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Incorporation of Cod liver oil to *Centella asiatica* (Gotu Kola) gummy candy as a cognitive enhancement supplement

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ABSTRACT: This study aimed to incorporate Cod Liver Oil (CLO) oil in *Centella asiatica* (Gotu kola) gummy candy and to evaluate its effect on cognitive function in the animal model. Cognitive impairment among children, particularly those identified as slow learners has been a concern for many parents including health authorities and the Federal Government. The current study explored the potency of Gotu kola and CLO combination in improving brain health. The gummy candy formulation was chosen to address children's taste and palatability issues. However, incorporating CLO into the formula might affect the physical properties of the gummy candy. This research uses the simplex lattice design (SLD) to determine the optimum proportion of cod liver oil and gelatine that produce good gummy physical characteristics. The physical characteristics of the gummies that have been evaluated include Moisture Content, Weight Uniformity, pH, and Elasticity. The studies also employed a texture analyzer to determine the gummy texture. Hedonic tests were performed to confirm the physical properties data. Pharmacological evaluation of the active ingredients included antioxidant activity tests using the DPPH assay, and cognitive tests using T-Maze assay. These studies suggested that a combination of 10% gelatine and 5% cod live oil produced the most acceptable physical characteristics. The addition of cod liver oil improved the antioxidant activity of Gotu kola. The optimum formula containing 4 grams of CLO and 4 grams of Gotu kola extract showed a strong antioxidant activity with an IC50 of 32.53 µg/ml. In the mouse memory test, administering 7 grams of CLO in Gotu kola extract gave a significant reduction in the time required to find food in the T-maze test. These studies suggest that fish oil can be incorporated into a gummy candy with acceptable physical properties to enhance memory.

KEYWORDS: Gummy candy; *Centella asiatica*; Cod liver oil; cognitive enhancement; slow learners; omega-3 fatty acids.

1. INTRODUCTION

Slow learning has been a concern for some developing countries. IQ values between 71 and 84 are used to identify slow learners [1]. Slow learners have some cognitive difficulties that tend to be left in terms of their ability to read, write, and do mathematics. A slow learner typically does poorly in every academic area and has a weak working memory. It is more likely to become socially isolated, drop out of school, and experience a decline in self-esteem. In the long term, slow learners are projected to be an economic burden for such countries since the parents spend more money to ensure the slow learner to catch up with the society. The economic burden of slow learners is enormous. Sixty-two percent of the costs were incurred indirectly. Of the total direct costs, 57.38%, 16.18%, and 10.30% were allocated to tuition, drugs, and remedial education, respectively. Slow learners cost INR 3,544,880 in total annually on average. The costs of the

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learning disability clinic averaged INR 2,250,194 a year. INR 57,951 was the average annual total cost per student [1].

To support the slow learners in developing their brain capacity, there are two processes that need to be protected which is adult neurogenesis and the blood-brain barrier (BBB). Adult neurogenesis is the process of maturing new neurons generated from stem cells in the hippocampus, influencing mood regulation, memory, and spatial learning [2]. Meanwhile, the BBB is a semi-permeable system that regulates the passage of substances to and from the brain. By keeping the pool of neuroactive substances between the peripheral tissues and the central nervous system (CNS) distinct, the blood-brain barrier (BBB) helps avoid signal crosstalk. When it comes to creating therapeutic compounds to treat brain disorders, the BBB may provide a challenge. If strategies to enhance medication delivery to the brain are discovered [3]. During the period of growth and development, nutritional aspects become very important for children's development. One of the nutrients that is important for children's brain development is omega-3 fatty acids. Omega-3 fatty acids are often used in therapy for children with brain disorders because their content is useful for the formation of sphingomyelin, a structural component of nerves. One product that contains omega-3 fatty acids is fish oil. Omega-3 fatty acids including ALA (alpha-linolenic acid), EPA (eicosapentaenoic acid), and DHA (docosahexaenoic acid) play a vital role in brain development. The three compounds contained in fish oil can penetrate the BBB and act as membrane builders in the BBB and the membranes of other brain cells. In addition, ALA, EPA, and DHA play a role in the development of brain nerve cells, signal neurotransmission processes, and protection against oxidative stress. Cod liver oil (CLO) is a nutritional supplement derived from the liver of cod fish. It contains extremely high concentrations of vitamins A, D, and E as well as high levels of the omega-3 fatty acids docosahexaenoic acid and eicosapentaenoic acid. For babies and kids, using cod liver oil supplements has a few health advantages [4]. Thus, cod liver oil can be used to supplement brain development in children.

Some herbal medicine that has been widely used to enhance brain function is *Centella asiatica* which has been known as Gotu kola. The plant has active compounds that are a bitter and have been associated with a compound known asiaticoside [5]. Asiaticoside has a high ability to cross the BBB, making it easily accessible to the brain [6]. The content of asiaticoside compounds in Gotu kola is 0.21-1.34% [7]. Additionally, Gotu kola has neuroprotective and neurotrophic properties, earning it the nickname "brain tonic" [8]. Now, Gotu kola products that are currently available in the marketplace include capsules, syrups, and pure extracts. However, children tend to dislike this product since it is difficult to use.

Gummy candy is one alternative to formulate the Gotu kola. Gummy candys' varied flavors and colors as well as their soft and chewy texture, also make them more attractive to children. We hypothesize that a combination of active compounds contained in Gotu kola and cod liver oil are potentially can improve children's brain function. Therefore, it is necessary to develop dossage forms that can carry Gotu kola extract and cod liver oil in a way that it is acceptable to children. One of the challenges in formulating children's supplements is the presence of bitter, salty, or sour tastes which tend to be disliked. The formulation approach with Gummy candy can be a solution to cover the problem.

Currently, many gummy candy formulations are on the market but most of them focus on multivitamins and minerals. None of the products marketed as both Gotu kola and cod liver oil in a single gummy candy. This research aimed to develop a gummy candy formula that can incorporate Cod Liver Oil and Gotu kola in a supplement to improve the cognitive function of the children. The research includes an animal study to assess the ability of these two ingredients to improve memories. The research includes the formulation of Gotu kola-based gummy candy with an evaluation of its physical characterization, antioxidant activity, and hedonic test among subjects.

2. RESULTS

2.1. Plant Determination

Plant determination was carried out at the Pharmacognosy-Phytochemistry Laboratory, Faculty of Pharmacy, Gadjah Mada University. It was confirmed that the sample belongs to the Apiciae family and was specially identified as *Centella asiatica* (L.) Urban (Document #21.19.12/UN1/FFA.2/BF/PT/2022) which is widely known as Gotu kola.

2.2. Extraction of *C.asiatica* (Gotu kola)

The maceration process yielded a crude extract, a concentrated extract weighing 248.72 g with a percentage of 56.53%. This outcome complies with the stipulations outlined in the Indonesian Herbal Pharmacopoeia 2nd Edition, which mandates a minimum yield of 7.3% for *C. asiatica* herbae extractum.

Utilizing the maceration method, reported the highest concentrations of madecassoside, asiaticoside, madecassic acid, and asiatic acid as 0.855%, 0.174%, 0.053%, and 0.025%, respectively [9].

2.3. Gummy candy Formulation

A series of eight experimental formulations were implemented, each involving distinct concentrations of gelatin and cod liver oil. Gelatin is widely used in food products, especially candy. Gelatin is produced from animal and fruit extraction [10]. The physical characteristics of the prepared samples underwent scrutiny through a pH value test, moisture content analysis, and weight uniformity examination. Texture profile analysis on the gummy candies provided comprehensive data on hardness (N), cohesiveness, adhesiveness (Nmm), springiness, gumminess (N), and chewiness (N). Hedonic tests were employed to assess the sensory appeal of the gummy candies, considering factors such as color, aroma, flavor, and texture. In general, the gummy candies were crafted into bear-shaped forms, exhibiting a brownish-purple hue and a slight degree of stickiness. Notably, variations in shape and stickiness were observed across formulations due to differences in gelatin and Cod liver oil concentrations.

2.4. Physical Characterisation Test

The pH value test revealed that the gummy exhibited a pH range of 4.46–6.61. Notably, the pH value, as illustrated in Figure 1, exhibited an upward trend with increasing fish oil content, attributed to the slightly neutral nature of cod liver oil. A study on the encapsulation of cod liver oil indicates the pH values ranged from 5.42 to 6.92 [11].

The study measured the moisture content of the eight formulations, ranging from 0.96% to 2.13%. The observed trend, as depicted in Figure 1, indicates that a decrease in gelatin content is associated with a lower moisture content. This trend is attributed to the inherent water-absorbing properties of gelatin, a hygroscopic material capable of absorbing moisture from the surrounding environment. Therefore, an elevated gelatin content is linked to reduced moisture content [12]

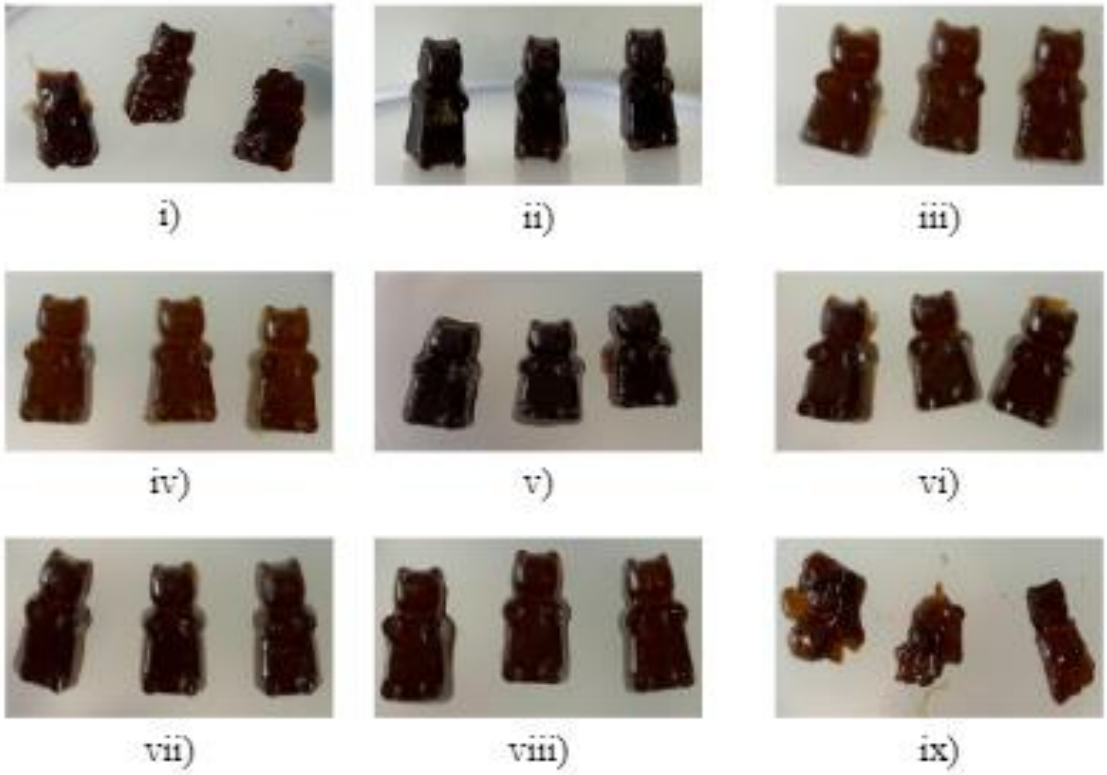

Figure 1. Result of The Gummy candy Formulations: i) Control; ii) F1; iii) F2; iv) F3; v) F4; vi) F5; vii) F6; viii) F7; ix) F8

The weight uniformity test was conducted to assess the consistency of the preparations and to ensure that each preparation contained a comparable amount of the extract. The procedure involved individually weighing 20 gummy candy samples for each formula and subsequently calculating the average

weight to determine the coefficient of variation (CV) value. Formula 5 exhibited the lowest CV value, specifically 2.69%, indicating that the weight uniformity achieved by Formula 5 was superior to that of other formulations.

This enhanced uniformity in Formula 5 can be attributed to the roles played by both cod liver oil and gelatin, constituting 5% (fish oil) and 10% (gelatin) of the gummy candy. This composition resulted in a more solid gummy texture compared to other formulas, which were less sticky and oily, minimizing the risk of separation and imparting a shiny appearance. The solid texture also facilitated the easy removal of the gummy candy from the mold, reducing the likelihood of physical damage due to scratches.

Conversely, formula 8 exhibited the highest CV value at 15.45%, signifying a lack of uniformity. Gummy candies from Formula 8 were stickier than those from other formulas, influencing the process of removing them from the mold and impacting the resulting weight uniformity. These findings suggest that the composition of gelatin and cod liver oil significantly influences both the texture and ease of removal from the mold in gummy candy preparations.

Overall, the results of the weight uniformity test indicated average weights for F1 to F8, fulfilling the weight uniformity requirements with an SD of 0.05 and a CV of 4.67%. The percentage coefficient of variation (CV) for each formula also met the stipulated criteria for weight uniformity percentage coefficient of variation, following the guidelines of the Indonesian Pharmacopoeia V, notably CV ≤ 5%.

Texture Profile Analysis (TPA) serves as a method for assessing the textural attributes of various materials, particularly focusing on food products. This technique involves subjecting a sample to two compression cycles, simulating the biting pattern observed in the human oral cavity. Parameters such as hardness (N), cohesiveness, adhesiveness (Nmm), springiness, gumminess (N), and chewiness (N) were measured to characterize the texture of the gummy candy. The aim is to use these parameters in formulating an optimal gummy candy composition that aligns with the preferences of panelists. The TPA test results, as presented in Table 1., reveal p-values of <0.001 for all parameters, indicating significant differences among formulations. These findings underscore the potential to discern distinctive textural characteristics in gummy candys, laying the groundwork for refining formulations to align more closely with panelists' preferences.

Table 1. The Result of *Texture Profile Analysis* (TPA) Test

Formula	Hardness (N)	Cohesiveness	Adhesiveness (Nmm)	Springiness	Gumminess (N)	Chewiness (N)
Control	22.82 ± 1.73	0.86 ± 0.02	0.24 ± 0.20	0.92 ± 0.01	19.67 ± 1.43	18.19 ± 1.48
1	11.64 ± 3.26	0.33 ± 0.04	0.86 ± 0.57	0.64 ± 0.02	3.84 ± 1.24	2.49 ± 0.85
2	14.65 ± 2.52	0.75 ± 0.03	0.31 ± 0.41	0.86 ± 0.01	11.00 ± 2.37	9.41 ± 1.99
3	22.92 ± 0.50	0.78 ± 0.02	0.16 ± 0.30	0.90 ± 0.01	17.95 ± 0.64	16.07 ± 0.77
4	16.52 ± 1.29	0.85 ± 0.05	0.50 ± 0.64	0.90 ± 0.03	13.95 ± 0.44	12.53 ± 0.39
5	22.08 ± 2.55	0.71 ± 0.04	1.13 ± 0.18	0.86 ± 0.01	15.67 ± 0.92	13.44 ± 0.90
6	13.42 ± 1.57	0.46 ± 0.02	1.18 ± 0.99	0.74 ± 0.02	6.22 ± 0.96	4.63 ± 0.73
7	21.42 ± 2.31	0.63 ± 0.03	0.82 ± 0.14	0.86 ± 0.01	13.37 ± 1.04	11.54 ± 0.82
8	7.70 ± 1.53	0.23 ± 0.15	2.87 ± 0.84	0.66 ± 0.02	1.72 ± 0.96	1.12 ± 0.61
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Based on Figure 1, the determination of moisture content, weight uniformity, and pH were found to vary between formulations. It was found that the pH value trend increased as the amount of fish oil increased due to the alkaline nature of fish oil. From Figure 1, Formula 5 and Formula 6 show values that are not significantly different from the pH value (ns). As well as the moisture content. Otherwise, the control formula and formula 8 show significantly different values of moisture content and pH values (*). If we look at the moisture content, the trend also seems to decrease as gelatin decreases. This is because gelatin can absorb water. Meanwhile, the weight uniformity of each gummy was uniform (SD 0.05; CV: 4.67%).

2.5. Optimum Formulation

After performing physical characteristic tests, the optimal formulation, identified through the analysis conducted using the Simplex Lattice Design (SLD) method in the Design Expert application, is

Formula 5. Comprising 5% fish oil and 10% gelatin in a total batch of 80 g, Formula 5 displays specific attributes, including an elasticity of 0.857, a moisture content of 1.42%, a pH of 6.12, and a weight of 1.02 g, as illustrated in Figure 2. The comparison of SLD predicted value and the formula optimum is illustrated in Table 2.

Table 2. Comparison of simplex lattice design (SLD) predicted value with optimum formula test results

Response	Predicted Value	Experiment Value	Sig.
Weight Uniformity	1.12	1.196	0.81
Elasticity	0.86	0.86	0.84
Moisture Content	1.69	1.61	0.99
Ph	5.98	5.69	0.93

2.6. Hedonic Test

Based on ethical clearance number KE/FK/1620/EC/2023 the hedonic test was carried out. Sensory evaluation is an important quality parameter because it determines whether a product is acceptable to consumers, in addition to its nutritional and functional aspects. Hedonic tests are designed to measure the degree of liking for a product. Category scales range from dislike very much, through neither like nor dislike, to like very much, with a varied number of categories used. Panelists indicate their degree of liking for each sample by choosing the appropriate category. The responses of panelists can be seen in Table 3. From Table 3, the color of formula 5 (gummy candy with 4 grams of cod liver oil and 8 grams of gelatine addition) has the highest average value (3.53) compared to other formulations. This shows that the panelists liked the gummy candies' color with formulation 5 the most. Moreover, formula 5 was significantly different from formula 8.

The aroma of formula 5 (gummy candy with 4 grams of cod liver oil and 8 grams of gelatine addition) in Table 3 has the highest average value (3.33) compared to other formulations. This shows that the panelists liked the gummy candies' aroma with formulation 5 the most. Moreover, formula 5 was significantly different from formula 1.

The flavor of formula 4 (gummy candy with 3 grams of cod liver oil and 9 grams of gelatine addition) has the highest average value (2.73) compared to other formulations. This shows that the panelists liked the gummy candies' flavor with formulation 4 the most. Moreover, formula 4 was significantly different from formula 8.

The texture of gummy candy can be seen in Table 3. It is formula 5 (gummy candy with 4 grams of cod liver oil and 8 grams of gelatine addition) that has the highest average value (3.73) compared to other formulations. This shows that the panelists liked the gummy candies' texture with formulation 5 the most. Moreover, formula 5 was significantly different from formulas 1, 2, 6, and 8.

Based on the result of the hedonic test, formula 5 has the highest average value for color, aroma, and texture. Formula 5 is not preferred on the flavor parameter. This is presumably due to the addition of Gotu kola and a small amount of sweetener causing a bitter taste to appear. Therefore, formula 5 was selected for further evaluation.

2.7. Antioxidant Activity Test using the DPPH Method

Based on the IC50 value, the antioxidant activity of Gotu kola extract and gummy candy combined with Gotu kola extract and cod liver oil is in a strong category. The IC50 value is said to be very strong if <50 µg/mL, strong 50-100 µg/mL, and low if >100 µg/mL [13]. The IC50 values of Gotu kola extract and the gummy candy combination of Gotu kola-cod liver oil extract respectively 29.88 µg/mL and 32.53 µg/mL. Antioxidant tests were performed on positive controls ascorbic acid, Gotu kola extract, and optimum formula of gummy candy (formula 5). The result of the antioxidant activity test is shown in Figure 3, it suggested that the antioxidant activity of Gotu kola extract was not significantly different from the results of the antioxidant activity of gummy candy combined with Gotu kola extract and cod liver oil which below 35 µg/mL. This suggested that the gummy candy exerts strong antioxidant activities.

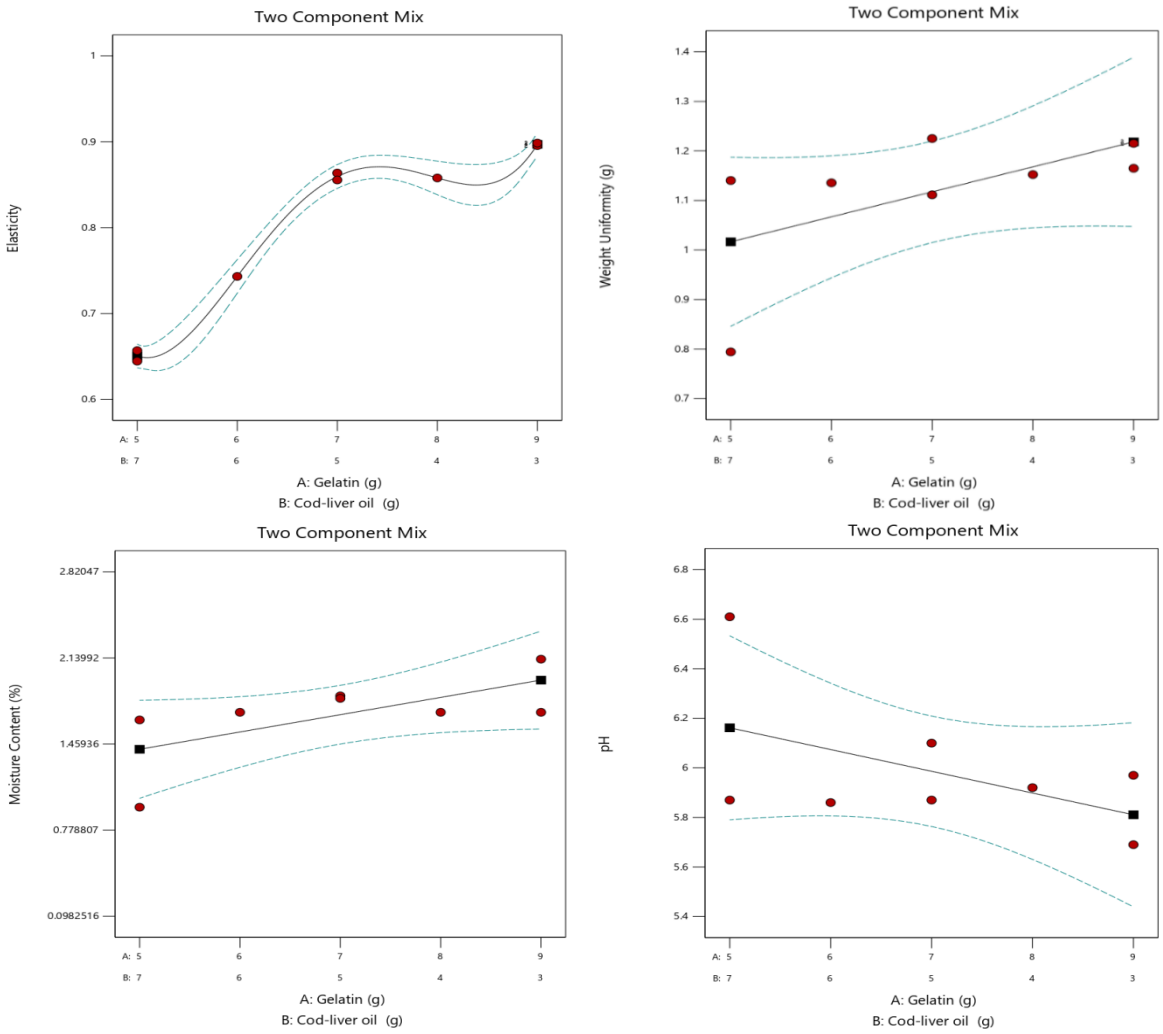

Figure 2. Moisture content, weight uniformity, pH, and elasticity in the optimum formula

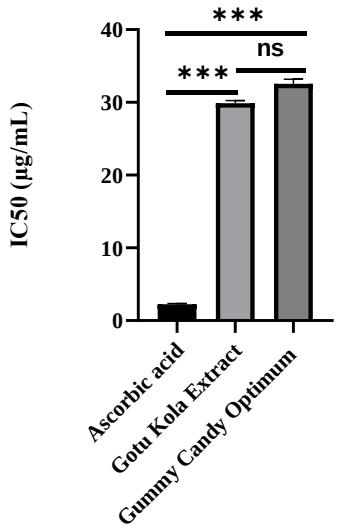

Figure 3. IC50 Value. Antioxidant activity of gummy candy combination *C.asiatica* (Gotu kola) and Cod Liver Oil compared to *C.asiatica* (Gotu kola) crude extract and ascorbic acid as a positive control. The analysis of antioxidants was performed using one-way ANOVA followed by a Post Hoc Test. Each bar represents mean ± SD, n = 3; *** = p-value <0.0001; ns= p-value 0.001

Table 3. The results of the hedonic test. Different superscripts in the same column showed significantly different ($p<0.05$) using One Way ANOVA. The scale given is as follows: 1. Dislike very much, 2. Dislike, 3. Average, 4. Like, 5. Like very much

Formulation	Color Score	Aroma Score	Flavor Score	Texture Score
1	3.23	2.73	2.13	1.93
2	3.10	2.93	2.40	2.90
3	3.27	3.00	2.63	3.50
4	3.50	3.13	2.73	3.27
5	3.53	3.33	2.63	3.73
6	3.47	2.97	2.37	3.00
7	3.43	2.80	2.40	3.67
8	2.87	2.77	1.97	1.43
	p-value<0.05	p-value<0.05	p-value<0.05	p-value<0.05

2.8. Memory Test

The animal studies were performed after the approval of an ethical committee of Gadjah Mada University number KE/FK/1620/EC/2023. The result of a memory test using a t-maze designed to evaluate the level of intelligence that measures spatial memory in rats is shown in Figure 4.

The results before and after Gotu kola and cod liver oil supplementation were analyzed statistically. Before supplementation, there is no significant difference between the control and treated groups in terms of the time required to find the food spot. However, the ability of the animal to find food was significantly improved after training. Without training, the time spent finding food in T-maze is around 200 seconds. While after training, it was shorter, 60 seconds in the control group. Scott Emulsion® is a cod liver oil supplement that is a source of omega-3 fatty acids (DHA+EPA). Therefore, in this research, Scott Emulsion® is used as a positive control. The positive control improves the ability of the animal to find food much less in 18 seconds. While the supplementation of Gotu kola and cod liver oil significantly shortened the time required to find food compared to the control group, there is a concentration-dependent where more cod liver oil administered is correlated with a shorter time for finding food. Administration of the Gotu kola and cod liver oil which is preferable in the hedonic test (7-gram cod liver oil and 4-gram Gotu kola results in the best memory performance among other supplementation groups. When compared to the positive control, the gummy candy product with a combination of CLO 7 GK 4 takes twice as long as the positive control. This may be caused by differences in the dose volume administered. In the positive control group, the cod liver oil administered is 1 ml emulsion, while in the treatment group, the amount of cod liver oil is much lower.

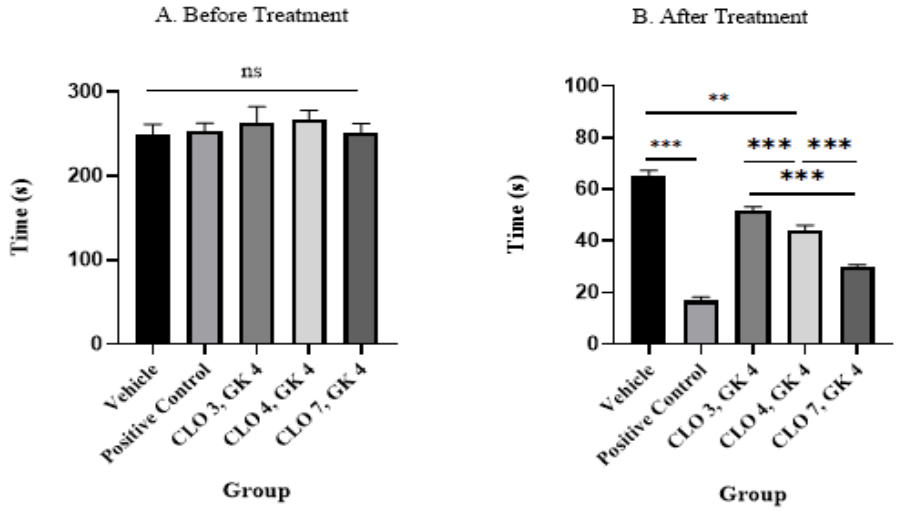

Figure 4. (a) Time difference before dosing treatment; (b) Time difference after dosing treatment for each group. Time is calculated based on the rat's ability to find its food. CLO = Cod Liver Oil, GK = Gotu kola Extract *(C.asiatica)*.

3. DISCUSSION

The main findings in this study are: 1) the optimized proportion of gelatin and cod liver oil is formula 5 with eight grams of gelatin and four grams of CLO. 2) Formula 5 showed the best result on texture analysis and hedonic test. 3) The gummy candy containing 4 grams of cod liver oil and 4 grams of Gotu kola exerts strong antioxidant activities evaluated with DPPH Assay. 4) There is a significant memory improvement after supplementation of 7-gram cod liver oil and 4 grams of Gotu kola extract examined using T-maze assay.

The ideal gummy candy contains 8 grams of gelatin and 4 grams of cod liver oil. The presence of cod liver oil has the potential to enhance the antioxidant activity of Gotu kola extract. Furthermore, 8 grams of gelatin can provide a desirable texture to gummy sweets. When compared to other formulas, the optimum recipe is more popular with panelists based on their preferences for color, texture, and style. This is due to the recent surge in customer demand for healthier, fortified foods [14].

Gummy candy with Gotu kola extracts and cod liver oil has an IC50 value of 32.53 µg/ml. This means that adding cod liver oil to Gotu kola extract gummy candy can reduce the IC50 value. A decrease in the IC50 value indicates that the antioxidant activity of gummy candy is getting better. Classification of the value as having very significant antioxidant activity (<50 µg/mL). Compared to [15], gummy candy containing a mixture of Gotu kola extract and cod liver oil shows greater antioxidant activity than Gotu kola gummy candy with an IC50 value of 166.50 µg/mL [16]. The high level of antioxidants in gummy candy is useful for improving brain memory. Based on a T-maze test for detect the level of intelligence that measures spatial memory in rats is appropriate [17], the study found that adding cod liver oil in gummy candy Gotu kola extract significantly improved memory and recall. This is demonstrated by the test animals' increased quickness in finding food, which went from 200 seconds to 60 seconds. This is showing the correlation between antioxidant actvity of the gummy candy increased the brain memory of the rats so that can be used for cognitive enhancement supplement.

The novelty of this research is the incorporation of fish oil into the Gotu kola to improve the memory of animals to find food. There is a concentration-dependent activity where the more cod liver oil, the shorter the time needed to find food. Further research is needed regarding the characterization of gummy candies with fish oil to produce gummy candy preparations with good characteristics and optimal memory improvement capabilities.

4. CONCLUSION

Based on this research, it can be concluded that the optimum gummy candy formula consists of 10% gelatin and 5% fish oil. This formula showed the best value of the texture profile and hedonic test. The antioxidant activity of the formula improved with the addition of fish oil. In the mouse memory test, the administration of fish oil and *Centella asiatica* (Gotu kola) reduced the time required to find food in the T-maze test. The higher the addition of fish oil, the greater the memory improvement.

5. MATERIALS AND METHODS

The materials of study were Gotu kola (PT. Omah Djamoe, Karanganyar, Indonesia), 70% ethanol (PT Progo Mulyo, Yogyakarta, Indonesia), cod liver oil (Mollers Pharma, Norwegia), gelatin (PT Pondasi Inti Sejahtera, Yogyakarta, Indonesia), pectin (PT Adimitra Karunia, Surabaya, Indonesia), sucrose (PT Sugar Group Companies, Cirebon, Indonesia), honey (PT Natura Alamindo Utama, Banten, Indonesia), Citric Acid (PT Pondasi Inti Sejahtera, Yogyakarta, Indonesia), natrium benzoate (PT Pondasi Inti Sejahtera, Yogyakarta, Indonesia), flavor and colorant blueberry (PT Pilarose, Banten, Indonesia), aquadest (PT Progo Mulyo, Yogyakarta, Indonesia), methanol (Merck Ltd, Darmstadt, Germany), ascorbic acid (Sigma Aldrich, USA), DPPH (PT Smart Lab, Indonesia), and aquadest (PT Progo Mulyo, Yogyakarta, Indonesia).

5.1. Plant Determination

Plant determination is used to determine plant species. Plant determination was carried out at the Pharmacognosy-Phytochemistry Laboratory, Faculty of Pharmacy, Gadjah Mada University.

5.2. Preparation of *C. asiatica* Extract (Gotu kola)

C. asiatica or Gotu kola simplicia was sieved (60 mesh) and weighed 440 g. Extraction was carried out by maceration (soaking the simplicia in filter fluid for 24 hours at room temperature and protected from light). A total of 440 grams of simplicia were macerated with 3080 ml of 70% ethanol (1:7). Then, re-

maceration was performed using 1320 mL of 70% ethanol (1:3). The extract was filtered using flannel cloth and filtered again using a sieve. The solvent is made into a thick extract using a water bath at a temperature of 50°C. The yield was calculated by dividing the total mass of the extracted sample by the total mass of the dry sample. Extract stored in the refrigerator [18].

5.3. Formulation of *Gummy candy*

Gotu kola concentration in gummy candy is 5%, there are 4 grams of active substance in 8 grams of total ingredients. A different formula was prepared based on Table 4.

The manufacturing procedure of gummy candy: honey and sucrose were mixed and heated at 80°C. In a different pot, gelatin and pectin were dissolved using several aquadest. The two solutions were mixed and stirred until homogeneous. Cod liver oil was added and heated at 80°C. Then, Gotu kola extract, citric acid, sodium benzoate, blueberry flavor, and essence, also aquadest were added to the solution and stirred until homogeneous. The gummy candy mixture was poured into a gummy candy molder and left for 24 hours at a cold temperature, then removed from the mold.

5.4. Physical Characterization of Gummy candies

Physical characterization tests include weight uniformity tests, pH value tests, moisture tests, and texture profile analysis tests. The weight uniformity test was carried out by weighing 20 gummy candy samples randomly and calculating the average for each gummy candy formulation. The pH value test was carried out by dissolving the gummy candy sample in 100 mL of distilled water, then the pH value was measured using a pH meter. The moisture was determined through thermogravimetry, involving the sample being subjected to an oven temperature of 105°C for 24 hours. The process included weighing the sample, and re-ovening it until a constant weight was achieved. Texture profile analysis assessments, including hardness, cohesiveness, adhesiveness, chewiness, gumminess, and springiness, were conducted using a Texture Analyzer instrument. The tests were performed with a Tekstur Analyzer Lloyd type TA1 at room temperature, applying a speed of 0.5 mm/s for 0.5 seconds [19]

Table 4. Gummy candy formulation

Material	Control	F1	F2	F3	F4	F5	F6	F7	F8
C. asiatica (Gotu kola) Extract (g)	4	4	4	4	4	4	4	4	4
Cod Liver Oil (g)	0	7	5	3	3	4	6	5	7
Gelatine (g)	12	5	7	9	9	8	6	7	5
Pectin (g)	2	2	2	2	2	2	2	2	2
Sucrose (g)	18	18	18	18	18	18	18	18	18
Honey (g)	23	23	23	23	23	23	23	23	23
Citric Acid (g)	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Natrium Benzoate (g)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Blueberry Flavor	qs	qs	qs	qs	qs	qs	qs	qs	qs
Essence Blueberry	qs	qs	qs	qs	qs	qs	qs	qs	qs
Purple Dye	qs	qs	qs	qs	qs	qs	qs	qs	qs
Aquadest (ml)	20	20	20	20	20	20	20	20	20

5.5. Hedonic Test

The hedonic test was carried out involving 30 untrained panelists aged 17-25 years. Panelists were presented with 8 samples according to the gummy candy formulation. Panelists were asked to rate how much they liked the color, aroma, taste, and texture of the gummy candy product. Ratings are carried out on

a scale of 1 (Dislike very much) to 5 (Like very much). This hedonic test meets the ethical principles outlined in International and National Guidelines regarding ethical standards and research procedures with humans with ethics committee approval No. KE/FK/1620/EC/2023.

5.6. Antioxidant Activity Using the DPPH Method

Antioxidant activity was assessed utilizing the DPPH method with modifications tailored to accommodate the specific characteristics of the sample under investigation [12]. It is important to measure antioxidant activity to determine the quality of Gotu kola which can be used as an ingredient in standard herbal medicine for health. The DPPH method earlier was chosen because it is a fast, simple, and inexpensive method for measuring antioxidant capacity involving free use radical, 2,2-Diphenyl-1-picrylhydrazyl (DPPH). DPPH is used to determine the ability of compounds to capture free radicals and evaluate the antioxidant activity of foods [20]. The preparation of the DPPH solution involved weighing 15.8 mg of DPPH and dissolving it in 100 mL of methanol to make a 0.4 mM solution. To determine the maximum absorption wavelength of DPPH, 1 mL of a 100 ppm DPPH solution was placed in a 10 mL measuring flask, incubated in the dark for 30 minutes, and the absorbance was measured at a wavelength of 515-517 nm. The gummy candy sample solution with Gotu kola herb extract was melted and dissolved in 10 mL of methanol (50 mg/mL). Concentration series ranging from 20 to 100 ppm were prepared. Absorption measurements were conducted using a UV-Vis spectrophotometer by pipetting 1 mL of the sample, 1 mL of DPPH, and methanol up to 5 mL. After a 30-minute incubation, the wavelength was measured, and the process was repeated three times. The percentage inhibition and IC50 value were determined by calculating the % inhibition and establishing the linear regression equation.

5.7. Memory Test Using T-Maze Test

The rat's memory test was carried out using the T-maze test to detect the level of intelligence that measures spatial memory in rats ([17]. This test uses a tool in the form of a T-shaped tunnel with food on one of the horizontal sides of the T. The memory test uses a pre-test and post-test 9 controlled design. Female Wistar rats aged 6 weeks with a body weight of 100—200 grams were used and divided into 5 groups. Each group consists of 5 rats. The five groups are control group 1 (aquadest), control group 2 (brain supplement products on the market, test group 1 (Gotu kola extract formulation 3/CLO 3-GK 4), test group 2 (Gotu kola extract formulation 8/CLO 4-GK 4), and test group 3 (Gotu kola extract formulation 5 as the best formulation based on the hedonic test/CLO-7-GK-4). The control group was given 1 mL and the test group was given a dose of 0.9 mg/gBW/day/rat. All groups were given training for 10 minutes/day/rat for 12 days. Furthermore, before and after training, the performance of each mouse was tested.

5.8. Statistical Analysis

The data obtained from each formula was analyzed quantitatively to obtain the optimum formula of the gummy candy preparation. The IC50 parameter used to determine the sample concentration was able to reduce 50% of DPPH free radicals. Analysis using One Way Anova on the T-Maze Test and hedonic test.

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