SOFT DRINK FORMULATION FROM CASCARA, LIME, AND STEVIA SWEETENER AND ITS USE AS A CANCER PREVENTIVE

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ABSTRACT

Cascara, the skin of coffee cherries, is often considered waste but has a unique fruity flavor when brewed. *Lime*, rich in vitamin C, is an antioxidant that supports health, while *stevia*, derived from *stevia* leaves, provides a low-sugar sweetener. This study aimed to explore the effects of varying *cascara*-to-*lime* ratios and different *stevia* concentrations. Using a Factorial Completely Randomized Design (CRD), the study tested two factors: *cascara*-to-*lime* ratio (100:0, 95:5, 90:10) and *stevia* concentration (0.1%, 0.2%, 0.3%, 0.4%, 0.5%). Results showed that the *cascara*-*lime* ratio significantly influenced antioxidant activity, pH, total acidity, vitamin C content, and sensory attributes like color, aroma, taste, and overall acceptance ($P<0.01$). *Stevia* concentration significantly affected antioxidant activity, pH, vitamin C content, sugar levels, and sensory properties ($P<0.01$). The optimal combination was the C_3S_5 treatment (90:10 *cascara*-to-*lime* ratio with 0.5% *stevia*). Further tests on the cancer-preventive potential of this combination showed no significant change in body weight ($P>0.05$) in mice, but it reduced inflammatory cells and necrosis in the brain, liver, and lungs due to oxidative stress induced by benzo(a)pyrene at doses of 10.5 and 21 mg/20g body weight.

Keywords: antioxidant; cancer; *cascara*; *lime*; *stevia* sweetener

ABSTRAK

Cascara merupakan kulit kopi yang sering dianggap limbah, tetapi memiliki cita rasa dan aroma khas yang fruity dan harum saat diolah menjadi minuman. Jeruk nipis adalah salah satu buah dari genus Citrus dan kaya akan vitamin C yang berfungsi sebagai antioksidan untuk menjaga kesehatan tubuh. Pemanis *stevia* berasal dari daun *stevia* memberikan rasa manis dengan kadar gula rendah. Penelitian ini bertujuan untuk mengetahui pengaruh perbandingan *cascara* dan jeruk nipis serta konsentrasi pemanis *stevia*. Rancangan yang digunakan adalah Rancangan Acak Lengkap (RAL) Faktorial dengan dua faktor perlakuan: perbandingan *cascara* dan jeruk nipis (C) (100:0; 95:5; 90:10) dan konsentrasi pemanis *stevia* (S) (0,1%, 0,2%, 0,3%, 0,4%, 0,5%). Hasil penelitian menunjukkan bahwa perbandingan *cascara* dan jeruk nipis memberikan pengaruh sangat nyata ($P<0,01$) terhadap aktivitas antioksidan, pH, total asam, kadar vitamin C, serta organoleptik warna, aroma, rasa, dan hedonik penerimaan umum. Penambahan konsentrasi pemanis *stevia* juga menunjukkan pengaruh sangat nyata ($P<0,01$) terhadap parameter yang sama. Perlakuan terbaik diperoleh dari kombinasi C_3S_5, dengan perbandingan 90:10 dan konsentrasi *stevia* 0,5%, yang kemudian dievaluasi untuk potensi preventif kanker. Pemberian zat uji pada mencit tidak menunjukkan pengaruh signifikan ($P>0,05$) terhadap berat badan, tetapi dosis 10,5 dan 21 mg/20g BB dapat mengurangi sel radang dan nekrosis akibat stres oksidatif di otak (*hippocampus*), hati, dan paru-paru yang diinduksi oleh *benzo(a)pyrene*.

Kata kunci: antioksidan, *cascara*, jeruk nipis, kanker dan pemanis *stevia*

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INTRODUCTION

According to data from the Ministry of Health of the Republic of Indonesia, in 2022, the incidence of cancer in Indonesia was 136 cases per 100,000 population, placing Indonesia eighth in Southeast Asia for the highest number of cancer patients. Cancer is a disease that arises due to oxidative stress, which leads to damage to human body cells, including proteins, carbohydrates, fats, and DNA. Damage to DNA can trigger the onset of cancer (Hikmah and Hardiany, 2021).

Oxidative stress is a condition of imbalance in the human body, where the level of antioxidants, which serve to protect body tissues, is lower than the level of free radicals. The presence of free radicals attacks the human body, leading to cell damage that can result in cancer. Therefore, it is essential for individuals to consume a variety of foods containing antioxidant compounds. This is because antioxidants can inhibit and prevent oxidation caused by free radicals, thereby reducing oxidative stress (Mulia et al, 2016).

Cascara, derived from the Spanish word meaning "skin," typically refers to the dried skin of coffee cherries. *Cascara*, or coffee husk, contains antioxidants that are beneficial for health. In addition to protecting body cells from damage caused by free radicals, the antioxidants in *cascara* also play a role in reducing the risk of degenerative diseases, inflammation, and in supporting heart health and the immune system. By utilizing *cascara* as a natural source of antioxidants, we can gain additional benefits from *cascara* beverages while also helping to reduce the waste generated from discarded coffee husks (Ochi, 2018).

Stevia contains diterpene glycosides with steviol as its aglycone. The stevioside present in *stevia* leaves is approximately 300 times sweeter than sucrose (table sugar). Despite this high sweetness, *stevia* has a negligible caloric content, making this sweetener safe for consumption and recommended for individuals with diabetes and obesity. *Stevia* sweeteners offer several advantages, including not causing cancer, not contributing to tooth decay, preventing obesity, inhibiting increases in blood sugar levels, and lowering blood pressure (Marlina and Widiastuti, 2018).

Lime contains various compounds that are beneficial for human health, such as citric acid, amino acids (tryptophan and lysine), essential oils (citral, limonene, and cadinene), glycosides, vitamin C, minerals (calcium, phosphorus, iron), and vitamin B1. Chemical compounds such as vitamin C, citric acid, and limonene possess antioxidant properties, which can help protect body tissues from oxidative stress, neutralize free radicals, and support overall health. Therefore, regular consumption of *lime* can enhance antioxidant intake and strengthen the immune system (Lestari et al, 2018).

MATERIALS AND METHOD

Materials

The research materials used in this study include *cascara* from Arabica coffee husks obtained from farmers in Karo Regency, *lime* sourced from traditional markets in Medan, liquid *stevia* sweetener branded "Royal *Stevia* Sweetener," and plastic PE bottles acquired from a plastic store in Medan. The reagents used in this study include 2.2-diphenyl-1-picrylhyldrazyl (Smartlab, Indonesia), ethanol (Smartlab, Indonesia), aquadest, phenolphthalein indicator (Smartlab, Indonesia),

NaOH (Smartlab, Indonesia), 2.6-dichlorophenol indophenol (Merck, Germany), $NaHCO_3$ (Merck, Germany), oxalic acid (Merck, Germany), ascorbic acid (Merck, Germany), glucose (Merck, Germany), phenol (Smartlab, Indonesia), sulfuric acid (Merck, Germany), benzo(α)pyrene (Merck, Germany), formalin (Merck, Germany), chloroform (Merck, Germany), xylene (Merck, Germany), and hematoxylin-eosin (Vector Laboratories, United States).

***Cascara* Preparation**

The extract is obtained by adding hot water at a temperature of 90°C to the *cascara* in a ratio of 1:30. The brewing time is conducted for 3 minutes. After the brewing process, the *cascara* extract is filtered from the *cascara*.

***Lime* Preparation**

The *lime* is washed, then cut in half, and the seeds are separated from the fruit flesh. After that, the *lime* is squeezed using a juicer and filtered through a strainer cloth.

Soft Drink Making with the Mixture of *Cascara* and *Lime* Juice with *Stevia* Sweetener

The soft drink formulation can be seen in Table 1. The brewed *cascara* is mixed with *lime* juice in the ratios of 100:0; 95:5; and 90:10, then *stevia* sweetener is added in measured amounts according to the treatment (0.1%, 0.2%, 0.3%, 0.4%, 0.5%) while stirring until dissolved. The *cascara* beverage is then packaged in plastic PE bottles. The *cascara* beverage is stored at refrigerator temperatures of 2-4°C, and chemical quality analysis such as antioxidant activity, pH based on (Yenrina, 2015), total acidity based on (Ranganna, 1978), vitamin C content based on (Apriyantono et al, 1989), sugar content based on (Apriyantono et al, 1989), and sensory attributes such as color, aroma, taste, and overall acceptance. The ratio of *cascara* extract to *lime* juice and the concentration of *stevia* sweetener that yield the best quality *cascara* beverage, based on antioxidant activity, are subsequently evaluated for their potential as a cancer preventive agent based on (Astriani, 2022).

Antioxidant Activity

The procedure began with the preparation of a DPPH solution, in which 5 mg of DPPH was weighed and then dissolved in a 100 ml volumetric flask using ethanol. Subsequently, the solution was stirred for 20 minutes.

Testing was conducted by preparing a stock solution, in which 25 mg of the sample was weighed and dissolved in a 50 ml volumetric flask using ethanol. Next, aliquots of the sample were taken to create concentration variations of 15 µg/ml, 25 µg/ml, 50 µg/ml, 100 µg/ml, and 200 µg/ml. DPPH solution was then added to each concentration at a volume of 1 ml. Following this, the solution was diluted with ethanol in a 5 ml volumetric flask. The samples were then incubated at 37ºC for 30 minutes. After incubation, the absorbance was measured against a blank, and the absorbance values were recorded using a spectrophotometer at a wavelength of 517 nm. Once the absorbance values were obtained, the percentage of inhibition or percentage of activity was calculated, and the IC_{50} value was determined.

$$Inhibition\ (\%) = \frac{Blank\ Absorbance - Sample\ Absorbance}{Blank\ Absorbance} \times 100\%$$

The calculation of IC_{50} involves inputting the values of the concentrations of the sample solutions (x-axis) and the percentage of inhibition against DPPH (y-axis) into the regression line equation.

Table 1. Soft Drink Formulation

Ingredient	S_1 (0,1%)	S_2 (0,2%)	S_3 (0,3%)	S_4 (0,4%)	S_5 (0,5%)
C_1 (100:0)	C_1S_1	C_1S_2	C_1S_3	C_1S_4	C_1S_5
C_2 (95:5)	C_2S_1	C_2S_2	C_2S_3	C_2S_4	C_2S_5
C_3 (90:10)	C_3S_1	C_3S_2	C_3S_3	C_3S_4	C_3S_5

Organoleptic Test

Organoleptic testing was conducted on the parameters of color, aroma, and taste for the *cascara* beverage. The assessment of overall acceptance was performed using a hedonic test on the *cascara* beverage. A sample of 20 panelists evaluated the samples, each assigned a different code corresponding to the organoleptic parameters of color, aroma, and taste. Additionally, a sample of 105 panelists, each assigned a different code, evaluated the samples based on the hedonic parameter of overall acceptance. The panelists who tested the samples were students from the Food Technology Study Program at the Faculty of Agriculture, University of North Sumatra. The scale used for the organoleptic testing can be seen in Table 2.

Evaluation of the Potential of *Cascara* Beverages as a Cancer Preventive Measure Against the Brain, Liver, and Lungs Tissue of Mice (*Mus musculus*) Induced by Benzo(α)pyrene.

Preparation of Test Animals

The test animals consisted of male mice (*Mus musculus L.*) with a body weight of approximately 35-39 grams. The mice were individually housed in a homogeneous environment within containers or cages equipped with food and water containers. Acclimatization of the mice was conducted for 7 days prior to the treatment. During the acclimatization period, the mice were provided with standard feed (pellets) and water ad libitum (as much as they desired). At the time of the study, each mouse was given approximately 14 grams of feed per day.

A total of 36 mice were used, with the control group (K1) consisting of 6 mice, the positive control group (K2) consisting of 10 mice, the treatment group with a dose of 10.5 mg/20g body weight (K3) consisting of 10 mice, and the treatment group with a dose of 21 mg/20g body weight (K4) consisting of 10 mice. The determination of the doses administered was based on similar research conducted by (Astriani, 2022). The number of mice used was determined using method by (Federer, 1963), which provides a formula to establish the required sample size. The calculations are as follows:

$$(n-1)\,(t-1) \geq 15$$
$$(n-1)\,(4-1) \geq 15$$
$$3n-3 \geq 15$$
$$3n \geq 18$$
$$n \geq 6$$

The conclusion from this calculation is that a minimum of 6 mice are required for each treatment group. To enhance the reliability of the study, an additional 4 mice were added to the treatment groups as backup samples.

Table 2. Scale of organoleptic parameter

Scale	Organoleptic Parameter			
	Color	**Aroma**	**Flavor**	**Overall Acceptance**
1	Very Clear	Very Not Fragrant	Very Sour	Very Disliked
2	Clear	Not Fragrant	Sour	Disliked
3	Little Clear	Little Not Fragrant	Little Sour	Little Disliked
4	Neutral	Neutral	Neutral	Netral
5	Little Cloudy	Little Fragrant	Little Sweet	Little Liked
6	Cloudy	Fragrant	Sweet	Liked
7	Very Cloudy	Very Fragrant	Very Sweet	Very Liked

Induction of the Carcinogenic Substance Benzo(α)pyrene and Administration of the Test Substance *Cascara* Beverage

A total of 0.3 mg of the carcinogenic substance Benzo(α)pyrene was dissolved in 0.2 ml of corn oil. This solution was then induced by injecting the Benzo(α)pyrene solution into the subcutaneous tissue of the mice in the neck region. All treatment groups (except for the negative control) were induced with Benzo(α)pyrene for 10 days while being fed the test substances for 15 days (except for the positive control). This was conducted to evaluate the effectiveness of *cascara* beverages as a preventive measure against oxidative stress that could lead to cancer. The test substances administered to the mice were 10.5 and 21 mg per 20 g of body weight per day for 15 days.

Measurement of Mouse Body Weight and Surgical Procedures on Mice

During the study, the body weight of the mice in each group was measured. The measurement of mouse body weight was divided into two phases: the first weighing on the initial day (baseline weight) and the weighing on day 15 (final weight) at the end of the study. At the conclusion of the study, the mice were anesthetized using chloroform applied to cotton and placed in a glass jar. Subsequently, surgery was performed to extract the brain, liver, and lungs of the mice.

Preparation of Histopathological Preparations of Mouse Organs

The process of preparing histopathological preparations consists of several stages, including the fixation stage, dehydration stage, embedding stage, cutting stage, staining stage, and mounting stage.

Fixation

The purpose of fixation is to rapidly halt cellular metabolism, thereby preventing autolysis or degradation of the tissue. Selected brain tissue samples are placed in a fixative solution, specifically 10% buffered formalin. Fixation is carried out for a minimum of 24 hours. After fixation, the mouse organs are rinsed with running water.

Trimming

The mouse organs are cut into smaller sizes of approximately 3 mm and then placed into embedding cassettes.

Dehydration, Clearing, and Impregnation

Dehydration is performed by immersing the mouse organs in graded alcohols of 70%, 80%, and 90% for 2 hours each. Subsequently, the organs are immersed in 96% alcohol, absolute alcohol I, II, and III for 1 hour each. To remove any residual alcohol, clearing is conducted by immersing the embedding cassettes in xylene solutions I, II, and III for 1 hour each. Impregnation is then carried out using paraffin I, II, and III for 2 hours each.

Embedding

The melted paraffin is poured into a stainless steel mold containing the mouse organs and covered with an embedding cassette until the organs are completely submerged, forming a block. The paraffin block is then cooled and placed in a freezer at a temperature of 4°C. The paraffin block is cut according to the location of the tissue using a scalpel and is prepared for sectioning with a microtome.

Cutting

The sectioning operation is conducted in a cold room. Thin sections with a thickness of 4 μm are prepared, resulting in very thin tissue slices. Subsequently, the fabric strips containing wrinkles are removed by floating them in water and then placed in a water bath for several seconds until they fully expand. After that, the tissue strips are placed on clean microscopic slides and treated with Meyer's albumin solution at the upper third of the slide. The slides containing the tissue are then placed in an incubator operating at a temperature of 37°C for 24 hours until the tissue is completely fixed.

Staining

Staining begins by immersing the tissue sections in Xylene solutions I, II, and III for 5 minutes each. Subsequently, the tissue is immersed in absolute alcohol I, II, and III for 5 minutes each. After that, the tissue is rinsed in distilled water for 1 minute. The organ sections are then placed in a hematoxylin-eosin staining solution for 20 minutes. Next, the tissue sections are rinsed in distilled water for 1 minute while gently shaking the organs. The tissue is then immersed in 56% acid alcohol for 2-3 minutes, followed by washing in distilled water for 15 minutes. After that, the tissue is placed in eosin for 2 minutes and then in 96% alcohol, followed by 96% alcohol again, and alcohol III and IV for 2-3 minutes each. Finally, the tissue sections are placed in xylene IV and V for 5 minutes.

Mounting

After the staining is complete, the tissue slides are placed on tissue paper on a flat surface and are then treated with a mounting medium, specifically Canada balsam, before being covered with a cover glass. During this process, it is essential to avoid the formation of air bubbles in the histopathological preparations of the mouse organs. The preparations are then observed using a microscope. The histopathological preparations of the brain are examined under a light microscope at a magnification of 400x. The method used for viewing the preparations is a double-blinded procedure.

RESULTS AND DISCUSSION

Antioxidant Activity

The result of study on *cascara*-to-*lime* ratio treatment on antioxidant activity (IC_{50}) can be seen in Figure 1. The ratio of *cascara* to *lime* has a significant effect ($p<0.05$) on antioxidant activity. The data labeled as aA, bB, dan cC indicate that each group is significantly different from one another. The highest antioxidant activity was observed in treatment C_3, with a *cascara* to *lime* ratio of 90:10, yielding an IC_{50} score of 34.33. In contrast, the lowest antioxidant activity was found in treatment C_1, with a *cascara* to

lime ratio of 100:0, resulting in an IC_{50} score of 100.37. This is attributed to the fact that *lime* contains vitamin C, which serves as an effective antioxidant in combating free radicals that damage cells and body tissues. Vitamin C can reduce or halt chain reactions by eliminating free radicals or inhibiting oxidation reactions. Additionally, vitamin C can neutralize oxidative stress through the process of electron donation, thereby reducing Reactive Oxygen Species (ROS) (Safnowandi, 2022).

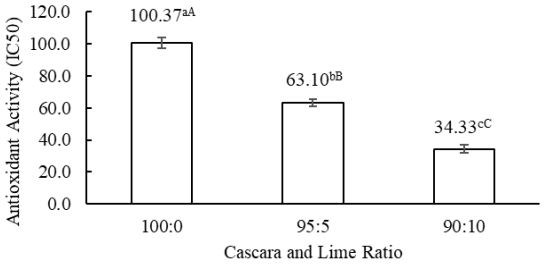

Figure 1. Effect of *cascara* and *lime* ratio on antioxidant activity (IC_{50})

The result of study on *stevia* sweetener concentration treatment on antioxidant activity (IC_{50}) can be seen in Figure 2. The concentration of *stevia* sweetener has a highly significant effect ($P<0.01$) on antioxidant activity. The data labeled as aA, bB, cC, dD, and eD indicate that each group is significantly different from one another. The highest antioxidant activity was found in treatment S_1 with a *stevia* sweetener concentration of 0.5%, yielding an IC_{50} score of 59.71, while the lowest antioxidant activity was observed in treatment S_1 with a *stevia* sweetener concentration of 0.1%, resulting in an IC_{50} score of 77.10. Antioxidant activity increases with the addition of *stevia* sweetener concentration. This is attributed to the presence of vitamin C and stevioside in *stevia*, which are significant antioxidants that play a crucial role in inhibiting cellular and tissue damage caused by free radicals (Nizori et al, 2023).

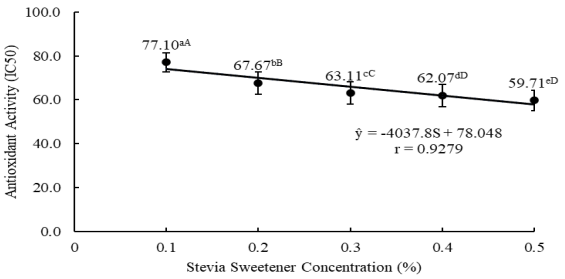

Figure 2. Effect of *stevia* sweetener concentration on antioxidant activity (IC_{50})

The result of study on the interaction between *cascara* and *lime* ratio and *stevia* sweetener concentration on antioxidant activity (IC_{50}) can be seen in Figure 3. The interaction between *cascara* and *lime* ratio and *stevia* sweetener concentration has a highly significant effect ($P<0.01$) on antioxidant activity (IC_{50}). The highest antioxidant activity (IC_{50}) was found in the C_3S_5 treatment with a *cascara* to *lime* ratio of 90:10 and a *stevia* sweetener concentration of 0.5% namely 30.68, while the lowest antioxidant activity was found in the C_1S_1 treatment with a *cascara* to *lime* ratio of 100: 0 and a *stevia* sweetener concentration of 0.1% namely 123.22.

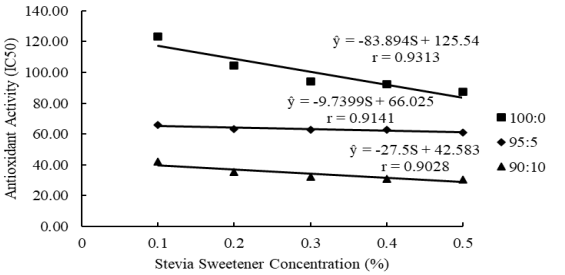

Figure 3. Interaction between *cascara* and *lime* ratio and *stevia* sweetener concentration on antioxidant activity (IC_{50}).

Based on Figure 3, The antioxidant activity in soft drink will increase as the amount of *lime* juice and *stevia* sweetener is added. This is consistent with the literature by (Hardiansyah et al, 2022) and

(Safnowandi, 2022), which states that the addition of *lime* juice and *stevia* sweetener will enhance antioxidant activity. This is due to the vitamin C content in *lime* juice and the stevioside content in *stevia* sweetener, which can inhibit free radicals from damaging cells and body tissues.

pH

The result of study on *cascara*-to-*lime* ratio treatment on pH can be seen in Figure 4. The ratio of *cascara* to *lime* has a highly significant effect (P<0,01) on pH. The data labeled as aA, bB, dan cC indicate that each group is significantly different from one another. Highest pH was observed in treatment C_1, with a *cascara* to *lime* ratio of 100:0, yielding a pH score of 4.02. In contrast, the lowest pH score was found in treatment C_3, with a *cascara* to *lime* ratio of 90:10, resulting in a pH score of 2.78. This is due to the citric acid content present in *lime*, as the concentration of *lime* juice increases, the citric acid content also rises, resulting in a lower pH value of the beverage (Kusumuwati et al, 2018).

The result of study on *stevia* sweetener concentration treatment on pH can be seen in Figure 5. The concentration of *stevia* sweetener has a highly significant effect (P<0.01) on pH. The data labeled as aA, bB, cC, dD, and eE indicate that each group is significantly different from one another. The highest pH score was found in treatment S_5 with a *stevia* sweetener concentration of 0.5%, yielding a pH score of 3.39, while the lowest pH score was observed in treatment S_1 with a *stevia* sweetener concentration of 0.1%, resulting in a pH score of 3.23. *Stevia* sweetener has the property of binding water, which results in a thicker solution and an increase in pH values. Additionally, *stevia* contains stevioside, which imparts sweetness to the sweetener, thereby raising the pH and reducing acidity in the soft drink (Rizki et al, 2023).

The result of study on the interaction between *cascara* and *lime* ratio and *stevia* sweetener concentration on pH can be seen in Figure 6. The interaction between *cascara* and *lime* ratio and *stevia* sweetener concentration has a highly significant effect (P<0.01) on pH. The highest pH value was found in treatment C_1S_5, with a ratio of *cascara* to *lime* juice of 100:0 and a *stevia* sweetener concentration of 0.5%, which was 4.11. The lowest pH value was found in treatment C_3S_1, with a ratio of *cascara* to *lime* juice of 90:10 and a *stevia* sweetener concentration of 0.1%, which was 2.68.

Based on Figure 6, The pH value of soft drink will decrease as the amount of *lime* juice is added, but the pH value of soft drink will increase as the amount of *stevia* sweetener is added. This is consistent with the literature by (Assalam et al, 2023), which states that the higher the amount of *lime* juice added, the higher the acidity produced, and consequently, the lower the measured pH value. This is due to the presence of citric acid in *lime*, which is a weak organic acid commonly found in citrus fruits. The addition of *stevia* sweetener to soft drink increases the pH value because stevioside, a natural sweetener, affects the beverage's pH (Sinta and Sumaryono, 2019).

Total Acidity

The result of study on *cascara*-to-*lime* ratio treatment on total acidity can be seen in Figure 7. The ratio of *cascara* to *lime* has a highly significant effect (P<0,01) on total acidity. The data labeled as aA, bB, dan cC indicate that each group is significantly different from one another. The highest total acidity was observed in treatment C_3, with a *cascara* to *lime* ratio of 90:10, yielding total acidity of 21.25%. In contrast, the lowest antioxidant activity was found in

treatment C_1, with a *cascara* to *lime* ratio of 100:0, resulting in total acidity of 5.31%. This is due to the citric acid content found in *lime*, which causes the total acidity levels to align with the decreasing pH results as the amount of *lime* is increased (Assalam et al, 2023). The concentration of stevia sweetener has no significant effect (P>0,05) on total acidity so the LSR test was not continued.

Vitamin C Content

The result of study on *cascara*-to-*lime* ratio treatment on vitamin C content can be seen in Figure 8. The ratio of *cascara* to *lime* has a highly significant effect (P<0,01) on vitamin C content. The data labeled as aA, bB, dan cC indicate that each group is significantly different from one another. The highest vitamin C content was observed in treatment C_3, with a *cascara* to *lime* ratio of 90:10, yielding total acidity of 4.25%. In contrast, the lowest vitamin C content was found in treatment C_1, with a *cascara* to *lime* ratio of 100:0, resulting in vitamin C content of 0.93%. The concentration of vitamin C will increase with the addition of *lime* juice. This is consistent with the literature by (Yulianto et al, 2022), which states that the addition of *lime* juice will enhance the functional properties of a beverage due to vitamin C being one of the antioxidants that can help maintain bodily health. The results of the vitamin C concentration are in line with the findings of antioxidant activity tests, where higher levels of vitamin C result in higher antioxidant activity.

The result of study on *stevia* sweetener concentration treatment on vitamin C content can be seen in Figure 9. The concentration of *stevia* sweetener has a highly significant effect (P<0.01) on vitamin C content. The data labeled as aA, bB, bcBC, cdBCD, and dD indicate that each group is significantly different from one another. The highest vitamin C content was found in treatment S_5 with a *stevia* sweetener concentration of 0.5%, yielding vitamin C content of 2.80%, while the lowest vitamin C content was observed in treatment S_1 with a *stevia* sweetener concentration of 0.1%, resulting in vitamin C content of 2.24%. The concentration of vitamin C will increase with the addition of *stevia* sweetener. This is consistent with the literature by (Simarmata et al, 2019), which states that the addition of *stevia* sweetener will increase the concentration of vitamin C and provide a sweet taste to the beverage. This is in line with the findings of antioxidant activity tests, where higher levels of vitamin C result in higher antioxidant activity.

Sugar Content

The ratio of *cascara* to *lime* has no significant effect (P>0,05) on sugar content so the LSR test was not continued. The result of study on *stevia* sweetener concentration treatment on sugar content can be seen in Figure 10. The concentration of *stevia* sweetener has a highly significant effect (P<0.01) on sugar content. The data labeled as aA, abAB, abcABC, dCD, and dD indicate that each group is significantly different from one another. The highest sugar content was found in treatment S_5 with a *stevia* sweetener concentration of 0.5%, yielding sugar content of 2.41%, while the lowest sugar content was observed in treatment S_1 with a *stevia* sweetener concentration of 0.1%, resulting in sugar content of 1.99%. *Stevia* does not raise sugar levels substantially but has a sweetness level that is 250 to 300 times higher than sugar commonly used. This is attributed to the presence of stevioside and rebaudioside, which impart the sweet flavor (Christine et al, 2022).

Sensory Attributes

Color

The result of study on *cascara*-to-*lime* ratio treatment on color sensory can be seen in Figure 11. The ratio of *cascara* to *lime* has a highly significant effect

(P<0,01) on color sensory score. The data labeled as aA, bB, dan cC indicate that each group is significantly different from one another. The highest color sensory score was observed in treatment C_3, with a *cascara* to *lime* ratio of 90:10, yielding a color sensory score of 6.44. In contrast, the lowest color sensory score was found in treatment C_1, with a *cascara* to *lime* ratio of 100:0, resulting in a color sensory score of 2.14. This is due to the dark red of *cascara* extract and the white extract of *lime*, which has a slight green tint. The combination of these two extracts results in a beverage product with a cloudy appearance (Hidayat et al, 2017).

The result of study on *stevia* sweetener concentration treatment on color sensory can be seen in Figure 12. The concentration of *stevia* sweetener has a highly significant effect (P<0.01) on color sensory score. The data labeled as aA, abAB, abAB, bAB, and cC indicate that each group is significantly different from one another. The highest color sensory score was found in treatment S_5 with a *stevia* sweetener concentration of 0.5%, yielding a color sensory score of 4.51, while the lowest color sensory score was observed in treatment S_1 with a *stevia* sweetener concentration of 0.1%, resulting in a color sensory score of 4.11. This is due to the presence of steroid and alkaloid compounds in *stevia* sweetener, which can influence the color of the resulting product (Cahyani et al, 2022).

Aroma

The result of study on *cascara*-to-*lime* ratio treatment on aroma sensory can be seen in Figure 13. The ratio of *cascara* to *lime* has a highly significant effect (P<0,01) on aroma sensory score. The data labeled as aA, bB, dan cC indicate that each group is significantly different from one another. The highest aroma sensory score was observed in treatment C_3, with a *cascara* to *lime* ratio of 90:10, yielding a aroma sensory score of 6.41. In contrast, the lowest aroma sensory score was found in treatment C_1, with a *cascara* to *lime* ratio of 100:0, resulting in a color sensory score of 4.05. The fresh aroma of *lime* comes from the presence of limonin compounds, which are typically found in fruits belonging to the *Citrus* genus (Assalam et al, 2023).

The result of study on *stevia* sweetener concentration treatment on aroma sensory can be seen in Figure 14. The concentration of *stevia* sweetener has a highly significant effect (P<0.01) on aroma sensory score. The data labeled as aA, abAB, bcABC, cdCD, and eE indicate that each group is significantly different from one another. The highest aroma sensory score was found in treatment S_5 with a *stevia* sweetener concentration of 0.5%, yielding a color sensory score of 5.49, while the lowest color sensory score was observed in treatment S_1 with a *stevia* sweetener concentration of 0.1%, resulting in a color sensory score of 4.98. The addition of *stevia* sweetener will have a significant impact on the aroma of the beverage due to the presence of steviol compounds in the sweetener. Steviol compounds contribute to both the sweet flavor and the pleasant aroma of the drink (Chungtai et al, 2020).

Flavor

The result of study on *cascara*-to-*lime* ratio treatment on flavor sensorycan be seen in Figure 15. The ratio of *cascara* to *lime* has a highly significant effect (P<0,01) on flavor sensory score. The data labeled as aA, bB, and bcBC indicate that each group is significantly different from one another. The highest flavor sensory score was observed in treatment C_1, with a *cascara* to *lime* ratio of 100:0, yielding a flavor sensory score of 4.63. In contrast, the lowest flavor sensory score was found in treatment C_3, with a

cascara to *lime* ratio of 90:10, resulting in a flavor sensory score of 3.51. As the addition of *lime* increases, the acidity score given by the panelists will also become more acidic. This sour taste is caused by the citric acid content present in *lime* (Seftiono et al, 2020).

The result of study on *stevia* sweetener concentration treatment on flavor sensory can be seen in Figure 16. The concentration of *stevia* sweetener has a highly significant effect (P<0.01) on flavor sensory score. The data labeled as aA, bB, cC, dD, and eE indicate that each group is significantly different from one another. The highest flavor sensory score was found in treatment S_5 with a *stevia* sweetener concentration of 0.5%, yielding a flavor sensory score of 6.13, while the lowest flavor sensory score was observed in treatment S_1 with a *stevia* sweetener concentration of 0.1%, resulting in a flavor sensory score of 1.90. *Stevia* sweetener contains stevioside and rebaudioside A, which provide flavor to foods and beverages, however *stevia* does not significantly raise blood sugar levels (Zahro et al, 2022).

The result of study on the interaction between *cascara* and *lime* ratio and *stevia* sweetener concentration on flavor sensory can be seen in Figure 17. The interaction between *cascara* and *lime* ratio and *stevia* sweetener concentration has a highly significant effect (P<0.01) on flavor sensory. The highest sensory taste was found in treatment C_1S_5 with a ratio of *cascara* to *lime* juice of 100:0 and a *stevia* sweetener concentration of 0.5%, which was 6.45 (sweet). The lowest sensory taste was found in treatment C_3S_1 with a ratio of *cascara* to *lime* juice of 90:10 and a *stevia* sweetener concentration of 0.1%, which was 1.45 (very sour).

Overall Acceptance

The result of study on *cascara*-to-*lime* ratio treatment on overall acceptance can be seen in Figure 18. The ratio of *cascara* to *lime* has a highly significant effect (P<0,01) on overall acceptance sensory score. The data labeled as aA, bB, dan cC indicate that each group is significantly different from one another. The highest overall acceptance sensory score was observed in treatment C_3, with a *cascara* to *lime* ratio of 90:10, yielding a overall acceptance sensory score of 6.25. In contrast, the lowest overall acceptance sensory score was found in treatment C_1, with a *cascara* to *lime* ratio of 100:0, resulting in a flavor sensory score of 6.00. This is because the addition of *lime* balances the sweetness from the *stevia* sweetener and provides a refreshing taste to the beverage. The addition of *lime* can impart a fresh aroma and flavor to drinks, making them more preferred by panelists (Mega et al, 2021).

The result of study on *stevia* sweetener concentration treatment on overall acceptance can be seen in Figure 19. The concentration of *stevia* sweetener has a high significant effect (P<0.01) on overall acceptance sensory score. The data labeled as a, ab, ab, abc, and d indicate that each group is significantly different from one another. The highest overall acceptance sensory score was found in treatment S_5 with a *stevia* sweetener concentration of 0.5%, yielding a flavor sensory score of 6.18, while the lowest flavor sensory score was observed in treatment S_1 with a *stevia* sweetener concentration of 0.1%, resulting in a flavor sensory score of 6.02. This is because the addition of *lime* will balance the sweetness from the *stevia* sweetener, resulting in a beverage that is neither too acidic nor too sweet. The appropriate addition of *stevia* sweetener enhances the sweetness of the product, thereby increasing the preference level among panelists (Zahro et al, 2022).

Evaluation of Cancer Preventive Potential

The Effect of Adding *Cascara* and *Lime* Juice Soft Drink on the Body Weight of Mice Induced with Benzo(a)pyrene (BaP).

The interaction of adding *cascara* and *lime* juice beverages on the body weight of mice showed no significant effect ($P > 0.05$), thus the LSR test was not continued. The induction of BaP in mice did not result in any significant changes in body weight. This is consistent with the literature by (Naby et al. 2023), which states that the induction of mice with BaP does not affect their body weight. The changes caused by BaP induction occur only at the microscopic histopathological level and not at the macroscopic level. BaP induction had no impact on either the body weight or dietary patterns of the observed mice.

The addition of *cascara* and *lime* juice soft drink has a significant impact on the histopathology of the hippocampus in mice induced with BaP.

The result of study on addition of *cascara* and *lime* juice soft drink on the histopathology of the hippocampus in mice induced with BaP can be seen in Table 3. The induction of BaP and the administration of the test substances, specifically *cascara* and *lime* juice soft drink, will result in differing conditions for each group of mice tested.

Table 3. Results of Histopathological Observations on the Brain Organ (Hippocampus) of Mice.

Organ	Parameter	
	Necrotic Cells	Inflammatory Cells
K1	-	-
K2	+	++
K3	+	+
K4	+	+

Based on Table 3, the induction of BaP in the brain organ (hippocampus) of mice in group K2 will lead to oxidative stress, resulting in damage to brain cells, as indicated by the presence of necrotic and inflammatory cells in the brain organ (hippocampus) of the mice. Necrotic cells appear purple with white spots, while inflammatory cells exhibit a dark purple color. In contrast, the mice in groups K3 and K4, which were administered the test substances of *cascara* and *lime* juice beverages, exhibited better conditions regarding necrotic and inflammatory cells compared to those in group K2. The results of the histopathological observations in the hippocampus can be seen in Figure 20.

Based on Figure 20, the histopathological condition in group K1 exhibited normal cells, with no growth of necrotic or inflammatory cells in the brain organ (hippocampus) of the mice. In contrast, group K2 displayed the poorest condition of brain cells (hippocampus), characterized by the proliferation of necrotic and inflammatory cells. Group K3 showed a better condition of brain cells (hippocampus) compared to group K2. Group K4 exhibited an improved condition of brain cells (hippocampus) compared to both groups K2 and K3, as group K4 received a higher dose of the test sample of *cascara* and *lime* juice beverages than group K3. It can be concluded that the administration of *cascara* and *lime* juice beverages can reduce oxidative stress in the brain organ (hippocampus) of mice. This is consistent with the literature by (Zhang et al, 2021), which states that vitamin C compounds can effectively alleviate nerve inflammation and cognitive impairments caused by oxidative stress. Additionally, vitamin C compounds can enhance the concentration of the HO-1 protein, which plays a crucial role in inhibiting oxidative stress.

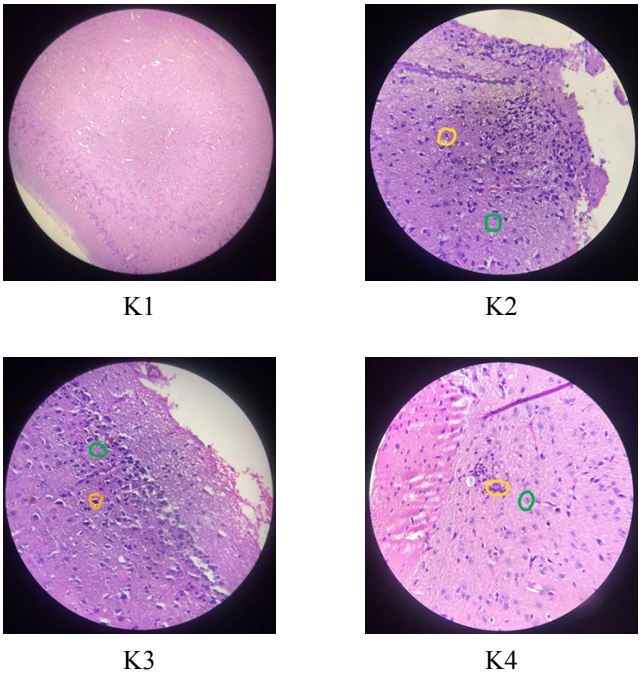

Figure 20. Histopathology of the brain (Hippocampus) of mice (Yellow = inflammatory cells, Green = necrotic cells)

The addition of *cascara* and *lime* juice soft drink has a significant impact on the histopathology of the liver in mice induced with BaP.

The result of study on addition of *cascara* and *lime* juice soft drink on the histopathology of the liver in mice induced with BaP can be seen in Table 4. Necrotic cells appear purple with white spots, while inflammatory cells exhibit a dark purple color. The induction of BaP and the administration of the test substances, specifically *cascara* and *lime* juice soft drink, will result in differing conditions for each group of mice tested.

Based on Table 4, the induction of BaP in the liver of mice in group K2 will lead to oxidative stress, resulting in the proliferation of necrotic and inflammatory cells in the liver. In contrast, the mice in groups K3 and K4, which were administered the test substances of *cascara* and *lime* juice beverages, exhibited better conditions regarding necrotic and inflammatory cells compared to those in group K2, particularly group K4, which was characterized by the absence of necrotic cells. The results of the histopathological observations in the liver can be seen in Figure 21.

Table 4. Results of Histopathological Observations on the Liver Organ of Mice.

Organ	Parameter	
	Necrotic Cells	Inflammatory Cells
K1	-	-
K2	++	+
K3	+	+
K4	-	+

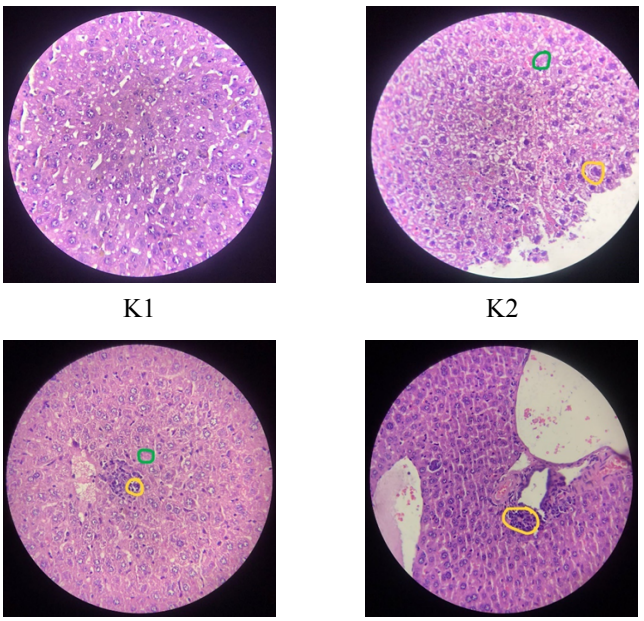

Figure 21. Histopathology of the liver of mice (Yellow = inflammatory cells, Green = necrotic cells)

Based on Figure 21, the histopathological condition of the liver in group K1 exhibited normal cells, with no proliferation of necrotic or inflammatory cells. In contrast, group K2 displayed the poorest condition of liver cells, characterized by the growth of necrotic and inflammatory cells. Group K3 showed a better condition of liver cells compared to group K2. Group K4 exhibited an improved condition of liver cells compared to both groups K2 and K3, characterized by the absence of necrotic cells and a minimal presence of inflammatory cells. It can be concluded that the administration of *cascara* and *lime* juice beverages can reduce oxidative stress in the liver of mice. This is consistent with the literature by (Nayila, 2020), which states that that the administration of vitamin C can lower serum ALT and AST activity, where elevated serum activity signifies liver damage. Additionally, vitamin C can reduce oxidative stress associated with abnormal liver function, and the addition of vitamin C may be beneficial in restoring the antioxidant defense system.

The addition of *cascara* and *lime* juice soft drink has a significant impact on the histopathology of the lungs in mice induced with BaP.

The result of study on addition of *cascara* and *lime* juice soft drink on the histopathology of the lungs in mice induced with BaP can be seen in Table 5. Necrotic cells appear purple with white spots, while inflammatory cells exhibit a dark purple color. The induction of BaP and the administration of the test substances, specifically *cascara* and *lime* juice soft drink, will result in differing conditions for each group of mice tested.

Table 5. Results of Histopathological Observations on the Lungs Organ of Mice.

Organ	Parameter	
	Necrotic Cells	Inflammatory Cells
K1	-	-
K2	++	++
K3	+	+
K4	+	+

Based on Table 5, The induction of BaP in the lungs of mice in group K2 will lead to oxidative stress, resulting in damage to lung cells, as indicated by the

presence of necrotic and inflammatory cells in the lung tissue. In contrast, the mice in groups K3 and K4, which were administered the test substances of *cascara* and *lime* juice beverages, exhibited better conditions regarding necrotic and inflammatory cells compared to those in group K2. The results of the histopathological observations in the lungs can be seen in Figure 22.

Based on Figure 22, The histopathological condition in group K1 exhibited normal cells, with no proliferation of necrotic or inflammatory cells in the lung tissue of the mice. In contrast, group K2 displayed the poorest condition of lung cells, characterized by the proliferation of necrotic and inflammatory cells. Group K3 showed a better condition of lung cells compared to group K2, while group K4 exhibited an even better condition of lung cells than both groups K2 and K3, as group K4 received a higher dose of the test sample of *cascara* and *lime* juice beverages compared to group K3.

Based on Figure 22, There are white circular structures that represent the alveoli of the lungs, where it can be observed that the alveoli in group K1 exhibit the best condition, with no inflammatory or necrotic cells present. In contrast, group K2 shows the poorest condition of alveoli, where the structure has deteriorated into necrotic and inflammatory cells. Group K3 presents a better condition of alveoli compared to group K2, while group K4 has alveoli that retain their shape with the least amount of necrotic and inflammatory cells compared to groups K2 and K3. It can be concluded that the administration of *cascara* and *lime* juice beverages can reduce oxidative stress in the lung tissue of mice. This is consistent with the literature by (Fowler, 2024), which states that the administration of vitamin C can help alleviate Acute Respiratory Distress Syndrome (ARDS) caused by free radicals, as vitamin C may mitigate the effects of inflammatory cells associated with ARDS in the alveoli of the lungs

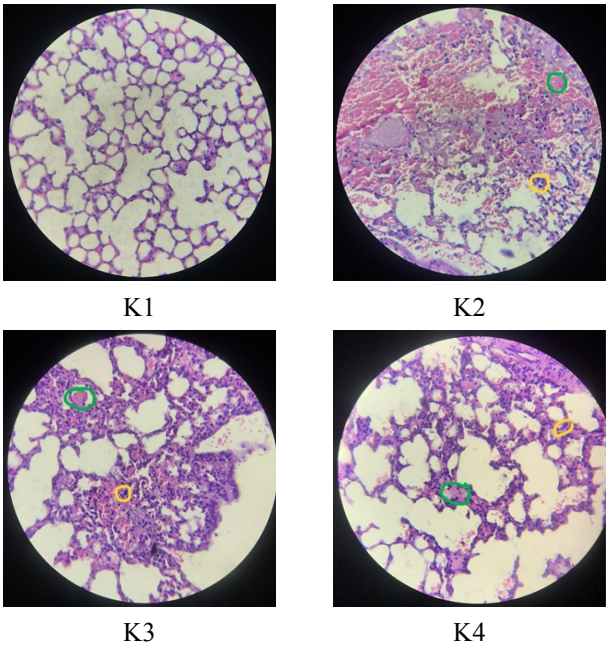

Figure 22. Histopathology of the lungs of mice (Yellow = inflammatory cells, Green = necrotic cells)

CONCLUSION

The comparison of *cascara* and *lime* juice has a highly significant effect ($P < 0.01$) on antioxidant activity, pH, total acidity, vitamin C content, organoleptic color, organoleptic aroma, organoleptic taste, and overall acceptance. The addition of *stevia* sweetener concentration has a highly significant effect ($P < 0.01$) on antioxidant activity, pH, vitamin C content, total sugars, organoleptic color, organoleptic aroma, and organoleptic taste. The best treatment was determined using the De Garmo method, specifically treatment C_3S_5, which involved a ratio of *cascara* to *lime* juice of 90:10 and a *stevia* sweetener concentration of 5%. The addition of cascara serves to enhance flavor, while the inclusion of lime and stevia plays a more significant role in cancer prevention. This optimal treatment was subsequently followed by an evaluation of its cancer preventive potential using mice. The administration of the test sample of *cascara* and *lime* juice soft drink has no significant effect ($P > 0.05$) on the weight of the mice. The administration of the test sample of soft drink at doses of 10.5 and 21 mg/20g body weight of the mice can reduce inflammatory and necrotic cells caused by oxidative stress in the brain (hippocampus), liver, and lungs of mice induced with benzo(a)pyrene.

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TABLES AND FIGURES

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