Vol 10 No. 2 (2025) 64-73 ISSN (Online) : 2502-0943

Jurnal Katalisator

ANTIOXIDANT ACTIVITY OF SOURSOP (ANNONA MURICATA L.) LEAF JELLY CONFECTIONS FORMULATED WITH MULTIPLE GEL MATRICES

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Detail Artikel

Diterima : 17 Oktober 2025
Direvisi : 30 Oktober 2025
Diterbitkan : 31 Oktober 2025

Kata Kunci

Jelly candy
Annona muricata Linn
Antioxidant activity
DPPH

Penulis Korespondensi

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ABSTRACT

Jelly confections represent promising nutraceutical products by incorporating bioactive compounds with health-promoting properties. Soursop leaves (Annona muricata L.) are recognized as a potential source of antioxidant and antibacterial agents, yet their application in functional foods remains limited. This study evaluated the antioxidant activity of soursop leaf jelly confections prepared with different gel matrix combinations. Formulation optimization was carried out using three gel matrices, namely carrageenan: agar (9:6), carrageenan: konjac (5:1), and konjac: agar (7:3). Antioxidant activity was assessed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay using a microplate reader spectrophotometer. The results demonstrated IC_{50} values of 28.95 µg/mL for carrageenan: agar, 8.82 µg/mL for carrageenan: konjac, and 30.33 µg/mL for konjac: agar formulations. Among these, the carrageenan: konjac (5:1) formulation exhibited the most potent antioxidant activity, indicating its potential for developing functional jelly-based nutraceuticals.

http://doi.org/10.22216/jk.v5i2.5717
Published by LLDIKTI Wilayah X

Jurnal Katalisator Vol 10 No. 2 (2025) 64-

INTRODUCTION

Nutraceuticals can be defined as foods or parts of foods that provide health benefits, including disease prevention. The term "nutraceutical" was first introduced by Stephen DeFelice in 1989, the founder and chairman of the Foundation for Innovation in Medicine (FIM) (Lia et al., 2019). One of the nutraceutical forms that has been widely developed today is jelly confection.

According to the National Standardization Agency of Indonesia (2008), jelly confection is a soft-textured candy processed by adding hydrocolloid components such as agar, konjac, carrageenan, and others, which are used to modify the texture to produce a chewy product. Konjac or glucomannan has the advantage of forming gels with high viscosity and serves as a soluble dietary fiber that benefits digestive health. In addition, konjac can increase gel elasticity when combined with carrageenan. However, its weakness lies in producing gels that tend to be weak, brittle, and prone to syneresis, thus requiring a combination with other hydrocolloids to form stable gels (Adrianus et al., 2014). Carrageenan is widely used in the food industry because it can produce strong, rigid, and stable gels in processed dairy and meat products. This hydrocolloid also has the advantage of being able to interact with proteins and other polysaccharides. Nevertheless, carrageenan has drawbacks, including instability in low (acidic) pH, the formation of brittle gels when used alone, and a slightly astringent taste in food products (Annisa Narulita et al., 2019). Agar, on the other hand, has the advantage of forming clear, strong, and heat-resistant gels, even at low concentrations. Due to these properties, agar is widely applied in microbiological media and food products. However, the disadvantage of agar lies in the gels being relatively hard and less elastic, prone to high syneresis, and unstable against repeated heating and cooling cycles (Teti D. Suryaningrum et al., 2016). Therefore, in the development of functional food products, the incorporation of active ingredients derived from herbal plants has become an important approach.

One of the herbal plants, soursop (Annona muricata Linn), has traditionally been used in the treatment of various diseases. The antioxidant compounds contained in it play a role in inhibiting the activity of reactive free radicals, which tend to attract electrons from surrounding molecules. The level of antioxidant activity is generally expressed as the IC_{50} value, which represents the sample concentration required to inhibit 50% of free radical activity (Oktriyanto et al., 2023). Antioxidant activity, particularly in South Sulawesi from the regions of Gowa, Takalar, and Pinrang, showed IC_{50} values of 70.509 µg/mL, 102.159 µg/mL, and 99.246 µg/mL, respectively (Faradiba et al., 2024). These findings indicate that local herbal plants, including soursop leaves, have great potential as natural antioxidant sources. Therefore, soursop leaves were selected as the active ingredient in the production of jelly confections with the aim of optimizing their ability to prevent free radical activity. The formulation process was carried out using variations in gelling agent concentrations to evaluate their effects on antioxidant levels as well as the physical form and texture quality of the resulting jelly confections.

Based on this background, a study was conducted on the formulation of jelly confections using different ratios of gelling agent combinations and the evaluation of their antioxidant activity.

http://doi.org/10.22216/jk.v5i2.5717
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MATERIAL & METHODS

Materials

The materials used in this study included soursop leaves (annona muricata l.), agar, konjac, carrageenan, sucrose, stevia, and soursop essence. Additional reagents were 96% technical ethanol (merck), 70% ethanol, dpph (2,2-diphenyl-1-picrylhydrazyl) (sigma), and quercetin (merck).

Methods

1. Preparation of Extract

Soursop leaves were washed and subjected to wet sorting. The samples were dried by air circulation without direct exposure to sunlight. Once dried, the simplicia was powdered, and 500 g of the powder was extracted using the maceration method with 2.5 L of 70% ethanol. The concentrated soursop leaf extract was obtained through maceration for 3 × 24 hours, with remaceration (replacement of solvent) every 24 hours, followed by evaporation using a rotary evaporator at 40 °C (Faradiba et al., 2024).

Table 1. Formula Design

Composition	Function	F1	F2	F3
Soursop leaf extract	Active compound	2%	2%	2%
Agar	Gelling agent	9%	–	7%
Carrageenan	Gelling agent	6%	5%	–
Konjac	Gelling agent	–	1%	3%
Sucrose	Sweetener	10%	10%	10%
Stevia	Sweetener	20%	20%	20%
Soursop essence	Flavoring agent	0.2 mL	0.2 mL	0.2 mL
Citric acid	Preservative	0.2%	0.2%	0.2%
Distilled water	Solvent	100 mL	100 mL	100

2. Preparation of Jelly Confections

The process began by dissolving stevia in water, heating until completely dissolved (mixture 1), and filtering. Agar and carrageenan were dispersed in a beaker and heated until dissolved (mixture 2). Mixture 1 was then added into mixture 2 and stirred until homogeneous and thickened at 50 °C. Sucrose was added (with gentle stirring to avoid foam formation), followed by 0.2 mL of soursop essence, and the mixture was stirred until uniform. The solution was poured into molds and allowed to solidify, then placed in

http://doi.org/10.22216/jk.v5i2.5717
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an oven for 2 × 24 hours. The same procedure was carried out for formulations F2 and F3 (Oktriyanto et al., 2023).

3. Antioxidant Assay

a. Preparation of DPPH Solution

A total of 2.5 mg of DPPH powder was weighed and dissolved in 50 mL of 96% ethanol in a volumetric flask to obtain a DPPH solution with a concentration of 50 ppm. The procedure for preparing the blank solution was carried out in a light-protected room, and the solution was prepared for immediate use (Wimpy & Harningsih, 2017).

b. Preparation of Quercetin Solution

A total of 2.5 mg of quercetin powder was weighed and dissolved in 50 mL of 96% ethanol in a volumetric flask to obtain a quercetin solution with a concentration of 50 ppm.

c. Determination of Maximum Wavelength

From the 50 ppm DPPH stock solution, 200 µL was pipetted into a microtube, homogenized using a vortex, and incubated in the dark at room temperature for 30 minutes. After incubation, the solution was transferred into a 96-well plate and the absorbance was measured at wavelengths ranging from 400–700 nm using a microplate reader.

d. Preparation of Stock Solution and Absorbance Measurement

Quercetin stock solution was prepared at a concentration of 50 ppm. From this stock, a series of concentrations was made: 1 ppm, 2 ppm, 3 ppm, 4 ppm, 5 ppm, and 6 ppm, each with a total volume of 5 mL. Each quercetin solution (150 µL) was pipetted into a microtube, then mixed with 150 µL of freshly prepared DPPH solution, homogenized using a vortex, and incubated in the dark at room temperature for 30 minutes. After incubation, 200 µL of each concentration was transferred into a 96-well plate, and absorbance was measured at 518 nm.

e. Preparation of Sample Concentration Series

A stock solution of 1,000 ppm was prepared by weighing 10 mg of each jelly candy formulation and dissolving it with 96% ethanol in a 10 mL volumetric flask up to the mark. From this stock, a series of concentrations (20, 40, 60, 80, and 100 ppm) was prepared, each diluted with 96% ethanol to a total volume of 5 mL (Zaddana et al., 2024).

f. Antioxidant Activity Test Using DPPH Method

The series of test solutions, positive control (quercetin), and blank solution were measured for absorbance at the maximum wavelength obtained (Zaddana et al., 2024).

RESULT AND DISCUSSION

Antioxidant Activity Test Using the DPPH Method

The antioxidant activity tested using the DPPH method is one of the most commonly applied approaches in antioxidant activity analysis, as it offers several important advantages.

Table 2. IC_{50} IC_{50} Values of Quercetin, Soursop Leaf Extract, and Jedy Formulas

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Sample	Concentration (ppm)	Blank Absorbance	Sample Absorbance	% Inhibition	IC_{50} (µg/mL
Quercetin	1	0,797	0,435	45,420	2,086
	2	0,797	0,408	48.808	
	3	0,797	0,365	54,203	
	4	0,797	0,327	58,971	
	5	0,797	0,296	62,860	
	6	0,797	0,273	65,746	
Soursop Leaf Extract (SLE)	10	0,797	0,382	52,070	5,777
	20	0,797	0,362	54,579	
	30	0,797	0,327	58,971	
	40	0,797	0,304	61,856	
	50	0,797	0,269	66,248	
	60	0,797	0,240	69,887	
Formula 1 (Carrageenan 9% : Agar 6%)	10	0,797	0,524	34,253	28,952
	20	0,797	0,449	43,663	
	30	0,797	0,393	50,690	
	40	0,797	0,324	59,347	
	50	0,797	0,264	66,875	
	60	0,797	0,209	73,776	
Formula 2 (Carrageenan 5% : Konjac 1%)	10	0,797	0,386	51,568	8,823
	20	0,797	0,365	54,203	
	30	0,797	0,320	59,849	
	40	0,797	0,297	62,735	
	50	0,797	0,261	67,252	
	60	0,797	0,243	69,510	
Formula 3	10	0,797	0,475	40,401	30,333
(Konjac 7% : Agar	20	0,797	0,442	44,542	

http://doi.org/10.22216/jk.v5i2.5717
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3%)	30	0,797	0,420	47,302
	40	0,797	0,342	57,089
	50	0,797	0,277	65,244
	60	0,797	0,221	72,271

Table 3. ANOVA Test of Average Antioxidant Activity

ANOVA

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	2426.091	11	220.554	8.150	.000
Within Groups	487.102	18	27.061		
Total	2913.193	29			

Table 4. Results of Tukey HSD Post-Hoc Test on Mean Antioxidant Activity

Formula	Mean ± SD
Quercetin	56,00 ± 7,97
Soursop Extract	58,74 ± 5,65
Formula F1	56,92 ± 14,63
Formula F2	60,90 ± 7,02
Formula F3	54,10 ± 12,55

The testing showed that the IC_{50} value of quercetin as a positive control was 2.086 µg/mL. The antioxidant activity assay revealed that pure soursop leaf extract had an IC_{50} value of 5.777 ppm, which is classified as very strong. After being formulated into jelly candies with different combinations of gelling agents, the IC_{50} values increased to varying degrees. Formula 2 (konjac–carrageenan) exhibited the lowest IC_{50} value of 8.823 ppm, whereas Formula 1 (carrageenan–agar) and Formula 3 (konjac–agar) showed IC_{50} values of 28.952 ppm and 30.333 ppm, respectively. In this study, each jelly candy formula was weighed at 10 mg, equivalent to 1 mg of soursop leaf extract, ensuring that the amount of extract tested was the same across all formulas. Therefore, the observed differences in IC_{50} values were not due to the amount of extract but rather influenced by the type and combination of gelling agents used in the formulations.

These differences occurred because the properties of each gelling agent affected the release of active compounds from the soursop leaf extract. The konjac–carrageenan

http://doi.org/10.22216/jk.v5i2.5717
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combination produced a more open gel structure with better porosity, enabling polyphenolic compounds in the extract to be more easily released and interact with free radicals. In addition, carrageenan contains negatively charged sulfate groups that may contribute slightly to antioxidant activity; thus, when combined with konjac, it helped maintain antioxidant potency close to that of the pure extract. In contrast, the combinations with agar (carrageenan–agar and konjac–agar) formed gels with tighter and stronger structures, entrapping more of the active compounds within the gel matrix. As a result, fewer antioxidants were available to react with free radicals, leading to higher IC_{50} values.

These findings are consistent with Aburizal et al. (2020), who reported that the formulation process of jelly candies can reduce vitamin C content and antioxidant activity due to interactions with gelling agents. Similarly, Mira et al. (2017) showed that the antioxidant activity of papaya juice decreased after being formulated into jelly candies, likely due to degradation of active compounds and entrapment of antioxidants in the gel matrix. Furthermore, Yuan et al. (2005) noted that the presence of sulfate groups in carrageenan may contribute to free radical scavenging activity, although its antioxidant capacity is relatively low compared to phenolic compounds. Thus, it can be concluded that although the concentration of gelling agents used was the same (2%), the combination of gelling agent types played a crucial role in the availability of active compounds. Formula 2 (konjac–carrageenan) proved to be more effective in maintaining the antioxidant activity of soursop leaf extract compared to carrageenan–agar or konjac–agar combinations.

Based on the One Way ANOVA analysis, the F-value was 8.150 with a significance value (p) = 0.000 (p < 0.000). These results indicate that there were significant differences in the mean inhibition among treatment groups. In other words, the treatments had a significant effect on inhibition activity. Further analysis using Tukey's test showed that Formula 2 and the pure soursop leaf extract exhibited higher inhibition values and were significantly different compared to some other formulas. Meanwhile, the inhibition values of quercetin, Formula 1, and Formula 3 were relatively not significantly different.

These findings indicate that Formula 2 has the best potential in enhancing inhibition activity compared to the other formulas, although its effectiveness is still comparable to that of the pure soursop leaf extract. Overall, these results demonstrate that formulation variations influence the resulting inhibition activity. The differences may be attributed to interactions with additional ingredients (such as gelling agents) as well as the stability of bioactive compounds within the formulation. Formula 2, which showed the highest inhibition value, is likely to possess a more optimal combination of gelling agents in maintaining or enhancing the activity of bioactive compounds from soursop leaf extract.

CONCLUSION

This study demonstrated that soursop leaf jelly confections exhibit varying antioxidant activities depending on the gel matrix combinations used. The carrageenan:konjac (5:1) formulation showed the lowest ic_{50} value (8.82 µg/ml), indicating the strongest antioxidant activity compared to other formulations. Therefore, this formulation has the potential to be developed as a nutraceutical product in the form of functional jelly confections.

ACKNOWLEDGEMENT

http://doi.org/10.22216/jk.v5i2.5717
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This research was funded by the ministry of higher education, science, and technology (kemdiktisaintek), republic of indonesia, supported by the research and community service institute (lp2s), universitas muslim indonesia, and the faculty of pharmacy, universitas muslim indonesia (umi).

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