

Development of gotu kola (*Centella asiatica* (L.) Urban) gummy candy and its evaluation on antioxidant activity

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ABSTRACT: Repetitive free radical exposure triggers the development of neurodegenerative disease. Currently, there is an increasing trend where neurodegeneration symptoms occur among younger individuals. This leads to the exploration of brain supplements that is suitable for all generation. Gotu kola (*Centella asiatica* (L.) Urban) extract has been used as a food supplement to neutralize free radicals and improve blood microcirculation in the brain. This supplement has been sold in the form of dried or liquid extract which is less practical. This study aimed to develop a gotu kola supplement in the form of gummy candy and evaluate its physical characteristics and antioxidant activity. *Centella asiatica* herb extract was prepared by maceration method using 70% ethanol. The extract was evaluated for its quercetin content using reverse-phase ultra-performance liquid chromatography (RP-UPLC). The antioxidant activity was examined using the DPPH reduction method. The gelling properties of beef gelatin and pectin were optimized in the studies. The physicochemical characteristics of the gummy candy were evaluated. Responses for each evaluation were analyzed and optimized using the simplex lattice design (SLD) method using software Design Expert version 13. The studies using RP-UPLC confirmed the presence of quercetin in the extract. The optimum formula was obtained with the proportion of bovine gelatin and pectin is 9.09 g (12.1%) and 0.91 g (1.22%), respectively. The physicochemical parameter of the optimum formula showed no significant difference between the predicted value and the experimental results for all parameters. After being formulated into gummy candy, the antioxidant activity (IC₅₀) of gotu kola is reduced from 129.63±1.9 µg/mL in the extract to 166.50±1.5 µg/mL in the gummy. These studies suggested that the optimum formula of gummy candy has been identified while in the same time efforts to prevent the reduction of gotu kola antioxidant activity need to be explored.

KEYWORDS: *Centella asiatica*, quercetin, gummy candy, antioxidant, gelling agent

1. INTRODUCTION

The increased incidence of chronic and degenerative diseases (non-communicable diseases), such as cancer, diabetes, cardiovascular disease, and Alzheimer's in many countries is largely due to lifestyle as smoking, alcohol drinking, obesity, stress, lack of physical activities, and persistent exposure to free radicals [1-3]. Consuming food supplements is one strategy to counteract the free radicals in the body. Gotu kola or *Centella asiatica* (L.) Urban is a popular medicinal plant used to maintain brain health. This plant belongs to the Apiaceae family and is commonly found in humid areas [4]. Studies reported that the most common bioactive compounds found in gotu kola are pentacyclic triterpenoid compounds called centelloids, such as madecassic acid, madecassoside, asiatic acid, and asiaticoside. In addition to centelloids, quercetin is known as the other major metabolite in gotu kola [5].

Gotu kola exhibits several pharmacological activities, such as antimicrobial [5], analgesic and anti-inflammatory [6], antidiarrheal [7], wound healing [8], and antioxidant [9,10]. A study by Rao et al [11] suggests that the gotu kola neuroprotective properties are associated with its antioxidant activity. Gotu kola is also shown to increase intelligence and memory and to improve the viability of brain cells [12].

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Due to its activity as neuroprotective and neurotropic [13–15], gotu kola is also known as a brain tonic [4]. Currently, gotu kola is widely available as a nutraceutical in the form of capsules and powder, which is less acceptable for younger ages. Pharmaceutical technology allows pharmaceutical scientists to formulate herbal medicine in more attractive products and is suitable for a wider range of consumer age. Therefore, this study is aimed to create an alternate product for gotu kola in the form of gummy candy. In this study, the effect of various combinations of bovine gelatin and pectin as gelling agents on physical characteristics is evaluated. In addition, the study also determined the optimum formula for gummy candy and evaluated the antioxidant activity of gotu kola before and after formulation. Gotu kola possesses many phytoconstituents and quercetin is one of them having antioxidant activity. Formulation of gotu kola supplements into gummy candy might increase the acceptance of consumers not only adults but also teenagers. This innovation will create a bigger opportunity and potential market for the gotu kola.

2. RESULTS AND DISCUSSION

2.1. Extraction of gotu kola extract

Plant determination performed by a botanical expert from the Pharmaceutical Biology Laboratory Gadjah Mada University suggested that the herb is truly *Centella asiatica* (L.) Urban) of the Apiaceae family. The extraction process yielded 22.73% of the crude extract. This value agrees with Indonesian Herbal Pharmacopoeia II which states that gotu kola crude extract should not be less than 7.3%. The extract *loss of drying* was $0.38 \pm 0.09\%$ and the spreadability value of 87.21 ± 0.38 mm.

2.2. Confirmation of quercetin compound in *Centella asiatica* extract

The qualitative analysis of quercetin in *C. asiatica* extract was determined using RP-UHPLC chromatography. The retention time of the quercetin standard was 4.833 min. Gotu kola extract also exhibited a peak on this retention time suggesting the presence of quercetin in the extract (Figure 1). This result agrees with the previous study that quercetin is one of the flavonoids in *Centella asiatica*. Quercetin has been reported to contribute to antioxidant activity [9]. This compound regulates glutathione levels in the body to manage free radicals and the hydroxyl group of quercetin binds to the active site of enzymes that are related to oxidative stress.

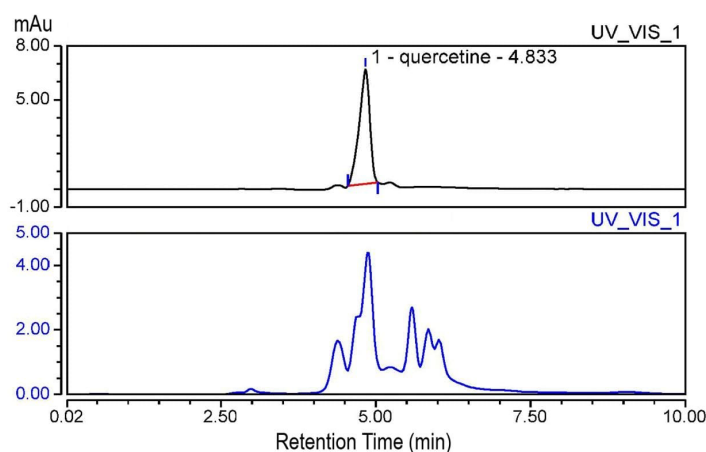


Figure 1. HPLC chromatogram of standard Quercetin (upper panel) and *C. asiatica* extract (lower panel). *Centella asiatica* at the concentration of 0.5 mg/mL is subjected to a 0.22 μm filter. The samples were separated using an RP-UHPLC instrument with the elution solvent MeOH:water (85:15) that contained 1% acetic acid. The stationary phase used was C-18 Thermo Hypersil Gold column (250x4.6mm, particle size 5 μm)

2.3. Formula Optimization for Gotu Kola Gummy Candy

In this study, the proportion of bovine gelatine and pectin was optimized using SLD and Design Expert software. Eight runs were prepared and tested for their physical properties. This included an organoleptic test, weight uniformity test, pH test, *loss of drying* test, and elasticity test. The results of organoleptic testing of gummy candy preparations of *Centella asiatica* herb extract had a dark-green color, a distinctive aroma of the extract, a heart shape, and a chewy texture (Figure 2).

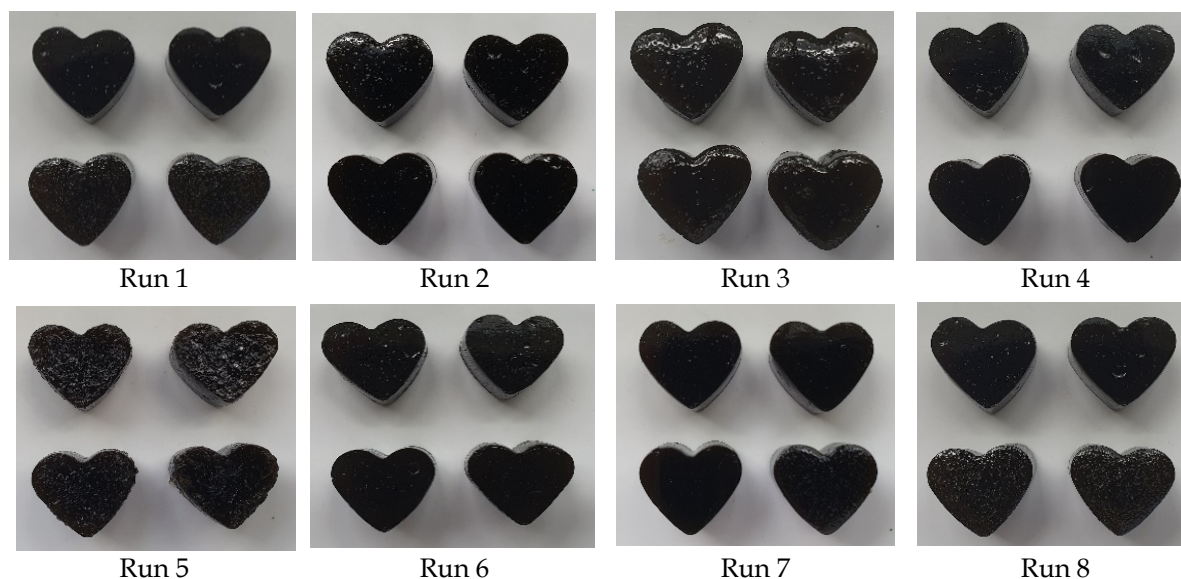


Figure 2. Gummy candy gotu kola herb extract. The gummy candies were prepared using bovine gelatine and pectin as a gelling agent. Sucrose and multiflora honey were used as a sweetener. Green color and melon essence were used for this gummy candy formula.

2.4. The Physical Properties of Gummy Candy Extract of *Centella asiatica*

The data on weight uniformity, *loss of drying*, and elasticity were used to determine the optimum formula. The correlation between the formula (run) and the physical properties is depicted in Table 1.

Table 1. The physical properties of gummy candy gotu kola herb extract

Run	Weight Uniformity	pH	Loss of drying	Elasticity
	CV (%)		X + SD (%)	X + SD (%)
1	2.02	4.60	7.79 ± 0.68	7.40 ± 0.18
2	1.77	4.60	3.91 ± 0.49	1.90 ± 0.22
3	2.04	4.60	7.92 ± 0.28	7.03 ± 0.41
4	1.76	4.80	3.34 ± 0.16	1.45 ± 0.22
5	1.84	4.60	6.39 ± 0.76	4.30 ± 0.24
6	1.82	4.60	4.51 ± 0.62	2.45 ± 0.22
7	1.77	4.80	2.25 ± 0.33	1.00 ± 0.25
8	1.70	4.80	2.16 ± 0.70	1.12 ± 0.45

The weight uniformity test was performed to evaluate if gummies produced on each run had uniform weights. Based on US Pharmacopeia on Chewable Gels Monograph in 2018, gummy candy can be identified as uniform in terms of weight if the weight deviation is less than 7.5% from the average weight of gummy candy. If there is one gummy that is outside the limit, the procedure can be repeated with the addition of 20 gummy candies. Gummy candy meets the requirements if there is no gummy with an average weight of more than 10%. The results of the gummy candy weight uniformity test for each run are shown in Figure 3a. All gummy candy in each run met the appropriate requirements in the USP Chewable Gels Monograph namely that there was not a single preparation whose weight deviated greater than 7.5% and the CV value <5%.

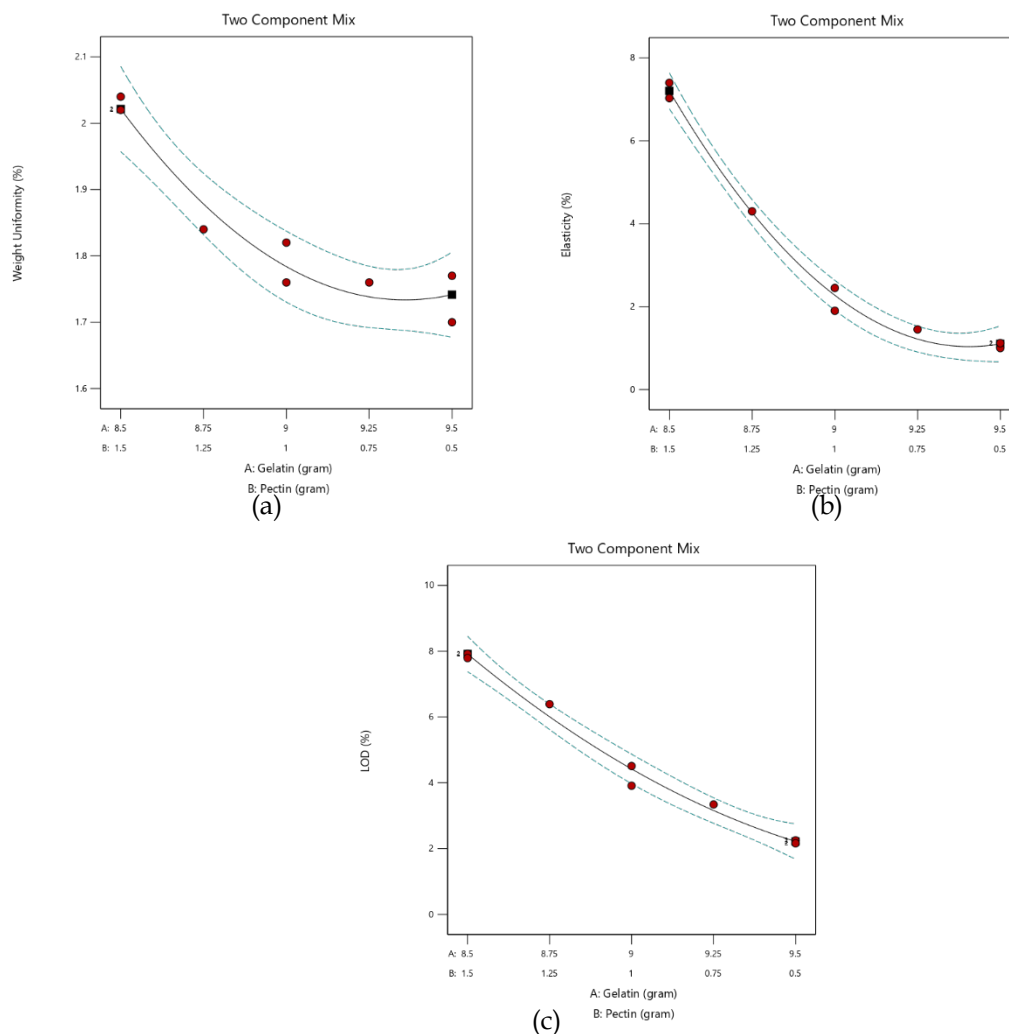


Figure 3. The relationship between bovine gelatin and pectin proportion toward (a) weight uniformity (b) elasticity (c) loss of drying (LOD) response. Bovine gelatine was used in the range of 8.5-9.5 gram/ 74.8 gram batch size. Pectin was used in the range of 0.5-1.5 grams for the 74.8-gram batch size. Graphs were prepared using Design Expert Ver 13.

Measuring the gummies' pH was aimed to support the preservatives used in this formula. In this study, the preservative used was sodium benzoate. Sodium benzoate has antimicrobial activity which can inhibit the growth of fungi in the product. However, sodium benzoate requires a pH range of 2-5 to perform its preservative activity [16-17]. Thus, pH identification is an important part of formulating gummy candy. The effect of the gelling agent on the each of runs is shown in Figure 3b. All runs exhibited a pH between 4.6 – 4.8 (Figure 3). This value falls within the range of sodium benzoate pH requirements for acting as a preservative.

Based on SNI 3547-2-2008 elasticity parameter is an important feature of gummy candy and is related to consumer acceptance. In this study, % elasticity is described by measuring the size before and after being given with particular weight. The smaller the % elasticity value indicates that the gummy candy preparation has a good elasticity which means that after being burdened by a particular strength, it can return to its original shape. The impact of the gelling agent proportion to the elasticity value is depicted in Figure 3c. Gotu kola gummy candies exhibited elasticity from 1 – 7% with the highest elasticity found in run 7 of 1.00% and the lowest elasticity found in run 1 of 7.40% (Figure 3c). The studies suggested that the higher gelatin proportion resulted in a better elasticity parameter. The reference product used in this study is Youvit® multivitamin with an elasticity value of 1.78%.

The LOD test describes the loss of water content in gummy candy preparations of *Centella asiatica* extract. LOD can provide a maximum limit on the amount of compound lost during the drying process. Water is an important component in a product because it can affect the appearance, texture, and taste of a

product [16]. The purpose of conducting the LOD test is to determine the residual water after the thickening or drying process. The LOD represents the evaporating water content, so the requirement for a good LOD is <10%. The results of the LOD test listed in Table VII show that the highest drying shrinkage is found in run 3 of 7.92%. This shows that the amount of water content and compounds lost during the drying process is 7.92%. The smaller the gelatin concentration, the greater the water content and missing compounds, meaning that the water content in the gummy candy preparation is also large [18].

2.5. Optimum Gummy Candy Formula with *Centella asiatica* Herb Extract

Optimization of the gummy candy formula for gotu kola extract was performed by analyzing the physical properties of the eight runs as a response to the Design Expert software version 13. The physical properties included were *loss of drying*, weight uniformity, and elasticity which were measured on the second day after removal from the mold. pH did not give significantly different results for each formula, so the pH was not included as a response to determine the optimum formula. The software showed that the optimum formula ideally contains 9,09 g of gelatin (12.1%) and 0.91 g of pectin (1.22%) for the batch size of 78.4 grams. The desirability value of the optimum formula was 0.870. The predicted value of each response offered by the software is 3.92% LOD, 1.76% weight uniformity, and 1.78% elasticity. The preparation of the optimum formula was conducted based on the software prediction. The gummy candies were further tested for elasticity, weight uniformity, and loss of drying and analyzed using one sample t-test. The statistical analysis suggested that there is no significant difference between predicted value and experimental data (Table 2)

Table 2. Comparison of the response of optimum formula between the predictive value and experiment

Response	Prediction	Experiment	Sig. (2-tailed)
Elasticity (%)	1.78	1.97	0.241
Weight Uniformity (%)	1.76	1.73	0.537
LOD (%)	3.92	4.38	0.936

2.6. Antioxidant activity of gotu kola extract and gummy candy

Gotu kola gummy candy in this study was evaluated for its antioxidant activity as previously mentioned the neuroprotective activities of gotu kola supplement are associated with the antioxidant properties of the herbs. Thus, the antioxidant activity of gotu kola was evaluated before and post being formulated into gummy candy. In addition, the activity was compared to ascorbic acid as a positive control. The IC₅₀ value of ascorbic acid is 3.60 µg/mL which suggests a very strong antioxidant category while *Centella asiatica* crude extract showed an IC₅₀ value of 129.63 µg/mL which suggests a moderate antioxidant category (Figure 4). The moderate antioxidant activity of *Centella asiatica* crude extract is influenced by several factors among others: the drying process with a particular temperature (50°C), the extraction process including the drying method, and the end point of the extraction.

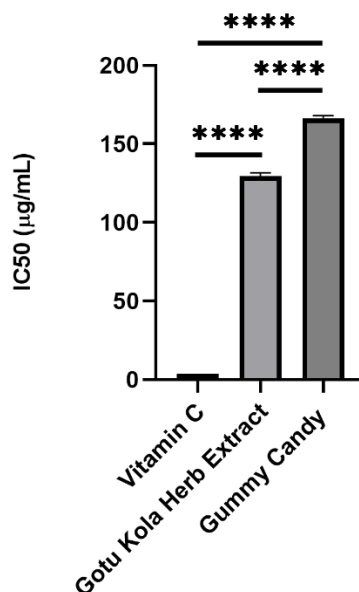


Figure 4. Graph of IC₅₀ comparison of vitamin C as a positive control, gotu kola (*Centella asiatica*) herb extract, and gotu kola gummy candy. The amount of sample examined was 25 mg. The gummy candy sample was calculated based on the amount of gummy candy that contained 25 mg of gotu kola extract. The antioxidant activity was evaluated using DPPH assay. N:3. 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 and *** represent p<0.001

Optimum gotu kola gummy candy showed an antioxidant IC₅₀ value of 166.50 µg/mL. Based on Molyneux [18], the IC₅₀ value of the gummy candy extract of *Centella asiatica* herb is included in the category of weak antioxidant. The decrease in the antioxidant value between gotu kola herb extract and gummy candy formulation might be a consequence of several factors, including the addition of excipients, the manufacturing process, temperature, and treatment which caused the antioxidant value to decrease.

In this study, an antioxidant activity test was also carried out on gummy candy preparations without gotu kola extract which served as a negative control. After testing, the IC₅₀ value in the negative control cannot be calculated because when reading the absorbance and calculating the IC₅₀ value there is no replication data from different concentration series whose value is above 50. Even though in calculating the IC₅₀ it must meet the requirements, namely having values above and below 50 It can be concluded that the negative control in the form of gummy candy without extract has no antioxidant activity or is said to be very weak.

The statistical analysis of antioxidant data suggested that gotu kola antioxidant activity in gummy candy is significantly reduced compared to those in crude extract before the production process. The studies on excipient also suggested that excipient is less likely to contribute to gummy candy antioxidant activity although honey has been added to the formula. Studies suggest that honey has some antioxidant activity [18]. However, this study did not improve the antioxidant activity of the gotu kola gummy candy. This might be because the amount of honey added is limited.

The reduction of antioxidant activity in the gummy product suggested that extract processing to a product will more likely affect the antioxidant activity. Thus, it is necessary to optimize the manufacturing process by reducing the process temperature as well as the *loss of drying*. In addition to that, it is imperative to create an alternative dosage form that can produce minimal water content which results in less reduction in antioxidant activity.

This current research poses a novelty in terms of creating a gotu kola gummy by providing information on the antioxidant activity of gotu kola in gummy candy that has never been reported previously. In this study, the antioxidant activity of the gotu kola gummy candy needs to be optimized for example by increasing the extract proportion in the formula and improving the production process to shorten the time as well as contact with heat and water.

The current studies also introduced honey as a sweetener. Approximately 28% of the gummy candy recipe was multiflora honey to substitute some part of the sucrose in the formula. The introduction of honey as a sweetener in these studies was due to its role as a natural sweetener that has antioxidant properties [19-20]. Honey contains various compounds, such as peptides, phenolics, organic acids, and enzymes. Honey is also reported as an alternative medicine for gastrointestinal, cardiovascular, and inflammation. In addition, it functions as an anti-cancer, anti-diabetic, and weight-regulating agent [18]. Although the data provide good physical characteristics, in future studies it is expected that honey can substitute the sweetener in the formula as compared to 28% in the current studies. With the addition of more honey, it is expected that the blank gummy candy itself already possesses an antioxidant activity compared to those that use sucrose as a sweetener.

3. CONCLUSION

In summary, the optimum gummy candy formula for gotu kola herb extract was obtained at the proportion of 12.1% bovine gelatin and 1.22% pectin. The optimum formula obtained has an elastic response, weight uniformity, and *loss of drying* that are not significantly different between the predicted value and the experiment. The antioxidant activity of gotu kola gummy candy suggested there is a reduction in antioxidant activity of gotu kola post formulation in gummy candy compared to those of crude extract. These studies suggested that improvement of the manufacturing process is required to prevent the loss of antioxidant activity during manufacturing.

4. MATERIALS AND METHODS

Materials: *Centella asiatica* extract, vitamin C (ascorbic acid, pharmaceutical grade, *Sigma-Aldrich*), ethanol (pro analysis), DPPH (2,2-Diphenyl-1-Picrylhydrazyl) (Smart Lab), distilled water (Progo Mulyo Inc.), yellow tip, blue tip, vortex, sonicator, centrifuge, 70% technical ethanol (pharmaceutical grade), gelatin (Gelita NZ LTD, type B with bloom 250, pharmaceutical grade), pectin (Adimitra Karunia Inc, pharmaceutical grade), citric acid (Sirsate Inc), sucrose (Point Inc), multiflora pure honey (Natura Alamindo Utama Inc.), melon essence (Gunacipta Multirasa Inc), light green dye (Gunacipta Multirasa Inc), sodium benzoate (Gunacipta Multirasa Inc), and distilled water (pharmaceutical grade). For RP-UPLC, the mobile phase methanol from Lichrosolv (Supelco) and water for injection (PT. Sampharindo Putramase) were used.

4.1. Preparation of *Centella asiatica* Extract

Centella asiatica simplicia was obtained from Omah Djamoé Arroyan Tasikmadu, Karanganyar, Central Java, Indonesia. Plant determination was performed at the Faculty of Pharmacy, Gadjah Mada University.

Before extraction, gotu kola was re-dried in the oven at 50°C for 2 hours and then powdered using a pollinator machine. Nine hundred grams of gotu kola herb was macerated using 6.3 L of ethanol 70% for two days. The macerate was then filtered and vacuumed using a Buchner funnel. The residue was re-extracted using 2.7 L of ethanol 70%. After all the macerates were filtered and vacuumed, the solvent was removed by heating the macerate over the pan installed inside the water bath with occasional stirring. The extract obtained was stored in the refrigerator until it was used.

4.2. The Qualitative analysis of quercetin using Reverse Phase Ultra-High-Performance Liquid Chromatography (RP-UHPLC)

Quercetin is one of the flavonoids that are present in many fruits, flowers, and leaves including *Centella asiatica*. To analyze quercetin in the *Centella asiatica* extract, RP-UPLC (Thermo Scientific) was used according to the protocol reported previously [21]. Briefly, to prepare a stock solution of 10 mg extract was dissolved in 10 ml of MeOH (Mass Spectrophotometer grade) resulting in 1mg/ml stock solution. The extract solution has then been diluted to 0.5 mg/mL, and subjected to a 0.22 µm filter. The RP-UHPLC instrument was set up as follows: the elution solvent used was MeOH (HPLC grade), and water (85:15) that contained 1% acetic acid. The injection volume was 10µL at the flow rate of 0.7 mL/min. Sample separation was analyzed using a UV-Vis detector at 370 nm. The stationary phase in this RP-UPLC was a C-18 Thermo Hypersil Gold column (250x4.6mm, particle size 5 µm). The separation was done using the isocratic system for 12 min with mobile phase MeOH: water (85:15) containing 1% acetic acid. Further, the chromatogram was analyzed using Chromeleon 7, Version 7.2.10.24543 (Thermo Fisher Scientific).

4.3. Evaluation of *Centella Asiatica* antioxidant activity

The antioxidant activity was evaluated using a DPPH reducing assay. A total of 500 mg of gotu kola herb extract (with various concentrations of 50, 75, 100, 125, and 150 ppm) plus 1.0 mL of 0.4 DPPH mM and ethanol p.a to 5.0 mL. The mixture was then vortexed and left for 30 minutes. This solution is then measured absorbance at wavelength 516.5 nm. Similar protocols were done for blank samples (without extract). The data on antioxidant activity was compared to vitamin C as a positive contr. The percentage of DPPH radicals inhibition was calculated by Equation 1

$$\text{Inhibition (\%)} = \frac{\text{Blank abs} - \text{Sample abs}}{\text{Blank abs}} \times 100\% \quad (\text{Equation 1})$$

4.4. Optimization of gotu kola gummy candy formula

In this study, the optimization was done on the gelling agents used, namely bovine gelatine and pectin. The bovine gelatin range was 8.5-9.5 g while the pectin range was 0.5-1.5 g for 74.8 g batch size (18). To create several runs, pectin and gelatine proportions were determined were processed using Design Expert version 13.0 following the Simplex Lattice Design method in Table 3.

Table 3. Runs determined by SLD and Design Expert software for gotu kola gummy candy

Ingredient	Run							
	1	2	3	4	5	6	7	8
Gotu kola extract (g)	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Gelatine(g)	8.50	9.00	8.50	9.25	8.75	9.00	9.50	9.50
Pectin (g)	1.50	1.00	1.50	0.75	1.25	1.00	0.50	0.50
Sucrosa (g)	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00
Multiflora honey (g)	22.00	22.00	22.00	22.00	22.00	22.00	22.00	22.00
Citric acid (g)	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Sodium benzoate (g)	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Essence	qs	qs	qs	qs	qs	qs	qs	qs
Colorant	qs	qs	qs	qs	qs	qs	qs	qs
Aquades (ml)	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Total	78.40	78.40	78.40	78.40	78.40	78.40	78.40	78.40

4.5. Physical Characteristic Test

Gummy candy was tested for physical characteristics, including organoleptic, pH, weight uniformity, elasticity, and loss on drying. For organoleptic assessment, gummy candies were evaluated on their color, scent, shape, and texture (18).

pH evaluation was done by dipping the pH paper into the liquid gel mass before the molding process. The pH value is measured by looking at the pH value listed on the universal pH indicator.

Evaluation of weight uniformity was conducted by sampling 20 gummy candies and weighing them one by one with a digital balance. The average value, SD, and CV of the gummy candy were calculated.

Evaluation of the elasticity test was performed by sampling 4 gummies for each run and placing them on the plate. Another plate of metal as well as 200 g of weights was placed on top of the second plate for 5 minutes. The difference in the height of the gummies before and after weight placement was recorded and evaluated according to Equation 2

$$\text{Elasticity (\%)} = \frac{T1 - T2}{T1} \times 100\% \quad (\text{Equation 2})$$

Information: T1 = early gummy candy thick
T2 = final gummy candy thick

To evaluate *loss of drying*, gummy candy was placed in a moisture analyzer (MB 120) with an initial temperature of 105°C until the weight was constant. The loss of drying was calculated based on Equation 3.

$$\text{LOD (\%)} = \frac{W1 - W2}{W1} \times 100\% \quad (\text{Equation 3})$$

Information: W1 = weight before heating
W2 = final weight

4.6. Optimum Gummy Candy Formula Antioxidant Test with *Centella asiatica* Herb Extract

A total of 4.04 gummy candy gotu kola herb extract were sampled. This sampling size was calculated for having a sample that has similarly 25 mg of *Centella asiatica* extract. Samples were diluted into various concentrations of 50, 100, 150, 200, 250 ppm). One milliliter of 0.4 DPPH mM and 5.0 mL ethanol p.a were added to the sample and then vortexed and left for 30 minutes. This solution is then measured absorbance at

wavelength 516.5 nm [22]. Done too blank absorbance measurement. The percentage of DPPH radicals inhibition was calculated by Equation 4.

$$\text{Inhibition (\%)} = \frac{\text{Blank abs} - \text{Sample abs}}{\text{Blank abs}} \times 100\% \quad (\text{Equation 4})$$

4.7. Statistical Analysis

Optimum formula verification is performed by comparing the response of the optimum formula from the experiment with predictive data using Design Expert Ver.13 software. Statistical analysis was performed using IBM SPSS Statistics 25 software with the one-sample t-test method. First, the data were analyzed for normality using the Shapiro-Wilk test as the number of data analyzed was less than 50. If the data showed sig.2 tailed > 0.05, this suggested the normality of the data and was amenable to a one-sample t-test. Antioxidant data were analyzed using One-way ANOVA followed by the Fisher post hoc test.

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