*, *17*(1), 161–194. https://doi.org/10.1007/s11101-017-9528-y
- Hannan, Md. A., Haque, M. N., Munni, Y. A., Oktaviani, D. F., Timalsina, B., Dash, R., Afrin, T., & Moon, I. S. (2021). Centella asiatica promotes early differentiation, axodendritic maturation, and synaptic formation in primary hippocampal neurons. *Neurochemistry International*, 144, 104957. https://doi.org/10.1016/j.neuint.2021.104 957
- Harwoko, Pramono, S., Nugroho, A. E. (2014). Triterpenoid-rich fraction of *Centella asiatica* leaves and in vivo antihypertensive activity. *International Food Research Journal*, 21(1), 149-154.
- Ioannou, I., Chekir, L., & Ghoul, M. (2020). Effect of heat treatment and light exposure on the antioxidant activity of flavonoids. *Processes*, *8*(9), 1078. https://doi.org/10.3390/pr8091078
- Jamil, S. S., Nizami, Q., & Salam, M. (2007). Centella asiatica (Linn.) urban: A review. *Natural Product Radiance*, 6(2).
- Jaradat, N., Hussen, F., & Ali, A. Al. (2015). Preliminary phytochemical screening, quantitative estimation of total flavonoids, total phenols, and antioxidant activity of Ephedra alata decne. *Journal of Materials and Environmental Science*, 6(6), 1771– 1778.
- Jeong, S.-M., Kim, S.-Y., Kim, D.-R., Jo, S.-C., Nam, K. C., Ahn, D. U., & Lee, S.-C. (2004). Effect of heat treatment on the antioxidant activity of extracts from citrus peels. *Journal of Agricultural and Food Chemistry*, *52*(11), 3389–

3393..https://doi.org/10.1021/jf049899k

Lin, Y., Zhang, L., Li, X., Zhai, C., Liu, J., & Zhang, R. (2024). Effect and characterization of konjac glucomannan on xanthan gum/κcarrageenan/agar system. *International Journal of Biological Macromolecules*, 257, 128639. https://doi.org/10.1016/j.jibiomac.2023.1

https://doi.org/10.1016/j.ijbiomac.2023.1 28639

- Long, H. S., Stander, M. A., & Van Wyk, B.-E. (2012). Notes on the occurrence and significance of triterpenoids (asiaticoside and related compounds) and caffeoylquinic acids in Centella species. *South African Journal of Botany,* 82, 53–59. https://doi.org/10.1016/j.sajb.2012.07.01 7
- Mohammad Azmin, S. N. H., & Mat Nor, M. S. (2020). Chemical fingerprint of Centella Asiatica's bioactive compounds in the ethanolic and aqueous extracts. *Advances in Biomarker Sciences and Technology*, *2*, 35–44. https://doi.org/10.1016/j.abst.2020.10.00
- Pindan, N. P., Saleh, C., & Magdaleni, A. R. (2021). Phytochemical test and antioxidant activity test of n-hexane fraction extract, ethyl acetate, and remained ethanol from the leaf of sungkai. Jurnal Atomik, 6, 22-27.
- Pittella, F., Dutra, R. C., Junior, D. D., Lopes, M. T. P., & Barbosa, N. R. (2009). Antioxidant and cytotoxic activities of Centella asiatica (L) Urb. International Journal of Molecular Sciences, 10(9), 3713–3721. https://doi.org/10.3390/ijms10093713
- Pramono, S., & Ajiastuti, D. (2004). Standardization of *Centella asiatica* (L.) urban herbal extract based on asiaticoside concentration by TLCdensitometry. *Majalah Farmasi Indonesia*, 15, 118-123.
- Rao, S., Chetana, M., & Umadevi, P. (2005). Centella asiatica treatment during postnatal period enhances learning and memory in mice. *Physiology & Behavior*, 86(4), 449–457. https://doi.org/10.1016/j.physbeh.2005.0 7.019
- Rusli, A., Metusalach, M., & Tahir, M. M. (2017). Characterization of carrageenan edible films plasticized with glycerol. *Jurnal Pengolahan Hasil Perikanan Indonesia*, *20*(2), 219. https://doi.org/10.17844/jphpi.v20i2.174 99
- Sathiyanarayanan, L., Paradkar, A. R., & Mahadik, K. R. (2010). Development and validation of a densitometric HPTLC method for simultaneous analysis of wedelolactone and asiaticoside in a polyherbal formulation. Acta Chromatographica, 22(4), 651–663.

https://doi.org/10.1556/AChrom.22.2010. 4.13

- Subathra, M., Shila, S., Devi, M. A., & Panneerselvam, C. (2005). Emerging role of Centella asiatica in improving age-related neurological antioxidant status. *Experimental Gerontology*, 40(8–9), 707–715. https://doi.org/10.1016/j.exger.2005.06.0 01
- Sunil, S., Sharma, D. U. K., & Arathy, S. A. (2020). Pharmaceutical jellies: A novel way of drug delivery. *J. Pharm. Sci.*, *12*. 322-327
- Susiloningrum, D., & Mugita Sari, D. E. (2021). Uji aktivitas antioksidan dan penetapan kadar flavonoid total ekstrak temu mangga (Curcuma mangga valeton & zijp) dengan variasi konsentrasi pelarut (Antioxidant activity test and determination of total flavonoid content of *Curcuma manga* valeton & zijp) extract with variation of solvent concentration). *Cendekia Journal of Pharmacy*, 5(2), 117–127. https://doi.org/10.31596/cjp.v5i2.148
- Suzery, M., Nudin, B., Nurwahyu Bima, D., & Cahyono, B. (2020). Effects of temperature and heating time on degradation and antioxidant activity of anthocyanin from Roselle petals (Hibiscus sabdariffa L.). *International Journal of Science, Technology* & Management, 1(4), 288–238. https://doi.org/10.46729/ijstm.v1i4.78
- Thommes, M., Baert, L., Van 'T Klooster, G., Geldof, M., Schueller, L., Rosier, J., & Kleinebudde, P. (2009). Improved bioavailability of darunavir by use of κ-carrageenan versus microcrystalline cellulose as pelletization aid. *European Journal of Pharmaceutics and Biopharmaceutics*, 72(3), 614–620. https://doi.org/10.1016/j.ejpb.2009.03.00 4
- Tunieva, E. K., Spiridonov, K. I., & Nasonova, V. V. (2021). A study on the synergetic interaction of kappa-carrageenan with konjac gum. *IOP Conference Series: Earth and Environmental Science*, 640(5), 052012. https://doi.org/10.1088/1755-1315/640/5/052012
- Umka Welbat, J., Sirichoat, A., Chaijaroonkhanarak, W., Prachaney, P., Pannangrong, W., Pakdeechote, P., Sripanidkulchai, B., & Wigmore, P. (2016). Asiatic acid prevents

the deleterious effects of valproic acid on cognition and hippocampal cell proliferation and survival. *Nutrients*, *8*(5), 303. https://doi.org/10.3390/nu8050303

- Uttara, B., Singh, A., Zamboni, P., & Mahajan, R. (2009). Oxidative stress and neurodegenerative diseases: A review of upstream and downstream antioxidant therapeutic options. *Current Neuropharmacology*, 7(1), 65–74. https://doi.org/10.2174/1570159097876 02823
- Wong, J. H., Barron, A. M., & Abdullah, J. M. (2021). Mitoprotective effects of Centella asiatica (L.) Urb.: Anti-inflammatory and neuroprotective opportunities in neurodegenerative disease. *Frontiers in Pharmacology*, 12, 687935. https://doi.org/10.3389/fphar.2021.68793 5
- Xu, M., Xiong, Y., Liu, J., Qian, J., Zhu, L., & Gao, J. (2012). Asiatic acid, a pentacyclic triterpene in Centella asiatica, attenuates glutamateinduced cognitive deficits in mice and apoptosis in SH-SY5Y cells. Acta Pharmacologica Sinica, 33(5), 578–587. https://doi.org/10.1038/aps.2012.3
- Yuliani, S., & Linar, N. (2019). Effect of gotu kola (Centella asiatica) extract toward expression of caspase 3 of hippocampus pyramidal cells on dementia model rats induced by trimethyltin. *Proceedings of the* Dahlan 2019 Ahmad International Conference Series on Pharmacy and Health Science (ADICS-PHS 2019). Yogyakarta, Indonesia. https://doi.org/10.2991/adicsphs-19.2019.12
- Zainol, N. A., Voo, S. C., Sarmidi, M. R., & Aziz, R. A. (2008). Profiling of Centella asiatica (L.) urban extract. *Malaysian Journal of Analytical Sciences*, *12*(2). 322-327
- Zin, Z. M., Sarbon, N. M., Zainol, M. K., Jaafar, S. N., Shukri, M. M., & Rahman, A. H. Ab. (2021). Halal and non-halal gelatine as a potential animal by-products in food systems: Prospects and challenges for Muslim community: *First International Conference* on Science, Technology, Engineering and Industrial Revolution (ICSTEIR 2020), Bandung, Indonesia. https://doi.org/10.2991/assehr.k.210312. 086