Development and Evaluation of Anti-Gout Jam Formulation from Jamaican Cherry (*Muntingia calabura)* and Stevia (*Stevia rebaudiana* Bertoni): Antioxidant Activity and Uric Acid Reduction Potential

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Received: April-15-2025; Accepted: Oktober-29-2025; Published: December-30-2025

Abstract

This study examines the potential of Jamaican cherry (JC) and stevia, a natural sweetener, as an anti-gout jam. It aims to evaluate the effects of JC leaf and stevia extracts on the jam's chemical, physical, and organoleptic qualities, finding the optimal formulation and ascertaining whether feeding JC jam lowers mice's blood uric acid levels. A complete block design with two factors was applied. The two factors were JC leaf extract (A1: 30%, A2: 50%, A3: 70%) and stevia extract (B1: 1%, B2: 2%, B3 3%). The findings indicate that, while there was no significant shift in the jam's viscosity, sugar reduction, color, flavor, aroma, or spreadability, adding more JC leaf extract did affect its pH and moisture content. Similarly, stevia extract did not affect flavor, color, spreadability, viscosity, and sugar reduction, but it significantly impacted pH, moisture content, and aroma. The ideal jam formulation was a blend of 70% JC leaf extract (A3) and 3% stevia extract (B3), with a preference score of 4.66 (neutral), a viscosity of 387.50 cP, a pH of 4.16, a moisture content of 59.38%, and a sugar reduction of 1.85%. According to the results of the in vitro test, the JC jam successfully lowered uric acid levels to normal conditions at 4.92 mg/dL.

Keywords: *gout; jam; jamaican cherry; stevia.*

1. INTRODUCTION

Healthy lifestyles are becoming less prioritized as human activity increases and times change. Fast food habits that include the use of preservatives can lead to various health issues. Among these, foods high in protein and those containing alcohol can increase blood uric acid levels. Purine, a component of protein, is metabolized by the kidneys to produce uric acid, which can lead to gouty arthritis, also known as gout. The kidneys regulate the body's ability to maintain stable levels of uric acid. Uric acid will build up in the tissues and joints and cause severe pain if the levels are too high and the kidneys are unable to control their stability. Long-term exposure to uric acid crystals can lead to the formation of kidney stones (Hananto & Setiadi, 2024; Johnson et al., 2023).

Nowadays, there are many medications and artificial sweeteners on the market that contain extra chemicals that are bad for the body. Since these medications and sweeteners have several adverse effects, a safer alternative for treating gout is needed. This alternative is the use of Jamaican cherry (*Muntingia calabura*) fruits and leaves. The flavonoids, tannins, triterpenes, saponins, and polyphenols found in Jamaican cherry (JC) fruit and leaves themselves exhibit antioxidant properties. By blocking the xanthine oxidase enzyme, which acts as a catalyst in the oxidation process of hypoxanthine to xanthine and subsequently to uric acid, flavonoids themselves can lower blood uric acid levels (Meiliza, 2013). It has been demonstrated that leaf decoction and JC fruit juice, as investigated by Meiliza (2013), lower uric acid levels (Ilkafah, 2018a). According to Kholifaturrokhmah and Purnawati (2016) the normal ranges for uric acid levels in men are 3.4–7.0 mg/dL and in women, 2.4–6.0 mg/dL.

Agroindustrial Journal Vol. 12 (No. 2), 2025, 66-74, doi: 10.22146/aij.v12i2.106016

People often use JC fruits and their leaves, which have a bitter flavor, either by eating them or by boiling them. For this reason, it is vital to have other processed goods that people enjoy, such as jam. In pre-diabetes, hyperuricemia is one of the metabolic abnormalities linked to hyperinsulinemia, which is why Siregar and Nurkhalis (2015) found a positive correlation between blood sugar levels and uric acid levels.

According to Nasrul and Sofitri (2012) insulin resistance and the hyperinsulinemia system mediate the link between uric acid and reduced sugar tolerance. With the contribution of oxygen and water, insulin resistance, hypoxia, and cell death can alter xanthine, which will then transform into uric acid and peroxide. The oxygen-free radical peroxide itself has the ability to alter the nitric oxide (NO) balance, which is crucial for maintaining the equilibrium of vascular tone. Furthermore, oxidative stress in metabolic syndrome is associated with elevated uric acid levels. As a result, pre-diabetic hyperinsulinemia will make the body reabsorb more, which will raise uric acid levels. Blood sugar levels may fluctuate after consuming fast food that is high in fat, sugar, or protein. Depending on the variables that affect blood sugar levels, alternative solutions are needed as a sugar substitute, including the use of natural ingredients like stevia.

An all-natural sweetener, stevia leaves (*Stevia rebaudiana* Bertoni) are 300 times sweeter than sucrose. The properties of stevia include antimicrobial, antifungal, antibacterial, antiviral, and anti-inflammatory properties. As a natural plant product, stevia's leaves yield the diterpene glycosides (stevioside and rebaudioside), which have been extensively tested on animals and used by humans without causing any adverse effects, as a sweetener to replace sucrose (cane sugar) and are advised for people with diabetes and those following a diet (Buchori, 2007). Based on the explanation above, to add additional value to JC leaves and fruits, their effect on uric acid must be investigated. This study aims to evaluate the effect of JC leaves and stevia extracts on the chemical, physical, and organoleptic quality of jam, to find the optimal formulation, and to determine whether giving JC jam can reduce uric acid levels in the blood of mice. The study will ensure that they have functional value as an anti-gout that can be tested in vivo and that individuals with high blood sugar levels can safely consume them.

2. MATERIAL AND METHODS

2.1 Equipment and Materials

Spoons, pans, scales, basins, knives, blenders, measurement cups, droppers, funnels, measuring flasks, test tubes, and cups were among the equipment used in the study. JC fruits and leaves, stevia leaves, sugar, citric acid, PB-acetate, arsenomolybdate reagent, Nelson reagent, white mice, chicken liver, and allopurinol were the components employed in this investigation.

2.2 Study Area and Period

This research was conducted at the Pilot Plan and Laboratory of the Faculty of Agricultural Technology, Institut Pertanian Stiper, with a research period of five months (March – July 2019).

2.3 Experimental Design

The addition of cherry leaf extract was based on a total weight of 200 grams of material. This means that the amount of cherry leaf extract added to each treatment was:

A1 (30%) = 60 grams of extract
A2 (50%) = 100 grams of extract
A3 (70%) = 160 grams of extract

The second factor was the amount of stevia addition, namely:

B1 = 1 %
B2 = 2 %
B3 = 3 %

2.4 Methods

The production of JC leaf extract is made from fresh leaves weighed according to treatments of 30%, 50%, and 70% of the total weight of the material. The JC leaves are ground, then 200 mL of

water is added, and the mixture is ground again. The ground mixture is then filtered. Stevia extract is made from stevia powder, which is mixed with water in a ratio of 1:3 (w/v), and then filtered using a vacuum filter.

Jam production begins with 200 grams of cherries as the main ingredient. The cherries are first washed to remove dirt and contaminants. Next, the leaf extract is added according to the treatment. The mixture of cherries and leaf extract is then blended to obtain a smooth consistency. Then, 20 grams of sugar and 10 mL of citric acid are added to the mixture, serving as additional sweeteners and acidity regulators. The next step involves adding stevia extract as an additional natural sweetener, as specified in the treatment. The mixture is then cooked until it thickens, forming the characteristic texture of jam. The final product, cherry jam, is packaged in sterile bottles to ensure quality and maintain its shelf life.

This study evaluated the following parameters: viscosity, pH, moisture content, reducing sugar content, organoleptic properties (taste, color, spreadability, and aromas), and an in vivo test using white mice (*Mus musculus*). The organoleptic evaluation was conducted using the Seven Hedonic Scale Different Test. A total of 20 semi-professional panelists-college students aged 17 to 25 years-participated in the sensory evaluation. Data analysis was performed using Analysis of Variance (ANOVA) to identify significant differences among treatment groups. When significant differences were found, Duncan Multiple Range Test (DMRT) was applied as a post-hoc test at the 5% significance level.

3. RESULTS AND DISCUSSION

3.1 Antioxidants in Jamaican Cherry Fruits and Leaves

It is well known that uric acid, a potent antioxidant in humans, can scavenge free radicals such as peroxynitrite, which may lead to oxidant imbalances and oxidative stress (Singh et al., 2022). JC fruits and leaves may be a source of antioxidants, which could help avoid these issues. The antioxidant activity of the JC fruits and leaves used in this study was analyzed, and the results are shown in Table 1, with values of 64.37% and 69.97%, respectively.

Table 1. Antioxidant activity of cherries and cherry leaves

Analysis	Fruits	Leaves
Antioxidant activity	64,37%	69,97%

Given this antioxidant content, it is anticipated that JC fruits and their leaves may be effective in treating gout. JC leaves' anti-inflammatory qualities can lessen joint discomfort by preventing inflammation in those locations. The total phenol content of JC fruit is 21.32 mg GAE/g, but the total phenol content of its leaf extract is 1.163 mg QE/g (Noorhamdani, 2014). Kholifaturrokhmah & Purnawat (2016) states that the flavonoids in JC fruit contain quercetin. Quercetin can lower blood uric acid levels on its own. By blocking the activity of the enzyme xanthine oxidase, which synthesizes uric acid, quercetin lowers uric acid levels.

3.2 Viscosity of Jamaican Cherry Jam

Viscosity analysis is the method of determining a liquid's thickness. Table 2 shows the results of a measurement of the JC jam's viscosity. The final JC jam's viscosity was not considerably impacted by the quantity of JC leaves used as an additive, according to the treatment given. In order to make jam, JC leaves are first produced from their extracted, to which the same amount of water is added. This ensures that the JC extract has a low level of dissolved solids. When up to 70% leaf extract was added, the jam's viscosity remained unchanged. Apriani (2013) asserts that the moisture content of a material also influences its viscosity.

Fruit jam is defined by the National Standardization Agency (1995) in SNI-01-3745-1995 as a semi-wet food made from fruit pulp that has been processed with a mixture of sugar that contains at least 45% by weight of fruit juice and 55% by weight of sugar. In contrast, just 10% sugar was used

in this JC jam study; instead, stevia juice was employed. When jam is made without sugar, the gel will be weaker (Winarno, 2008).

Table 2. Mean Viscosity of JC Jam (cP).

Jamaican cherry leaf extract	Stevia Extract			Mean
	B1 (1%)	B2 (2%)	B3 (3%)	
A1 (30%)	225.00	345.00	325.00	298.33
A2 (50%)	267.50	366.25	363.75	332.50
A3 (70%)	332.75	377.50	387.50	365.92
Mean	275.08	362.92	358.75	298.33

3.3 pH Jamaican Cherry Jam

Table 3 indicates that the mean pH value tends to decrease as the concentration of JC leaf extract arises. The decline is attributed to the presence of flavonoid concentration in the leaf extract. These findings support the idea by Alvianti and Fitri (2018) that higher concentrations of JC extract result in more acidic formulations. It is well known that the flavonoids included in JC leaves raise the acidity of solutions.

The average pH value increased to 4.98%, 4.95%, and 4.64%, respectively, with the addition of 1%, 2%, and 3% stevia extract. The finding is consistent with Buchori's (2007) research, which found that the pH of the jam decreases with increasing stevia extract concentration. Because both JC leaves and stevia extract contain flavonoid components, their pH values interact. Flavonoids are the largest class of slightly acidic phenol chemicals (Alvianti & Fitri, 2018). According to the average results, the pH level becomes more acidic or lower as the amount of stevia and Jamaican cherry extracts increases.

Table 3. Mean pH of JC Jam.

JC leaf extract	Stevia Extract			Mean
	B1 (1%)	B2 (2%)	B3 (3%)	
A1 (30%)	5.00a	4.94a	4.82a	4.92p
A2 (50%)	4.95a	4.93a	4.94a	4.94q
A3 (70%)	5.00a	4.99b	4.16b	4.71p
Mean	4.98p	4.95p	4.64p	

Notes: Means followed by the same letter in the same column indicate no significant difference with the Duncan test at 5% level.

3.4 Moisture Content of Jamaican Cherry Jam

Moisture content is the percentage of water that is present in a material. According to Harefa and Pato (2017) water has a significant impact on the taste, texture, and presentation of food. Table 3 shows the findings of the examination of the water content of JC jam.

Table 4. Moisture Content of JC Jam (Percentage).

Jamaican cherry leaf extract	Stevia Extract			Mean
	B1 (1%)	B2 (2%)	B3 (3%)	
A1 (30%)	54.17 c	55.99 c	54.88 c	55.01 p
A2 (50%)	56.26 bc	55.57 c	53.21 c	55.01 p
A3 (70%)	54.78 c	62.88 c	59.38 b	59.01 q
Mean	55.07 c	58.15 p	55.82 p	

Notes: Means followed by the same letter in the same column indicate no significant difference with the Duncan test at 5% level.

Table 4 shows that the moisture content of the jam product is affected by the addition of JC leaf extract at 30%, 50%, and 70%. Since JC leaf extract has a high moisture content, increasing its

proportion results in a higher moisture content in the jam. The finding is supported by Ahmad et al. (2018), who reported a 5.26% rise in moisture content as the concentration of JC leaf extract increased. The moisture content of JC jam is affected by the addition of stevia extract. To prevent dissolved particles from influencing the jam's moisture content, stevia extract was filtered before processing.

The addition of stevia and JC leaf extracts suggests that the two ingredients interact and significantly affect the jam's moisture content. A study by Laswati (2018) found that both liquid extracts contain the main ingredient of JC, with a water content of 80.43%. This result is consistent with the assertion by Murtadha et al. (2012) that moisture content is influenced by fruit maturity. Wirawan and Mushollaeni (2008) state that there is a correlation between cooking duration and moisture content, with the cooking process contributing to both the increase and decrease of moisture levels. Based on Table 4, the A3B2 treatment had the highest average moisture content, whereas the A2B3 treatment had the lowest. This difference is likely due to the lack of a standardized cooking time, as the endpoint of cooking was determined by visual appearance, specifically the thickening of the food.

3.5 Reduce Sugar Content

Sugars that can act as reducing agents are known as reducing sugars. The existence of free ketone or aldehyde groups is the cause of this. Metal oxidizers, such as Cu(II) compounds, serve as oxidizing agents or reductants in these reactions. Glucose, mannose, fructose, lactose, maltose, and other sugars are examples of reducing sugars (Fadhilah et al., 2024). In contrast, sucrose is classified as a non-reducing sugar (Andragogi et al., 2018).

Table 5 presents the analysis of the reduced sugar content of JC jam. The results show that variation in the addition of JC and stevia extracts did not have a significant effect on lowering sugar levels. This is attributed to the absence of sugar content in JC extract. These findings are consistent with the study by Laswati et al. (2018), which also reported a lack of sugar content in JC leaves.

Table 5. Mean of JC Jam Sugar Reduction (Percentage).

Jamaican cherry leaf extract	Stevia Extract			Mean
	B1 (1%)	B2 (2%)	B3 (3%)	
A1 (30%)	2.15	2.18	2.37	2.24
A2 (50%)	1.66	2.25	2.41	2.11
A3 (70%)	2.69	2.51	1.85	2.35
Mean	2.17	2.31	2.21	

Furthermore, based on Table 5, the addition of stevia extract itself did not affect the reducing sugar levels, which may be related to non-enzymatic browning reactions, such as the Maillard reaction. The Maillard reaction can reduce the concentration of reducing sugars as they are converted into new brown compounds called melanoidins (Rosida, 2011). Melanoidins are substances formed during food processing and preservation through reactions between reducing sugars and proteins or amino acids in the later stage of the Maillard reaction (Wang et al., 2011)

3.6 Organoleptic Test

To evaluate the color, aroma, spreadability, and flavor of JC jam, an organoleptic test was performed. After completing a questionnaire, participants were given a score: 1 for Very Dislike, 2 for Somewhat Dislike, 3 for Dislike, 4 for Neutral, 5 for Somewhat Like, 6 for Like, and 7 for Very Like. Table 5 presents the total organoleptic test findings. Meanwhile, Table 6 presents the interaction of the organoleptic test for two factors and three levels.

Based on Table 6, the addition of JC extract did not have a significant effect on the jam's color preference. This intense green color of the JC extract may have contributed to a lower level of acceptance by the panelists. Furthermore, the addition of stevia extract also had no significant effect on the panelists' preference for jam color. The finding is likely due to the small amount of stevia extract used, which did not noticeably alter the appearance of the jam.

Table 6. Overall test of JC jam.

Experimental treatment	Color	Aroma	Spreadability	Flavor	Mean	Description
Jamaican cherry extract						
A1 (30%)	4.48	4.36	4.49	4.38	4.42	Neutral
A2 (50%)	4.33	4.30	4.48	4.45	4.38	Neutral
A3 (70%)	4.48	4.44	4.36	4.67	4.48	Neutral
Stevia extract						
B1 (1%)	4.53	4.25q	4.39	4.35	4.37	Neutral
B2 (2%)	4.38	4.43p	4.52	4.58	4.47	Neutral
B3 (3%)	4.38	4.42p	4.43	4.57	4.44	Neutral

Notes: Means followed by the same letter in the same column indicate no significant difference with the Duncan test at 5% level.

Table 7. Overall test of JC jam.

Experimental treatment	Color	Aroma	Spreadability	Flavor	Total	Mean
A1B1	4.32 c	4.08 d	4.45	4.50 ab	17.35	4.34
A2B1	4.52 abc	4.35 bc	4.53	4.45 ab	17.85	4.46
A3B1	4.73 a	4.33 bc	4.20	4.10 b	17.36	4.34
A1B2	4.42 abc	4.5 ab	4.55	4.43 b	17.90	4.48
A2B2	4.35 c	4.4 ab	4.58	4.68 ab	18.01	4.50
A3B2	4.37 bc	4.4 ab	4.43	4.63 a	17.83	4.46
A1B3	4.70 ab	4.5 ab	4.48	4.20 b	17.88	4.47
A2B3	4.10 d	4.15 c	4.35	4.23 ab	16.83	4.21
A3B3	4.32 c	4.60 a	4.45	5.28 a	18.65	4.66

Notes: Means followed by the same letter in the same column indicate no significant difference with the Duncan test at 5% level.

Since JC is intensely green and stevia extract is blackish-brown, it can be argued that the addition of both extracts alters the jam's color. These findings are consistent with a study by Islamika et al. (2023), which reported that JC leaf extract has a blackish-brown color, and with Joshi et al. (2022), who found that stevia extract is dark brown in color. The panelists preferred the brown hue of the JC jam, which resulted from the mixing and heating process. The most favorable jam was obtained by experimental treatment A3B3, which had the highest mean score of 4.66. The mean scores for color, flavor, spreadability, and aroma were 4.32, 4.60, 4.45, and 5.28, respectively. A3B3 did not score the highest on the color test, but it was the panelists' top preference in terms of flavor and aroma. The spreadability test revealed no significant differences among the nine experimental treatments, indicating similar preferences.

The aroma of the JC jam was not significantly affected by the addition of Jamaican leaf extract. The result may be due to the fact that panelists did not enjoy the leafy aroma imparted by the extract. However, the aroma was significantly influenced by the use of stevia extract, possibly due to its distinctive and pleasant aroma. This is because panelists may be drawn to the unique and pleasant aroma of stevia extract. As shown in Table 5, the addition of 3% stevia extract resulted in the highest average aroma score. According to Hidayanto et al (2017) a richer aroma correlates with a higher concentration of stevia. The interaction between Jamaican leaf and stevia extracts contributed to a unique aroma, combining the aroma of stevia with that of JC leaf extract, which resembles the sweet, fruity aroma of the JC fruit itself.

The jam's spreadability was not significantly affected by the addition of JC leaf extract. It is possible because panelists perceived the leaf extract as having high moisture content, which they did not favor. Similarly, the addition of stevia extract did not significantly affect the jam's spreadability. This could be due to the filtration process during stevia extract preparation, which removes most dissolved particles. As such, incorporating up to 3% stevia extract did not noticeably impact the jam's spreadability.

The flavor of the jam was also unaffected by the addition of JC leaf extract. This may be attributed to the astringent and bitter taste of the extract, which the panelists found unappealing. Furthermore, the addition of stevia extract did not significantly influence flavor preference, likely because panelists did not favor its overly sweet taste. However, the overall taste of JC jam was influenced by the combination of stevia and JC leaf extracts. Increasing the concentrations of both extracts enhanced the panelists' preference for the jam's flavor. While stevia provides sweetness, JC extract is naturally astringent and bitter. The combination created a balance between sweetness and bitterness that panelists found appealing. Among the tested formulations, as shown in Table 6, the A3B3 treatment received the highest preference across all organoleptic tests.

3.7 In Vivo Test of Jamaican Cherry Jam as an Anti-Gout Agent

Figure 1 presents the result of the in vivo test on JC jam for its anti-uric acid activity. The x-axis shows the uric acid levels of the treated mice, while the y-axis shows the treatment given to the mice. The table shows that before treatment, the rats had uric acid levels of 1.18 mg/dl. After treatment with chicken liver, the rats' uric acid levels were 8.56 mg/dl, and after treatment with cherry jam, the rats' uric acid levels were 4.92 mg/dl.

According to Kholifaturrokhmah and Purnawati (2016), the normal range of uric acid levels is 3.4–7.0 mg/dL in men and 2.4–6.0 mg/dL in women. The in vivo experiment demonstrated that the administration of JC jam was effective in reducing uric acid levels to 4.92 mg/dL, which falls within the normal physiological range. This indicates that the jam can be considered a functional food for preventing gout.

Although not as potent as the chemical drug allopurinol, JC jam is considered a much safer natural alternative. This aligns with the findings of Ilkafah et al. (2018b), who stated that traditional medicines are generally safer due to their natural origin and milder effects compared to synthetic drugs. Allopurinol primarily functions to lower elevated blood uric acid levels in gout patients, but does not address the pain or inflammation during acute gout attacks, necessitating the use of additional analgesic medications (Arthritis Australia, n.d.). In contrast, JC leaves have demonstrated effectiveness in managing both uric acid levels and joint discomfort.

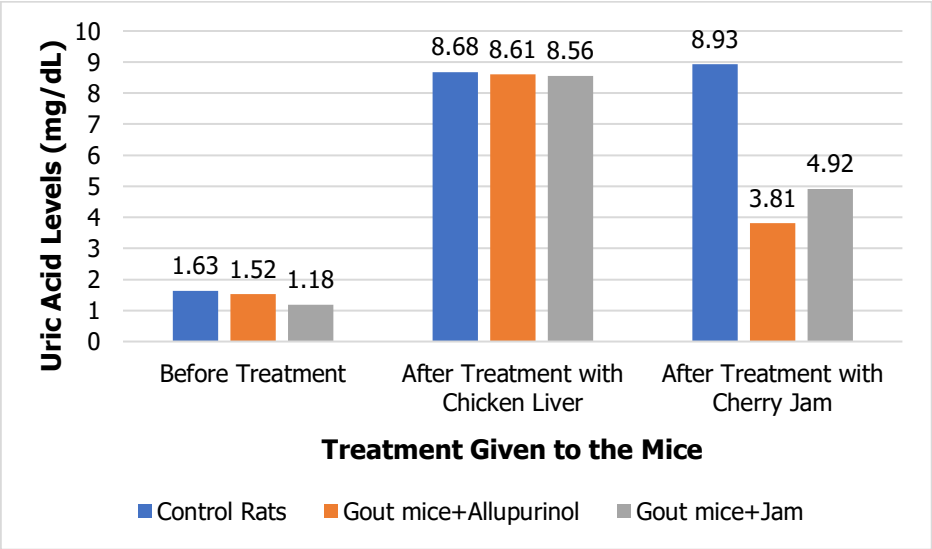

Figure 1. In vivo test results of JC jam as an anti-gout

4. CONCLUSIONS

The JC leaf extract used had a significant effect on the jam's pH and moisture content. However, it did not significantly affect viscosity, reducing sugar content, or organoleptic characteristics (color, scent, taste, and spreadability). Similarly, the addition of stevia extract did not affect the viscosity, reducing sugar content, color, spreadability, or taste of the jam. Meanwhile, it did have a notable impact on pH, moisture content, and aroma. The highest overall acceptability was observed in the A3B3 treatment, with an average of 4.66, corresponding to a neutral description. This formulation consisted of 70% JC leaf extract (A3) and 3% stevia extract (B3), supported by a viscosity of 387.50 cP, a pH of 4.16, a moisture content of 59.38%, and a reducing sugar content of 1.85%. Furthermore, in vivo testing revealed that JC jam was capable of lowering uric acid levels to within the normal range (4.92 mg/dL), indicating its potential for gout prevention.

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