Borneo Journal of Pharmacy
Vol 7 Issue 3 August 2024
Pages 297 – 305

https://journal.umpr.ac.id/index.php/bjop/article/view/4640
DOI: https://doi.org/10.33084/bjop.v7i3.4640
e-ISSN: 2621-4814

Research Article

Formulation of Nutraceutical Jelly Candy from a Combination of *Cucurbita moschata* Puree and *Averrhoa carambola* Juice as Antioxidant

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Keywords:
Antioxidant
Jelly candy
Sweet starfruit
Yellow pumpkin

Abstract

Nutraceutical candy jellies are gaining popularity as a potential approach to deliver antioxidants in a palatable form. This study investigated the antioxidant activity of *Cucurbita moschata* puree combined with *Averrhoa carambola* juice, formulated into jelly candies. Design-Expert software V.13 was used to optimize the jelly candy base formula. The combined *C. moschata* puree and *A. carambola* juice exhibited strong antioxidant activity (IC_{50} = 29.580 ppm) at variation V1. The optimal base formula B3 consisted of 12% gelatin and 4% carrageenan. The formulated jelly candy possessed very strong antioxidant activity (IC_{50} = 44.771 ppm). These findings suggest the potential of *C. moschata* puree and *A. carambola* juice as ingredients in functional jelly candies.

Received: January 19th, 2023
1st Revised: March 23rd, 2023
2nd Revised: July 18th, 2023
3rd Revised: June 3rd, 2024
Accepted: June 20th, 2024
Published: August 30th, 2024

INTRODUCTION

Indonesia's rich biodiversity includes numerous medicinal plants with potential therapeutic applications. Many of these plants contain secondary metabolites that could serve as valuable components in pharmaceutical preparations[1]. Pharmaceutical preparations encompass a wide range of products, including chemical drugs, traditional medicines, cosmetics, and health supplements. Among the essential compounds required by the human body are antioxidants, which play a crucial role in neutralizing harmful free radicals. Free radicals are highly reactive molecules capable of causing complex cellular damage, leading to oxidative stress and contributing to the development of degenerative diseases[2].

Yellow pumpkin or *Cucurbita moschata*, a widely cultivated horticultural crop in Indonesia, holds potential as a source of bioactive compounds. Characterized by its taproot, hollow stems, five-lobed leaves, and yellow unisexual flowers, *C. moschata* has been a significant agricultural commodity in Indonesia, with production reaching 369,846 tons in 2010[3]. Despite its abundance, the full potential of *C. moschata* remains largely untapped.

Cucurbita moschata is rich in beta-carotene, a provitamin-A with potent antioxidant properties. Its antioxidant activity, measured at 30.75 ppm, is significantly higher than that of chayote, another *Cucurbita* species with an IC_{50} of 440.26 ppm[4]. Beyond beta-carotene, *C. moschata* contains a diverse array of secondary metabolites, including polyphenols, tannins, phenolic acids, and flavonoids such as quinic acid, p- coumaric acid, trans-cinnamic acid, cirsiliol, and luteolin, which further contribute to its antioxidant potential[5]. Additionally, *C. moschata* is a source of essential nutrients like carbohydrates, proteins, lipids, vitamins (including vitamin C and niacin), and minerals (Ca, K, Cu, Na, P, Fe, Zn)[6,7].

Sweet starfruit or *Averrhoa carambola*, a tropical fruit widely cultivated in regions like Indonesia, also holds potential for pharmaceutical applications. Characterized by its distinctive star shape, the fruit exhibits a yellow to light yellow hue,

How to cite: Soraya AF, Kawareng AT, Agustina R. Formulation of Nutraceutical Jelly Candy from a Combination of Cucurbita moschata Puree and Averrhoa carambola Juice as Antioxidant. Borneo J Pharm. 2024;7(3):297-305. doi:10.33084/bjop.v7i3.4640

translucent texture, and a juicy flavor reminiscent of oxalic acid[8]. The *A. carambola* tree reaches heights of 6-9 meters, featuring numerous branches and compound leaves with 3-6 pairs of leaflets[9].

Phytochemical analysis of *A. carambola* reveals a rich composition of bioactive compounds. Notably, it contains flavonoids (4.22%) and saponins, demonstrating its antioxidant potential[10]. The juice of *A. carambola* exhibits a strong antioxidant activity of 78.797 ppm, attributed in part to its vitamin C content of 1.232 mg/mL[11]. Additionally, the fruit contains gallic acid (0.96%), protocatechuic acid (0.05%), and quercetin (0.40%)[8]. Other essential nutrients include calcium, phosphorus, iron, sodium, potassium, zinc, vitamin C, and thiamin[7].

The pharmaceutical industry has witnessed significant growth, driven by the development of products that enhance health and prevent diseases. Nutraceuticals, derived from the words "nutrition" and "pharmaceutical," represent a class of functional foods with potential health benefits beyond their nutritional value[12]. These compounds have gained prominence as alternative or complementary therapies for various health conditions, including degenerative, non-degenerative, and neurodegenerative diseases. The appeal of nutraceuticals lies in their generally mild side effects and nutritional value, making them attractive options for health-conscious individuals seeking natural remedies[13].

Nutraceutical jelly candies offer a convenient and appealing delivery method for bioactive compounds. While they are susceptible to melting at room temperature, their attractive sensory properties, including color, smell, taste, and texture, make them suitable for all ages[14]. Previous studies have demonstrated the successful formulation of nutraceutical jelly candies using *C. moschata* as the active ingredient, with products meeting Indonesian National Standards (SNI 3547.2-2008)[15]. Similarly, *A. carambola* has been incorporated into jelly candies alongside pineapple fruit, resulting in favorable sensory evaluations[16]. Building upon these findings, this study aimed to develop nutraceutical jelly candies incorporating both *C. moschata* puree and *A. carambola* juice. The antioxidant properties of these ingredients were evaluated using the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) method.

MATERIALS AND METHODS

Materials

Cucurbita moschata and *A. carambola* specimens were procured from Samarinda City and authenticated at the Dendrology Laboratory of the Faculty of Forestry, Universitas Mulawarman, Samarinda, East Borneo, Indonesia. The specimens were assigned the numbers 188/UN17.4.08/LL/2022 and 189/UN17.4.08/LL/2022, respectively. For experimental purposes, a blender, centrifuge, hotplate, mixers, and a UV-Vis spectrophotometer (Genesys 10S) were utilized. The materials employed included distilled water, DPPH, gelatin, carrageenan, methanol, sodium propionate, sorbitol, and *A. carambola* flavoring. **Figure 1** depicts the *C. moschata* and *A. carambola* specimens.

a **b**

Figure 1. *Cucurbita moschata* (**a**) and *A. carambola* (**b**).

Methods

Sample preparation

Cucurbita moschata fruits were peeled, deseeded, and washed under running water. The flesh was then cut into 2 x 2 x 2 cm cubes and steamed for 35 minutes at 45-50°C. The steamed flesh was subsequently mashed using a mixer. Ripe *A. carambola* fruits were peeled, deseeded, washed, and cut into small pieces before being blended. The resulting pulp was filtered using a filter cloth to remove any remaining solids.

Optimization of active substances

The main ingredients are combined and varied into three concentrations as shown in **Table I**. Then the antioxidant activity of each variation using the DPPH method with the aim of obtaining the optimal formula that has the strongest antioxidant activity. Stock solutions of the active substances were prepared by dissolving them in methanol to a concentration of 200 ppm in a 100 mL volumetric flask. Serial dilutions were then performed to obtain test solutions at concentrations of 20, 40, 60, 80, and 100 ppm in 5 mL volumetric flasks. Two milliliters of each test solution were transferred to aluminum foil-covered test tubes and mixed with 2 mL of a 40 ppm DPPH solution. The mixtures were incubated in the dark at room temperature for 30 minutes.

The absorbance of the samples was measured at the maximum wavelength of DPPH using a UV-Vis spectrophotometer. Measurements were performed in triplicate. The percentage of DPPH inhibition and IC_{50} values were calculated using the linear regression equation $y = bx + a$, where y represents the probit percent inhibition (5), x represents the concentration of the test solution, and IC_{50} is the antilog of the x-intercept. The equation for calculating percent inhibition is provided in **Equation 1**, and the equation for calculating IC_{50} is provided in **Equation 2**, in which a and b represent intercept and slope from the linear regression equation, respectively.

Table I. Variation in concentration of active ingredients.

Formula	*Cucurbita moschata* puree (g)	*Averrhoa carambola* juice (g)	Ratio
V1	1	3	1 : 3
V2	3	1	3 : 1
V3	2	2	1 : 1

$$\%inhibition = \frac{(absorbance_{control} - absorbance_{sample})}{absorbance_{control}} \text{x} 100\% \quad [1]$$

$$IC_{50} = \frac{5 - a}{b} \quad [2]$$

Optimization of jelly candy formula base

Simplex Lattice Design (SLD) using Design Expert software (version 13) was employed to optimize the jelly candy base formulation. Gelatin and carrageenan were selected as independent variables, while water content served as the response variable. The experimental design parameters were inputted into Design Expert, and the generated design matrix was used to prepare jelly candy base formulations with varying gelatin and carrageenan concentrations. The water content of each formulation was measured using a moisture analyzer. The resulting data were analyzed using Design Expert to determine the optimal jelly candy base formulation.

Jelly candy formulation

Gelatin and carrageenan, serving as gelling agents, were dissolved in hot water (80°C) under constant stirring to prepare the jelly candy base. Sorbitol, a sweetener and plasticizer, and sodium propionate, a preservative, were then incorporated into the base. The main ingredients, *C. moschata* puree and *A. carambola* juice, were added at an optimal concentration (previously determined) to the base at a temperature of 45-50°C to form the jelly candy dough. The dough was poured into molds and allowed to set at room temperature for 1 hour, followed by refrigeration for 24 hours. Finally, the jelly candies were removed from the refrigerator and allowed to stand at room temperature for 1 hour to obtain the finished product (**Figure 2**).

Antioxidant activity test of jelly candy

Jelly candy samples (1 g each) were dissolved in methanol p.a. and water, followed by centrifugation at 3500 rpm for 10 minutes. The supernatant was collected and diluted with methanol p.a. to a final concentration of 200 ppm, creating a stock solution. Serial dilutions were prepared from this stock solution to obtain concentrations of 20, 40, 60, 80, and 100 ppm in 5 mL measuring flasks. The antioxidant activity of the test solutions was assessed using the DPPH method. Two milliliters of each test solution and 2 mL of DPPH solution were mixed in a test tube, sealed, and shielded from light with aluminum foil. The absorbance of the samples was then measured at the maximum wavelength of DPPH.

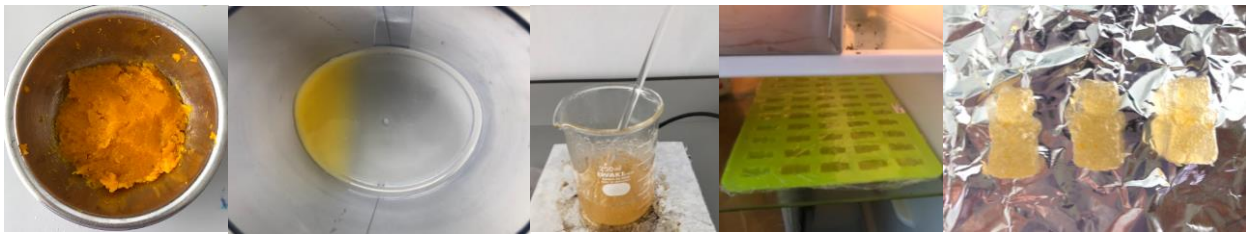


Figure 2. Representative images of the jelly candy preparation process. *Cucurbita moschata* puree (**a**), *A. carambola* juice (**b**), heated (**c**) and refrigerated jelly candy dough (**d**), and final jelly candy product (**e**).

Data analysis

Antioxidant activity was assessed using the DPPH method with three replicates. The percentage inhibition of DPPH and IC_{50} values were calculated using linear regression analysis, where the equation $y = bx + a$ was employed. The variable y was set to 5, and the antilog of x represented the IC_{50} value.

RESULTS AND DISCUSSION

The antioxidant activity of the combined *C. moschata* puree and *A. carambola* juice extracts were evaluated using the DPPH method. The selected *C. moschata* fruits were ripe, characterized by their orange skin and flesh, as previous research by Majid[17] demonstrated that mature *C. moschata* exhibits higher beta-carotene content (3.915 µg/g) compared to less ripe varieties (1.742 µg/g). The *A. carambola* fruits used were greenish-yellow, a color associated with higher vitamin C content (25.9 mg/100 g) compared to raw (25.2 mg/100 g) or fully ripe (23.4 mg/100 g)[18]. The IC_{50} values obtained from the combined extracts at various concentrations (V1= 1 : 3, V2= 3 : 1, V3=: 1 : 1) were 29.580±0.06, 38.905±0.03, and 55.463±0.08 ppm, respectively. Both V1 and V2 exhibited very strong antioxidant activity (<50 ppm), while V3 demonstrated strong antioxidant activity (50-100 ppm)[19]. A comparison of these IC_{50} values is presented in **Figure 3**.

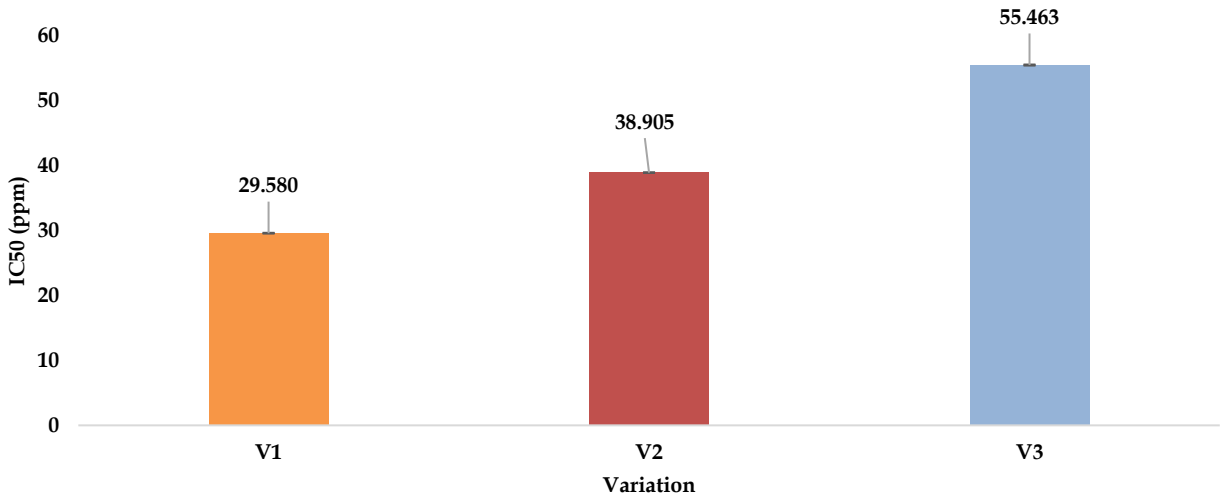


Figure 3. Antioxidant activity test of *C. moschata* puree and *A. carambola* juice extracts combination.

The DPPH method, a widely employed technique for evaluating antioxidant activity, was chosen due to its simplicity, rapidity, and ease of spectrophotometric measurement[20]. The method relies on the principle of DPPH free radicals scavenging electrons from antioxidant compounds, leading to a reduction in the characteristic purple color of DPPH. The IC_{50} value, representing the concentration of the test compound required to reduce DPPH free radicals by 50%, serves as a quantitative measure of antioxidant activity. A lower IC_{50} value indicates stronger antioxidant activity[21]. Based on our analysis, the optimal concentration ratio was determined to be V1. This formulation demonstrated the highest antioxidant activity as evidenced by the lowest IC_{50} value, suggesting its superior ability to scavenge free radicals.

The antioxidant activity of the *C. moschata* and *A. carambola* combination is attributed to the synergistic interaction of their respective phytochemical constituents. *Cucurbita moschata* contains a notable concentration of beta carotene (14.59%)[4] and various polyphenols (53.02 ± 1.56 mg GAE/g), including tannins (25.65 ± 0.08 mg CE/g), phenolic acids (quinic acid, p-coumaric acid, trans-cinnamic acid), and flavonoids (cirsiliol, luteolin)[6]. Similarly, *A. carambola* is characterized by a high content of flavonoids (4.22%)[10], vitamin C (1.232 mg/mL)[11], gallic acid (0.96%), protocatechuic acid (0.05%), and quercetin (0.40%)[8]. The combined presence of these antioxidant compounds in the two ingredients likely contributes to their enhanced free radical scavenging capacity.

Gelatin and carrageenan emerged as the optimal gelling agents in this study, contributing significantly to the jelly candy's texture and stability. Gelatin, a well-known gelling agent, forms a thermally reversible gel, meaning it can transition between sol and gel states depending on temperature[22,23]. This property makes gelatin preferable to irreversible gelling agents like pectin and gum Arabic[24]. While both gelatin and carrageenan can function as stabilizers, thickeners, and gel formers, carrageenan-based gels tend to be more brittle and less elastic. Therefore, combining gelatin and carrageenan can create a more desirable texture, with gelatin providing the necessary elasticity and carrageenan offering additional stability and structure[25].

Optimization of jelly candy formulation was conducted using Design Expert. Gelatin and carrageenan concentrations were identified as independent variables, while water content served as the dependent variable. Experimental formulations were prepared and analyzed for water content. The resulting data were evaluated using SLD method, yielding five optimal formulations presented in **Table II**.

Table II. Recommendations formula of jelly candy base.

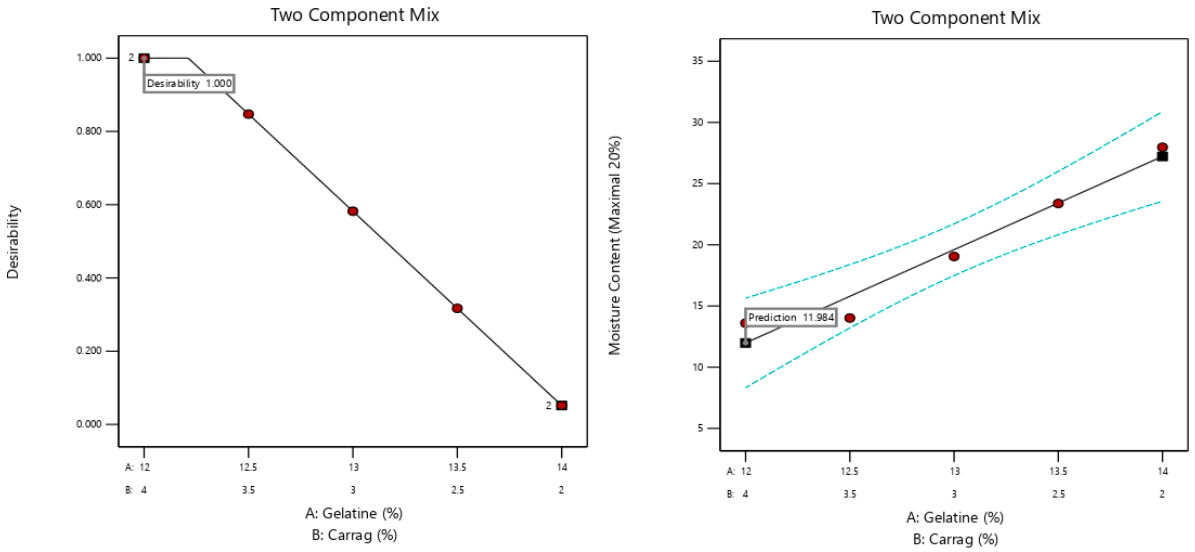
Material	Formula (%)					Material function
	B1	B2	B3	B4	B5	
Gelatin	14	13.5	13	12.5	12	Gelling agent
Carrageenan	2	2.5	3	3.5	4	Gelling agent
Sorbitol	10	10	10	10	10	Sweetener and plasticizer
Sodium propionate	0.3	0.3	0.3	0.3	0.3	Preservative
Averrhoa carambola flavour	0.25	0.25	0.25	0.25	0.25	Flavoring
Distilled water	ad 100	ad 100	ad 100	ad 100	ad 100	Solvent

Table III summarizes the water content analysis results for the various jelly candy formulations. Water content is a crucial factor in determining product quality and durability, as excessive moisture can lead to microbial growth and spoilage, while insufficient moisture can compromise texture and prevent microbial growth[26]. Two formulations, B1 and B2, were found to exceed the maximum water content limit of 20% as specified in SNI 3547.2-2008. This deviation was attributed to the higher gelatin content in these formulations compared to the others. The increased gelatin concentration likely facilitated the formation of a denser three-dimensional network within the gel matrix, capable of binding and retaining a greater amount of water[22].

Table III. Water content of jelly candy formula base.

Formula	Water content (%)			Average	Parameter
	R1	R2	R3		
B1	28.41	29.02	26.48	27.97±1.33	Max. 20%[27]
B2	25.99	21.35	22.79	23.38±2.37	
B3	19.08	19.04	19.03	19.05±0.03	
B4	13.61	14.1	14.34	14.02±0.37	
B5	13.46	13.59	13.74	13.60±0.14	

Optimization using SLD resulted in the counter-plot graphs depicted in **Figure 4**. The optimal emulgel formulation was identified as B5, containing 12% gelatin and 4% carrageenan. This formulation exhibited a desirability value of 1.00, indicating its optimal alignment with the desired response criteria. Desirability values range from 0 to 1, with values closer to 1 representing formulations that are closer to the desired target[28]. **Figure 4** illustrates the prediction results, demonstrating a direct correlation between increasing gelatin concentration and a corresponding increase in water content. The SLD-derived solution, consisting of 12% gelatin and 4% carrageenan, is presented in **Table IV**.



a b

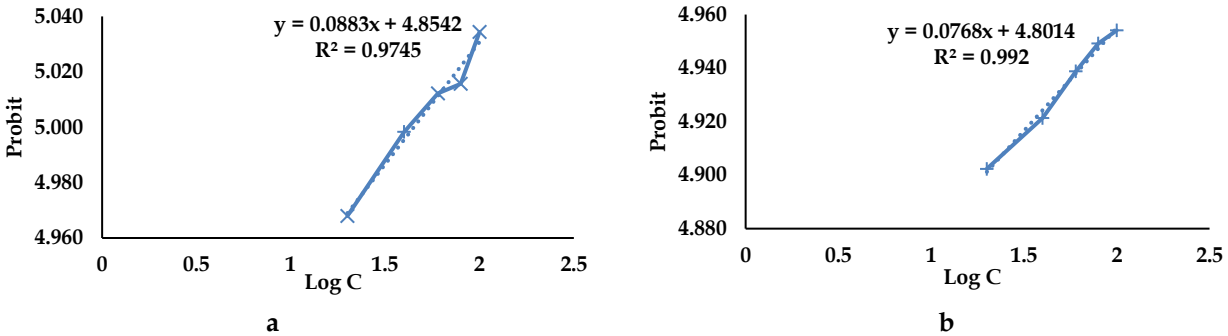
Figure 4. Desirability results from base optimization (**a**) and predicted moisture content results of jelly candy base using the SLD method (**b**).

Table IV. Optimal jelly candy base solution based on SLD method.

Gelatin (%)	Carrageenan (%)	Desirability	Decision
12	4	1.000	Selected

The antioxidant activity of the developed jelly candy formulations was evaluated using the linear regression equation depicted in **Figure 5**. The IC_{50} values, which indicate the concentration required to inhibit the DPPH radical by 50%, were determined for each formulation. The results demonstrated that the jelly candy formulations exhibited a strong antioxidant activity, with an IC_{50} value of 44.771 ppm. In contrast, the jelly candy base alone exhibited a very weak antioxidant activity, with an IC_{50} value of 385.478 ppm[19].

Interestingly, the IC_{50} values of the jelly candy formulations were slightly higher than those of the combined *C. moschata* puree and *A. carambola* juice (IC_{50} = 29.580 ppm). This suggests that the jelly candy base might have a modest inhibitory effect on the antioxidant activity of the combined ingredients. However, the jelly candy formulations still maintained a very strong antioxidant activity category.



a b

Figure 5. Desirability results from base optimization (**a**) and predicted moisture content results of jelly candy base using the SLD method (**b**).

CONCLUSION

Our findings demonstrate that *C. moschata* puree and *A. carambola* juice can be effectively combined to create nutraceutical jelly candies with potent antioxidant properties. The use of gelatin and carrageenan as gelling bases further enhances the formulation's stability and texture.

ACKNOWLEDGMENT

The authors would like to express their sincere gratitude to the Tropical Farmaka Laboratory, Faculty of Pharmacy, Universitas Mulawarman, for their invaluable support in conducting this research. A portion of this work was presented at the 16th Mulawarman Pharmaceuticals Conference held on November 15-17, 2022, at Universitas Mulawarman, Samarinda, Indonesia.

AUTHORS' CONTRIBUTION

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Funding acquisition: -
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Methodology: Andi Tenri Kawareng, Risna Agustina
Project administration: -
Resources: Andi Tenri Kawareng, Risna Agustina
Software: -
Supervision: Andi Tenri Kawareng, Risna Agustina
Validation: Andi Tenri Kawareng, Risna Agustina
Visualization: -
Writing - original draft: Alya Fajrina Soraya
Writing - review & editing: Andi Tenri Kawareng, Risna Agustina

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

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