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Fortification of Gotu Kola (*Centella asiatica* (L) Urban) Gummy Candy with Sancha Inchi Oil (*Plukenetia Volubilis* L.): Antioxidant Activity and Formula Development

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ABSTRACT

Nutraceuticals have become increasingly popular in society due to growing health awareness and the rising demand for health supplements. Reactive oxygen species (ROS) are compounds that trigger cellular inflammation, leading to tissue damage and contributing to the development of neurovascular diseases. Commercially available gotu kola and sacha inchi oil products are typically limited to capsules, soft gels, and virgin oil. To enhance consumer acceptance, the combination of gotu kola extract and sacha inchi oil (SIO) offers potential for formulation as gummy candy. This study aims to identify the optimum proportion of sacha inchi oil and pectin in gummy candy formulation and to evaluate the antioxidant activity of these substances, both individually and in combination. Antioxidant activity was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. A Simplex Lattice Design in Design Expert software was employed to prepare eight formulations. The physical properties of the gummy candies were evaluated using a Texture Profile Analyzer (TPA). The findings reveal that gotu kola extract exhibits strong antioxidant activity, with an IC_{50} value of 38.44 µg/mL. The combination of sacha inchi oil and gotu kola also demonstrated potent antioxidant activity, with an IC50 value of 100.83 ± 2.16 µg/mL. Different proportions of sacha inchi oil and pectin significantly influenced gummy candy attributes such as gumminess, chewiness, springiness, and cohesiveness. The optimum formulation was identified as containing 7.3% sacha inchi oil and 0.33% pectin. These results suggest that incorporating sacha inchi oil may serve as a viable alternative to fish oil in the development of gummy candy supplements.

Keywords: gummy candy, *Centella asiatica*, *sacha inchi* Oil, antioxidant, omega-3 fatty acids

INTRODUCTION

Developing countries in tropical regions are known to experience a significant insufficiency of omega-3, particularly among children and adolescents. Inadequate levels of omega-3 highly unsaturated fatty acids (HUFAs) in the diet can adversely affect brain development and neurodevelopmental outcomes (Gow & Hibbeln, 2016). Studies on omega-3 long-chain polyunsaturated fatty acid (LCPUFA) consumption indicate that fewer than 20% of the global population consumes at least 250 mg of omega-3 polyunsaturated fatty acids (PUFAs) from seafood daily. However, several factors limit the accuracy of estimating dietary intake of EPA and DHA (Eicosapentaenoic Acid and Docosahexaenoic Acid). These limitations include variations in the availability of accurate and up-to-date nutritional

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data across different countries and challenges in reporting dietary data accurately (Stark et al., 2016). *Plukenetia volubilis L.* and *Centella asiatica* (L.) Urban have long been popular as traditional herbal medicines and are widely utilized for medicinal purposes in various countries. Edible oils are commonly used to enhance the nutritional value of supplements and vitamins.

Sacha inchi seed oil is a naturally occurring substance anticipated to gain significant popularity in the coming years due to its high omega-3 content, which constitutes 48% of its total fatty acids. In comparison, argan oil and olive oil contain considerably lower omega-3 levels, at less than 0.5% and 0–1.5%, respectively. Sacha inchi oil is also rich in vitamins A, D, and E, along with bioactive compounds such as terpenoids and flavonoids, which exhibit potent antioxidant properties (Miklavčič et al., 2020). Vegetable oils like sacha inchi oil serve as natural sources of polyunsaturated fatty acids (PUFAs), comprising 92–93% of its fatty acid content. The α-linolenic acid (ALA, C18:3n-3) content of sacha inchi oil is comparable to that of linseed oil (approximately 47%) and is roughly six times higher than that of soybean oil (approximately 8%) (Fanali et al., 2011). The oil's n-6/n-3 PUFA ratio ranges from 0.81 to 1.12, which closely aligns with the optim ratio for human health, around 1:1 (Wang et al., 2018). Due to its high PUFA content and favorable n-6/n-3 ratio, sacha inchi oil is highly attractive to the functional food, pharmaceutical, and cosmetic industries (Wang et al., 2018; Cardenas et al., 2021). It has been proposed for medical interventions targeting various conditions, including cardiovascular diseases, diabetes, arthritis, inflammatory skin disorders, and attention deficit hyperactivity disorder (ADHD).

Gotu kola is a traditional plant widely used in various Asian countries for its medicinal properties. Its therapeutic value is attributed to the presence of triterpenoids, carotenoids, glycosides, flavonoids, and alkaloids. Sacha inchi oil (SIO) has also demonstrated anticonvulsant effects in experimental epilepsy models. In a study using mice, seizures were induced with pentylenetetrazole (PTZ) and subsequently treated with SIO. The mechanism by which SIO protects against PTZ-induced seizures is linked to the enhancement of the neurotransmitter GABA and its cumulative antioxidant effects (Herrera-Calderon et al., 2019). Another compound known to enhance antioxidant activity is asiaticoside, which is found in gotu kola extract. The bioactive constituents of gotu kola may play a significant neuroprotective role, with asiatic acid demonstrating therapeutic potential in treating cerebral ischemia. This effect is thought to be related to the reduction of blood-brain barrier permeability and the mitigation of mitochondrial damage (Orhan, 2012).

In 2014, the Functional Food Center (FFC) defined functional food products as "natural or processed foods containing safe and efficient levels of bioactive compounds, which offer a scientifically proven and documented health advantage by utilizing specific biomarkers to prevent, manage, or alleviate chronic diseases or their symptoms." Despite variations in definitions, there is a general consensus among scientists that functional foods contain bioactive components that offer health benefits beyond those of standard dietary supplements (Baker et al., 2022). Gummy candy represents an innovative alternative for formulating nutraceutical products, particularly for individuals with dysphagia. The incorporation of oil into gummies is a common practice, as seen in flavored fish oil gummies. Substituting fish oil with edible plant-based oils is a novel approach in the manufacturing process of nutraceuticals. Gummy candies are typically made using gelling agents such as gelatin and pectin, resulting in a desirable texture and sweet taste that effectively masks the bitterness of active ingredients.

The taste of edible oil, such as sacha inchi oil (SIO), is expected to help mask the bitter and astringent taste of tannins found in gotu kola extract. However, to date, no studies have specifically investigated the incorporation of SIO into gummy candy, while most available research has focused on the addition of fish oil to gummy formulations. Therefore, this study aims to optimize the incorporation of sacha inchi oil into gotu kola gummy candy. The study involves formulating gummy candy with sacha inchi oil and gotu kola extract, followed by an evaluation of the antioxidant activity of the combined compounds.

MATERIALS AND METHOD

The materials used in this research included *Centella asiatica* (L.) Urban and *Plukenetia volubilis* (L.) oil (PT. Golden Omega Indonesia). Other materials were beef gelatin (Bloom 250, Gelita NZ Ltd, Type B), citrus pectin (PT. Adimitra Karunia, pharmaceutical grade), ceiba nectar honey (PT. Natura Alamindo Utama, Tangerang, Indonesia), crystal sucrose (PT. Gulaku Sugar Group Company), citric acid (PT. Multiverse Anugerah Chemindo), sodium benzoate (PT. Gunacipta Multirasa),

blueberry essence, and distilled water (CV Progo Mulyo). Additional chemicals included ascorbic acid (Sigma-Aldrich, USA), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (PT. Smart Lab Indonesia), methanol p.a. (Merck Ltd, Darmstadt, Germany), and Amos 4D Gummy Fruit (AmosCandy™, China).

Plant Determination

Plant determination is a crucial initial step in the research process to verify the authenticity of the plant species. In this study, plant determination was conducted at the Pharmacognosy-Phytochemical Laboratory, Department of Pharmaceutical Biology, Universitas Gadjah Mada, Indonesia (Immanuela, 2024).

Preparation of Gotu Kola Extract

Approximately 1,500 g of dried *Centella asiatica* (L.) Urban herbs were placed in an oven at 110°C for 3 hours. The dried herbs were then ground using a Cross Beater Mill (Retsch Milling Machine). The subsequent step involved macerating the herbs with 7,000 mL of 70% ethanol at room temperature for 48 hours. The herbs were re-macerated with 3,000 mL of 70% ethanol for an additional 24 hours. After maceration, the macerate was obtained by filtration using a sieve cloth and a vacuum pump. The solvent was evaporated in a stainless steel pan on an electric stove at 40–50°C until a concentrated extract was obtained. The ethanolic extract yield of gotu kola was calculated as a percentage (w/w) using the following formula: Yield [%] = [total extracted sample mass:dried herbs mass] x 100 (Rahmasari, et al, 2024).

Gotu Kola Extract Characterization Using Thin Layer Chromatography (TLC)

Thin Layer Chromatography (TLC) was performed to identify asiaticoside, the major constituent of *Centella asiatica* (gotu kola). The TLC analysis was conducted using silica gel 60 F_{254} coated on aluminum foil as the stationary phase. A small spot of the ethanolic extract was applied 1 cm above the bottom edge of the plate, marked with a line. Three different mobile phases were used consecutively for separation: ethyl acetate:methanol:distilled water (8:2:1 v/v), chloroform:methanol:distilled water (65:25:4 v/v), and hexane:ethyl acetate:diethylamine (80:20:2 v/v). The plate was placed in an isolated chamber containing the mobile phase until the solvent front reached 1 cm below the top edge of the plate. The plate was then carefully removed and air-dried. Triterpenoid compounds were visualized by spraying the plate with Liebermann-Burchard reagent and heating it in an oven at 100°C for 5–10 minutes. The asiaticoside spot was clearly visible under UV light at 365 nm (Indonesian Herbal Pharmacopeia, 2017).

Gummy Candy Formulation

The optimum formula was determined using the Simplex Lattice Design (SLD) method, which ensures that the selected formulation meets the criteria for good physical properties of the gummy candy (Laksitorini et al., 2019). Various formulations of gotu kola and sacha inchi oil gummy candy were prepared (Table I). The preparation steps for the gummy candy are described as follows: Pectin and gelatin were bloomed in hot water, followed by the addition of sweetening agents such as sucrose and honey, which were heated at 70–80°C until fully dissolved and mixed. Subsequently, the oil was gradually poured into the solution along with the gotu kola extract and additives (citric acid, sodium benzoate, and distilled water), and the mixture was heated to its boiling point for several minutes. The mixture was then allowed to cool to 40°C before being poured into molds and left to set for 24 hours. The gummy candies were removed from the molds and stored at room temperature for 1–2 days (Immanuela, 2024).

Table I. Formulation of gummy candy

Ingredient (% b/b)	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
Gotu Kola Extract	4	4	4	4	4	4	4	4
Sacha Inchi oil	4	5.5	6	5	6	4	5	4.5
Pectin	2	0.5	0	1	0	2	1	1.5
Gelatine	10	10	10	10	10	10	10	10
Sucrose	18	18	18	18	18	18	18	18
Honey	20	20	20	20	20	20	20	20
Citric acid	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Sodium benzoate	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Essence	qs	qs	qs	qs	qs	qs	qs	qs
Aquadest	15	15	15	15	15	15	15	15
	73.5	73.5	73.5	73.5	73.5	73.5	73.5	73.5

Physical Properties of Gummy Candies

The organoleptic properties of the gummy candies were evaluated based on their shape, color, odor, and texture. Physical characteristics were analyzed using a Texture Profile Analyzer (TPA) by comparing them with a commercial gummy candy product. The TPA applied force to the gummies at a

specific rate, performing multiple compressions to assess their physical attributes. The Texture Profile Analyzer measured several parameters, including hardness, springiness, gumminess, chewiness, adhesiveness, and cohesiveness. In addition, weight uniformity tests were conducted on all gummy samples from each formula, ensuring the relative standard deviation (RSD) for each run remained below 5%.

Moisture content evaluation was performed using a halogen moisture balance set at 110°C for the warm-up process. The instrument displays the moisture content value as a percentage, indicating the proportion of water present in the gummy preparation.

Antioxidant Activity Using DPPH Method

The DPPH scavenging activity was evaluated for each sample. A 0.4 mM DPPH solution was prepared using methanol p.a. Approximately 500 mg of gotu kola extract was weighed and dissolved in 10 mL of methanol p.a., then diluted 100 times. For sacha inchi oil, 50 mg of oil was dissolved directly in methanol p.a. without dilution and prepared in various concentrations: 110, 220, 330, 440, and 500 ppm. For the mixture of sacha inchi oil and gotu kola extract, the components were combined according to the optimum formula ratio of 5.7:4 (sacha inchi oil:gotu kola, b/v), with each compound weighing 500 mg. The mixture was prepared in concentrations of 30, 60, 90, 120, and 150 ppm. Each sample or standard solution was mixed with 1 mL of DPPH solution, followed by the addition of methanol p.a. to bring the total volume to 5 mL. The mixtures were incubated in complete darkness for 30 minutes. The absorbance of each sample was measured at a wavelength of 516.5 nm. The percentage of DPPH free radical scavenging activity was calculated using the following formula:

$$\%\ \text{DPPH Scavenging Rate} = \frac{A_{blank} - A_{sample}}{A_{blank}}\ x\ 100\%$$

The absorbance of the blank is represented as A_{blank}, while the absorbance of the sample is denoted as A_{sample}.

Statistical Analysis

The optimum gummy candy formula was determined by analyzing the responses for each physical attribute—gumminess, chewiness, springiness, and cohesiveness—using the Simplex Lattice Design (SLD) method with Design Expert software version 13. Subsequently, the results for each response were statistically analyzed using a one-sample t-test and one-way ANOVA ($p < 0.05$) via IBM SPSS Statistics version 25, followed by a post-hoc comparison test.

The statistical analysis indicated that the null hypothesis was correct based on the given values, demonstrating that the responses were statistically significant. A one-sample t-test compares the mean of a single sample to a known or hypothesized population mean to determine whether the sample mean is significantly different.

RESULTS AND DISCUSSION

Extraction and Characterization of Gotu Kola extract

The determination of plant species was conducted at the Pharmacognosy-Phytochemical Laboratory, Department of Pharmaceutical Biology, Universitas Gadjah Mada. The herb was confirmed to belong to the *Apiaceae* family and identified as *Centella asiatica* (L.) Urban (Document No. 42.27.11/UN1/FFA.2/BF/PT/2023).

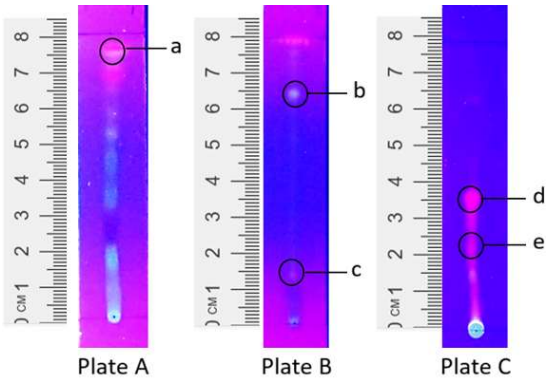


Figure 1. Thin Layer Chromatography (TLC) Results of Gotu Kola Extract. Silica gel F254 was used as the stationary phase, while the mobile phases were ethyl acetate:methanol:distilled water (8:1:1 v/v), chloroform:methanol:distilled water (65:25:4 v/v), and hexane:ethyl acetate: diethylamine (80:20:2 v/v) for Plate A, Plate B, and Plate C, respectively.

The crude extract of the herb yielded 28.81% and met the requirement stated in the Indonesian Herbal Pharmacopeia 2nd edition, which specifies a minimum yield of 7.3% for *Centella asiatica*. The extract had a dark greenish-brown color and an astringent odor. Thin Layer Chromatography (TLC) was performed based on the principle of separating chemical components through adsorption and partition mechanisms, influenced by both the stationary phase

(adsorbent) and the eluent. The extract was diluted with ethanol p.a. to a concentration of 20 mg/mL and applied to three different silica gel adsorbent plates with varying mobile phases. The plates were heated in an oven and examined under UV light at 365 nm.

The identification results from Thin Layer Chromatography (TLC) were expressed as Rf values, with each plate representing a standard reference from the Indonesian Herbal Pharmacopeia 2nd edition. The Rf value for asiaticoside was 0.33, denoted as **d**. Other triterpenoid saponin constituents were also detected on each plate: **a** indicating asiatic acid, **b** representing madecassic acid, and **d** denoting asiaticoside (Harwoko et al., 2014; Indonesian Herbal Pharmacopeia, 2017). These previously mentioned compounds play a crucial role as antioxidant-supportive substances.

Formulation of Gotu Kola and Sacha Inchi Oil

The gummy candy in this study was formulated using a combination of edible oil and the plant-based gelling agent citrus pectin. The optimum formula was determined by adjusting the concentrations of pectin and sacha inchi oil, using the Simplex Lattice Design (SLD) method and Design Expert software. Eight different formulations were developed with varying pectin and oil concentrations. The physical attributes of each sample were evaluated through organoleptic testing, weight uniformity analysis, texture profile analysis, and moisture content measurement. Organoleptic evaluation identified the gummy candies as bear-shaped with a dark-brownish color and a distinctive aroma of sacha inchi oil and gotu kola extract. All samples were chewy with a minimal oil odor.

The content uniformity test was conducted to assess the consistency of the gummy preparations and ensure that each sample contained a comparable quantity of the extract. This test aimed to evaluate the consistency of both the weight and the active substances within the gummies (Hidayah et al., 2023). The test was performed by analyzing 20 randomly selected gummies and calculating the acceptance value. The requirements were met by determining the lower and upper limits of the gummy's average weight for each run. The results showed that none of the formulas exceeded the specified minimum or maximum value range. Content uniformity was evaluated following the United States Pharmacopeia (USP) guidelines for solid dosage forms with multiple components, where the ratio of the active substance is greater than 25%. According to these guidelines, the relative standard deviation (RSD) of the final dosage unit must not exceed 2%. All formulas met this requirement, ensuring the content uniformity as specified in the Pharmacopeia.

The Texture Profile Analyzer (TPA) provided several responses for each formula, evaluating parameters such as gumminess, chewiness, springiness, and cohesiveness. The gumminess values ranged from 8,518 N to 14,246 N, while chewiness ranged from 7,548 N to 13,833 N (Figures 2b and 2c). Formula 6 exhibited the lowest gumminess and chewiness, which was attributed to the higher amount of pectin used, leading to increased water content in the gummy. The hydrogen bonds formed between pectin molecules help stabilize the gel structure, which results in a softer texture. In contrast, sacha inchi oil, with a moisture content of up to 0.9% w/w (based on its Certificate of Analysis), contributed to Formula 3 showing the highest gumminess and chewiness values. Regarding the gelling agent, a lower pectin concentration resulted in firmer and chewier gummies.

The most critical evaluation for gummy candy is the moisture content. The lowest moisture content was observed in Formula 3, with a value of 4.42% (Figure 2a). The addition of pectin to a gelatin-based gummy resulted in higher moisture content. In systems containing pectin, moisture levels can range from less than 10% in pectin powder to as high as 90% in pectin gel. Incorporating approximately 1% pectin helps maintain a relatively high moisture content, up to 50%. The addition of sacha inchi oil had an insignificant effect on the gummy's moisture content due to the low water content in sacha inchi oil (moisture and impurities = 0.9% w/w). In contrast, pectin played a more dominant role in increasing moisture content. Pectin, as a gelling agent, thickened and stabilized the gummy's rheology and internal structure. The other gelling agent, gelatin, was used at a stable concentration of 10 g and contributed to the gummy's physical attributes by providing firmness. Pectin's structural backbone consists of d-galacturonic acid molecules linked by α-1,4 glycosidic bonds with various methyl ester groups. High-methoxy pectin forms a gel under acidic conditions and in the presence of high sugar content. For a stronger gel network, pectin requires a lower pH to promote hydrogen bond formation (Efe & Dawson, 2022).

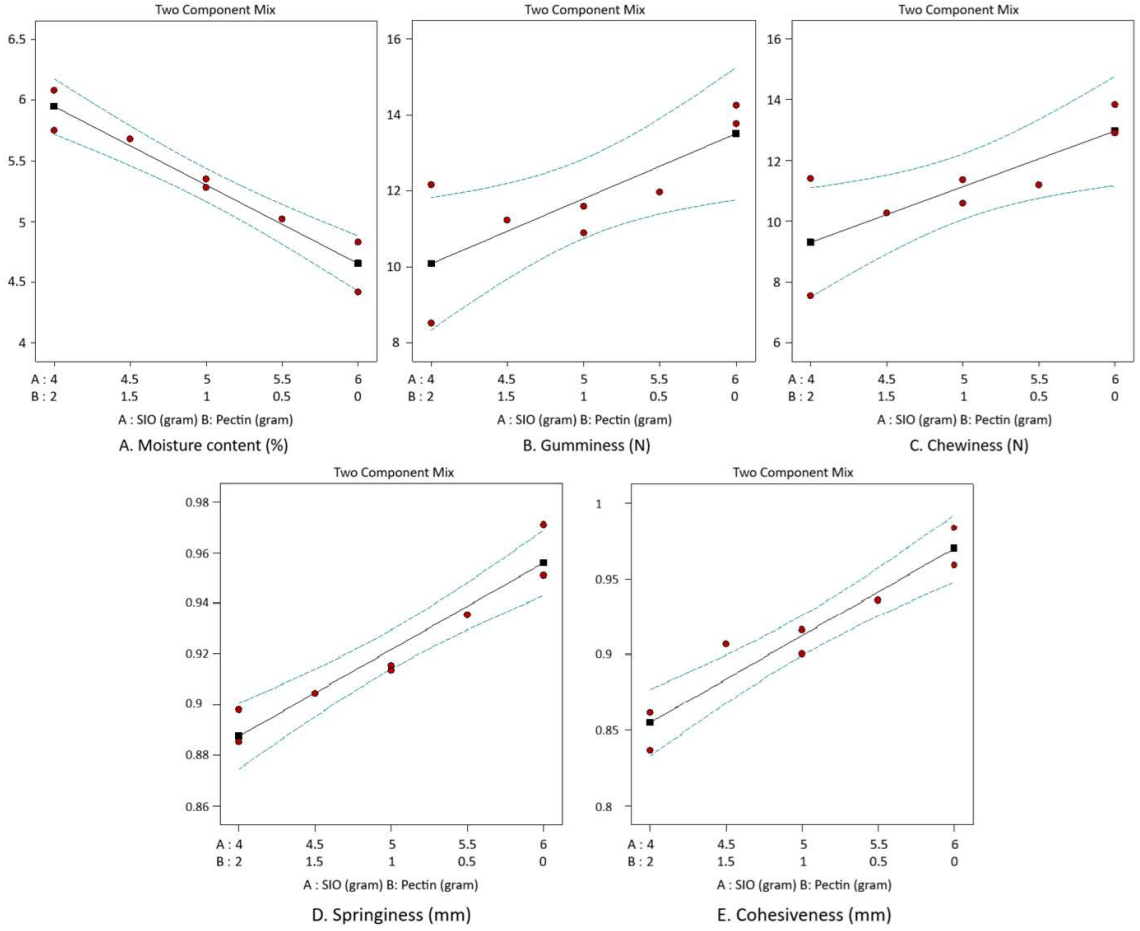


Figure 2. Profile of physical properties of gummy candy based on Simplex Lattice Design as the two component mix were SIO vs pectin were expressed in grams unit.

Springiness represents the elasticity of the gummy, indicating its ability to stretch and return to its original shape. The results show that lower pectin concentrations result in higher springiness values. In the formulation, gelatin serves as the primary gelling agent, supporting the physical structure of the gummy candy. Pectin, on the other hand, tends to create a softer, more jelly-like texture. Therefore, the addition of pectin to a gelatin-based gummy reduces firmness, leading to less springy gummies. Formula 3 and Formula 5 exhibited the highest springiness values, with measurements of 0.97 and 0.95, respectively (Figure 2d). This indicates that higher pectin concentrations reduce elasticity, resulting in lower springiness values.

Cohesiveness was another parameter evaluated in this study (Figure 2e). It refers to the robustness of internal connections within the gummy, indicating its ability to withstand repeated deformation compared to the initial state (Tireki et al., 2021). An increase in sacha inchi oil concentration made the gummies more resistant to deformation by strengthening internal bonds, thereby enhancing cohesiveness. Cohesiveness reflects the forces of attraction between similar molecules, contributing to the structural integrity of the gummy (Siegwein, 2010). Vegetable oils, such as sacha inchi oil, can act as natural surfactants due to their molecular structure, containing both hydrophilic (head) and hydrophobic (tail) groups. The surface-active properties, oxidative stability, and thermal stability of vegetable oil-based surfactants are attributed to their unique fatty acid compositions (Alsabagh et al., 2021; Kumar et al., 2016).

Verification of Optimum Formula

The evaluation of the optimum gummy candy formula containing sacha inchi oil and gotu kola was conducted using Design Expert software version 13. The responses analyzed included moisture content, gumminess, chewiness, springiness, and cohesiveness. The software predicted a desirability value of 0.786. According to the analysis, the ideal formula consisted of 5.74 g of sacha inchi oil and 0.26 g of pectin for a total batch size of 78.3 g, corresponding to 7.3% sacha inchi oil and 0.33% pectin. The predicted values for each response compared to the experimental values (Table II).

Table II. Comparison of Simplex Lattice Design (SLD) Predicted Values with the Actual Response Evaluation of the Optimum Formula.

Response	Predicted Value	Experiment Value	Sig.
Moisture content	4.65	13.147	ns
Gumminess	13.06	13.15	ns
Chewiness	12.49	12.60	ns
Springiness	0.96	0.96	ns
Cohesiveness	0.97	0.96	ns

ns = not significant

Evaluation of the Antioxidant Activity of Sacha Inchi Oil and Gotu Kola Extract

Analyzing the antioxidant value of active ingredients in a nutraceutical product is crucial and must be conducted using specific techniques. The crude extract of gotu kola is known for its high antioxidant capacity due to its various triterpenoid compounds. Meanwhile, sacha inchi oil contains essential constituents rich in fatty acids, including omega-3, omega-6, and omega-9. Several studies have also reported the presence of bioactive compounds in sacha inchi oil, such as vitamins A, D, and E, as well as flavonoids, steroids, and triterpenoids, all of which may exhibit antioxidant activity (Maya et al., 2023). The combination of these two materials could potentially exhibit synergistic antioxidant effects, enhancing their overall health benefits.

The DPPH radical scavenging assay is widely recognized as one of the simplest methods for determining antioxidant activity, measured by the IC_{50} value of the sample. The assay is based on the reduction of the purple color produced by DPPH radicals through the transfer of hydrogen atoms, which results in a color change from violet to pale yellow. When an antioxidant compound reacts with DPPH, the free radical nature of the molecule is neutralized, causing this color shift. The ethanolic extract of gotu kola demonstrated the ability to reduce the oxidation process of DPPH (1,1-diphenyl-2-picrylhydrazyl), with the IC_{50} value (Table III), which serves as a parameter to categorize antioxidant activity. The remaining purple color of the DPPH molecule was measured using a UV-Vis spectrophotometer at a wavelength range of 515–520 nm to determine the antioxidant activity of the tested compound (Sirivibulkovit et al., 2018). In this study, ascorbic acid was used as a positive control due to its well-known ability to donate electrons and neutralize free radicals.

Table III. Antioxidant activity of each sample using DPPH assay.

Sample	IC_{50} value ± SE (µg/mL)
Ascorbic acid	2.98±0.06
Gotu kola extract (GK)	38.44±0.31
Sacha inchi oil (SIO)	314.07±2.25
Combination of GK and SIO	100.83±1.26

The IC_{50} value of ascorbic acid, used as a positive control, was 2.98 ± 0.06 µg/mL. The IC50 values for gotu kola extract and sacha inchi oil were 38.44 ± 0.31 µg/mL and 314.07 ± 2.25 µg/mL, respectively (Table III). For the mixture of sacha inchi oil and gotu kola extract (in a 5.7:4 ratio), the IC_{50} value was 100.83 ± 1.26 µg/mL. Sacha inchi oil exhibited weak antioxidant activity, as reported by Setha et al. (2013) and Jumina et al. (2019). However, the combination of sacha inchi oil and gotu kola extract demonstrated stronger antioxidant activity compared to sacha inchi oil alone. The statistical analysis revealed a significant difference between the IC_{50} value of sacha inchi oil and the IC_{50} values of gotu kola extract and sacha inchi oil (Sig. < 0.05). Ascorbic acid showed the strongest antioxidant activity due to its highly effective free radical scavenging ability. In contrast, the antioxidant activity of gotu kola extract, sacha inchi oil, and their mixture was less potent due to the varied compounds present, which do not exhibit the same level of radical scavenging efficiency as ascorbic acid. Antioxidants—present in the form of vitamins, minerals, and plant-derived secondary metabolites—neutralize free radicals by donating one or two electrons (Maya et al., 2023). It remains uncertain whether the gummy candy

maintains high antioxidant activity, as previous studies have shown a reduction in antioxidant activity in similar formulations (Syaifabila et al 2024). This reduction may be due to antioxidant compounds in the extract interacting with and protecting the gelling agent from oxidation, thereby limiting their availability to react with DPPH free radicals and causing a decrease in measured antioxidant activity.

The novelty of this research lies in the determination that sacha inchi oil can be incorporated into gummy candy formulations at an optimum concentration of up to 7.4% w/w. Future research should focus on optimizing the production process and enhancing the robustness of key process parameters to ensure consistency and scalability.

CONCLUSION

In summary, the combination of gotu kola extract and sacha inchi oil demonstrated strong antioxidant properties. The optimum gummy candy formula was determined to contain 7.3% sacha inchi oil and 0.33% pectin. This formula exhibited consistent physical properties—moisture content, gumminess, chewiness, springiness, and cohesiveness—that closely matched the predicted values and experimental data. The antioxidant activity of the sacha inchi oil and gotu kola mixture was strong, with an IC_{50} value of 100.83 ± 2.16 µg/mL.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Alsabagh, A. M., Aboulrous, A. A., Abdelhamid, M. M., Mahmoud, T., Haddad, A. S., & Rafati, R. (2021). Improvement of heavy oil recovery by nonionic surfactant/alcohol flooding in light of the alkane carbon number and interfacial tension properties. *ACS Omega*, *6*(29), 18668–18683. https://doi.org/10.1021/acsomega.1c01373

Baker, M. T., Lu, P., Parrella, J. A., & Leggette, H. R. (2022). Consumer acceptance toward functional foods: A scoping review. *International Journal of Environmental Research and Public Health*, 19(3), 1217.

Cárdenas, D.M., Gómez Rave, L.J., Soto, J.A. (2021). Biological activity of sacha inchi (Plukenetia volubilis linneo) and potential uses in human health: a review, *Food Technology and Biotechnology*, 59, 253–266.

Efe, N., & Dawson, P. (2022). A Review: Sugar-based confectionery and the importance of ingredients. *European Journal of Agriculture and Food Sciences*, 4(5), 1–8. https://doi.org/10.24018/ejfood.2022.4.5.552

Einhorn-Stoll, U. (2018). Pectin-water interactions in foods – From powder to gel. *Food Hydrocolloids*, *78*, 109–119. https://doi.org/10.1016/j.foodhyd.2017.05.029

Fanali, C., Dugo, L., Cacciola, F., Beccaria, M., Grasso, S., Dachà, M., Dugo, P., Mondello, L., 2011. Chemical characterization of sacha Inchi (Plukenetia volubilis L.) oil, *Journal of Agricultural and Food Chemistry*, 59, 13043–13049.

Gow, R. V., & Hibbeln, J. R. (2014). Omega-3 fatty acid and nutrient deficits in adverse neurodevelopment and childhood behaviors. Child and adolescent psychiatric clinics of North America, 23(3), 555–590. https://doi.org/10.1016/j.chc.2014.02.002

Hidayah, A. N., Wati, A. A., Yuniarti, N., & Laksitorini, M. D. (2023). The development of alternative dosage form for creatine monohydrate: A Floating Tablet. *In J.Food Pharm.Sci* (Vol. 2023, Issue 3). www.journal.ugm.ac.id/v3/JFPS

Immanuela, A. (2024). Development of gummy candy extract gotu kola (Centella Asiatica (L.) Urban) fortified sacha inchi oil (Plukenetia volubilis L.) : Loading optimization and antioxidant strength evaluation. *Undergraduate Thesis*, University of Gadjah Mada.

Laksitorini, M. D., Yathindranath, V., Xiong, W., Hombach-Klonisch, S., & Miller, D. W. (2019). Modulation of Wnt/β-catenin signaling promotes blood-brain barrier phenotype in cultured brain endothelial cells. *Scientific Reports,* 9(1). https://doi.org/10.1038/s41598-019-56075-w

Maya, I., Winardi, D. O., Amalia, E., Mita, S. R., Kusumawulan, C. K., Putriana, N. A., & Sriwidodo, S. (2023). Physicochemical

characteristics, fatty acid profile, and in vitro antioxidant activity evaluation of sacha inchi seed oil from Indonesia. *Cosmetics*, *10*(6). https://doi.org/10.3390/cosmetics10060171

Miklavčič, M. B., Taous, F., Valenčič, V., Elghali, T., Podgornik, M., Strojnik, L., & Ogrinc, N. (2020). Fatty acid composition of cosmetic argan oil: provenience and authenticity criteria. *Molecules*, *25*(18), 4080.

Jumina, Siswanta, D., Zulkarnain, A. K., Triono, S., Priatmoko, Yuanita, E., Imawan, A. C., Fatmasari, N., & Nursalim, I. (2019). Development of C-Arylcalix[4]resorcinarenes and C-Arylcalix[4]pyrogallolarenes as antioxidant and UV-B protector. *Indonesian Journal of Chemistry,* 19(2), 273–284. https://doi.org/10.22146/ijc.26868

Rahmasari, F. S., Sahid, M.N.A., Siswanti, D.U., Darsih, C., Utami, I.D., Alam, L.P.M., Laksitorini, M.D. (2024). The development of antioxidant nutraceuticals containing Chrysanthemum indicum L. gummy candy. *Tradisional Medicine Journal, 29(1),* 37-45 https://doi.org/10.22146/mot.87112

Setha, B., Gaspersz, F.F., Idris, A.P., Rahman, S.A., & Mailoa, M.N. (2013). Potential of seaweed Padina Sp. as a source of antioxidant. *International Journal of Scientific & Technology Research*, 2, 221-224.

Sirivibulkovit, K., Nouanthavong, S., & Sameenoi, Y. (2018). Paper-based DPPH assay for antioxidant activity analysis. *Analytical sciences : the international journal of the Japan Society for Analytical Chemistry*. 34(7), 795–800. https://doi.org/10.2116/analsci.18P014

Siegwein A.M. 2010. The effects of soy protein isolate addition on the physicochemical properties of gummi confections. M.Sc. *Thesis*, Graduate Program in Food Science and Nutrition, The Ohio State University

Stark, K. D., van Elswyk, M. E., Higgins, M. R., Weatherford, C. A., & Salem, N. (2016). Global survey of the omega-3 fatty acids, docosahexaenoic acid and eicosapentaenoic acid in the blood stream of healthy adults. In *Progress in Lipid Research* (Vol. 63, pp. 132–152). Elsevier Ltd. https://doi.org/10.1016/j.plipres.2016.05.001

Syaifabila, I., Yuniarti, N., Darsih, C., & Laksitorini, M. D. (2024). Development of gotu kola (Centella asiatica (L.) Urban) gummy candy and its evaluation on antioxidant activity. *Journal of Research in Pharmacy*, *28(4)*(28(4)), 1047–1056. https://doi.org/10.29228/jrp.787

Tireki, S., Sumnu, G., & Sahin, S. (2021). Correlation between physical and sensorial properties of gummy confections with different formulations during storage, *Journal of Food Science and Technology*, *58*(9), 3397–3408, https://doi.org/10.1007/s13197-020-04923-3

Wang, S., Zhu, F., Kakuda, Y., 2018. Sacha inchi (Plukenetia volubilis L.): Nutritional composition, biological activity, and uses, *Food Chemistry*, 265, 316–328

USP. 2016. Weight Variation <905>. 32(6) Sixth Interim Revision Announcement: <905> UNIFORMITY OF DOSAGE UNITS. In: USP-NF. Rockville, MD: USP; Nov 21, 2016. Pharmacopeial Forum : Volume No. 32(4) Page 1201