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Antioxidant Activity and Organoleptic Tests on Functional Drinks Made from Soursop Leaves (*Annona Muricata)* with a Combination of Sappan Wood *(Biancaea Sappan*)

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Abstract: Functional beverages are popular because they have bioactive compounds that contribute to body health. This study aimed to determine the effect of combining soursop leaves (*Annona muricata*) and sappan wood (*Biancaea sappan*) on total phenolic content, flavonoid content, antioxidant activity, and sensory characteristics of a functional beverage. The study employed a Complete Randomized Design (CRD) with one factor consisting of seven treatment combinations, which represented different proportions of soursop leaves and sappan wood. The results showed that the 70% soursop leaves and 30% sappan wood combination (A7B7) produced the highest total phenolic content of 3335.97 mg GAE/100g, the flavonoid content of 2180.35 mg QE/100g, and the lowest IC50 value of 62.02 ppm, indicating strong antioxidant activity. Sensory tests showed that treatment (A1B1) in color parameters showed a significant difference ($p<0.05$), and treatment (A2B2, A3B3, A4, B4, A5B5, and A6B6) in taste, aroma, texture, and preference parameters did not show a significant difference ($p<0.05$). The A7B7 combination was identified as the best formulation based on antioxidant activity and panelist preferences.

Keywords: Antioxidant; Organoleptic Test; Sappan Wood; Soursop Leaves.

Introduction

Functional beverages are in increasing demand among the public due to their content of bioactive substances that contribute to the body's health. Functional beverages can offer additional health benefits, including enhanced immunity, protection against degenerative diseases, and defence against oxidative stress induced by free radicals [1]. Oxidative stress is recognised as one of the primary causes of various chronic diseases, including diabetes, cancer, and cardiovascular disease; therefore, the development of beverages with high antioxidant activity is a crucial step in supporting a healthy lifestyle [2]. Soursop leaf (*Annona muricata*) is one of the natural ingredients that has potential as a source of antioxidants. Antioxidants are compounds that can fight free radicals, which are unstable molecules that can damage body cells through the oxidation process. The high antioxidant activity in soursop leaves makes it a potential ingredient for preventing or reducing the risk of diseases caused by oxidative stress, such as cardiovascular disease, cancer, diabetes, and neurodegenerative disorders [3]. Various studies have shown that soursop leaves contain vitamins A, B, and C, as well as bioactive compounds such as flavonoids, tannins, saponins, polyphenols, and alkaloids, which exhibit antioxidant [4], anticancer, anti-inflammatory, antihypertensive [5], and antimicrobial activities [6].

Several researchers have innovated by combining soursop leaf infusion with various herbal ingredients such as ginger [7] and cinnamon [8] to optimize the antioxidant activity of the infusion. This combination has been shown to increase the content of bioactive compounds, such as flavonoids and total phenolics, which play a crucial role in scavenging free radicals. However, research on combining soursop leaf infusion with other herbs with strong phenolic compounds is still limited. One potential natural ingredient that has not been widely explored is sappan wood (*Biancaea sappan*). Sappan wood is known to be rich in antioxidant compounds, such as brazilin, flavonoids, and polyphenols [10], which play a crucial role in scavenging free radicals and preventing oxidative stress. The antioxidant activity of sappan wood is relatively high, with an IC_{50} value of 74.44 µg/mL, while vitamin C, a positive control, had an IC_{50} of 3.04 µg/mL. Based on the grouping of antioxidant activity, sappanwood extract is included in the strong activity category (IC_{50} = 50–100 µg/mL) [11].

Although the antioxidant potential of soursop leaves and sappanwood has been widely reported separately, few studies have examined their combined formulation functional beverage. Most previous studies have focused solely on single extractions or the use of a single herbal ingredient without considering the synergistic effects between these natural ingredients. However, the combination of these two ingredients has the potential to generate positive interactions between bioactive compounds, such as flavonoids and polyphenols, which can enhance overall antioxidant activity.

The combination of soursop leaves and sappan wood in a functional drink formulation is expected to produce a product with optimal health benefits, as both contain high levels of bioactive compounds. This research aims to

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develop a soursop leaf-based functional drink formulation with the addition of sappan wood and to evaluate the antioxidant activity and sensory quality (color, taste, aroma, and texture) of the resulting product. The results of this study are expected to contribute to the development of functional drinks based on natural ingredients that benefit public health while also encouraging the optimal use of local resources.

Research Methods

The study employed a Complete Randomized Design (CRD) with one factor consisting of seven treatment combinations, which represented different proportions of soursop leaves and sappan wood, namely:

1. A1B1 (100% soursop leaf)
2. A2B2 (95% soursop leaves: 5% sappan wood)
3. A3B3 (90% soursop leaves: 10% sappan wood)
4. A4B4 (85% soursop leaves: 15% sappan wood)
5. A5B5 (80% soursop leaves: 20% sappan wood)
6. A6B6 (75% soursop leaves: 25% sappan wood)
7. A7B7 (70% soursop leaves: 30% sappan wood)

Each treatment was carried out three (3) times, resulting in 21 experimental units. The data obtained were analyzed using the Analysis of Variance (ANOVA) statistical method and continued with Duncan's Multiple Range Test if there was a significant difference ($P \leq 0.05$) between treatments.

Preparation of Soursop Leaves

Soursop leaf powder (*Biancaea sappan*) preparation begins with sorting the soursop leaves. The leaves are cleaned using running water and separated from the stalk. After that, it is washed, then cut to a size of 2 cm. After cutting, the material is dried using a dehydrator at 50 °C for 6 hours, until the leaves become dry. It is then ground and sieved through a 30-mesh sieve [12].

Preparation of Sappan Wood

This processing involves peeling the bark off the sappan wood and then cleaning it of its outer skin. After that, the sappan wood will be split into pieces measuring 3-4 cm in width. The skin will then be cleaned and dried using a dehydrator for 6-8 hours at a temperature of 50-55 °C. Next, the grinding process is carried out using a flouring tool, and then sieved using a sieve with a 30-mesh size [13].

Preparation of brew

The brewing process begins by mixing soursop leaf powder and sappan wood, with a weight of 3 grams in each treatment, and then wrapping the mixture in a tea bag (Nilo tea filter). Then, the preparation is brewed with 150 ml of hot water at a temperature of 80 °C - 90 °C for 2 minutes [14].

Analytical Procedures

Phenol Total Test

The Folin–Ciocalteu reagent is reduced by phenolic compounds and other reducing agents to a blue complex; the color intensity at a wavelength of ~765 nm is directly proportional to the amount of phenol. Results are expressed as gallic acid equivalents (GAE) [15].

Flavonoid Total Test

300 µL of the sample was mixed, and 4.0 aquabides and 0,03 mL of 10% NaNO2 were added for 6 min. The mixture was added to 0,30 mL of $AlCl_3$ (10%) and allowed to stand for 5 minutes. Then, 4.0 mL of 10% NaOH was added, followed by 1.1 mL of aquabides. Readings were taken using a UV-Vis spectrophotometer with an absorbance of 510 nm. Total flavonoids were expressed in mg RE g-1 of extract [16].

Antioxidant Activity Test (IC_{50} Method)

A 0.1 mM DPPH solution was prepared by dissolving 0.004 g of DPPH in a 100 mL volumetric flask, followed by the addition of methanol to the mark. Sample concentrations were varied at 0, 100, 200, 300, 400, and 500 mg/mL. From each concentration, 0.5 mL was taken and mixed with 1 mL of 0.1 mM DPPH solution. Then, 4 mL of methanol was added, the mixture was vortexed until homogeneous, and incubated for 30 minutes. Absorbance was measured at a wavelength of 517 nm [17].

Organoleptic Test

The organoleptic test used the hedonic method, which aims to analyze consumers' level of preference for the samples presented. The hedonic test was conducted using 15 untrained panelists, with parameters including taste, aroma, texture, and color. In the hedonic test, panellists were presented with samples in turn and asked to complete a questionnaire to score their preferences [18].

Results and Discussion

Total phenol

Based on the results of the ANOVA test, the combination formulation of soursop leaves and sappan wood in brewed products had a significant effect on total phenols ($P < 0.05$). The average total phenol of the soursop leaf and sappan wood combination is presented in Table 1

Table 1. Total Phenols of the Soursop Leaf and Sappan Wood Beverage

Formulation	Mean Total Phenol (mgGAE/100gr)
A1B1	2550.30[a]
A2B2	2700.57[b]
A3B3	3008.47[c]
A4B4	3034.66[d]
A5B5	3110.75[e]
A6B6	3235.87[f]
A7B7	3335.97[g*]

Description:

1. Different notations indicate that the interaction between treatments has a significant effect ($P< 0.05$).
2. The value of total phenolics is the mean value of 3 replicates.
3. * sign indicates the highest antioxidant activity

Based on Table 3.1, the highest average total phenol content was found in treatment A7B7 (70% soursop leaves: 30% sappan wood), at 3335.97 mg GAE/100 g. Meanwhile, the lowest average was found in the control treatment (100% soursop leaves), at 2550.30 mg GAE/100 g. These results indicate that the greater the proportion of sappan wood added, the higher the total phenol content. This increase is attributed to the presence of phenolic compounds in the sappan wood powder used. These findings align with research reporting the addition of sappan wood extract to soursop leaf herbal drinks, which showed an increase in total phenol content along with an increase in the proportion of sappan wood in the formulation. At the addition of 10% sappan wood, the total phenol content was recorded at 2675.42 mg GAE/100 g, while at the addition of 30% the total phenol content increased significantly to 3335.97 mg GAE/100 g [19].

This finding is also in line with research on the addition of sappan wood powder (*Caesalpinia sappan* L.) to roselle tea (*Hibiscus sabdariffa*). The study reported that increasing the concentration of sappan wood resulted in an increase in total phenol levels. At a 5% sappan wood addition, the total phenol level was recorded at 82.40%, while at a 15% concentration, it increased to 91.80% [20]. Another showed similar results, where the addition of sappan wood extract to functional drinks significantly increased phenol levels, namely from 41.2 mg GAE/L to 63.9 mg GAE/L. The consistency of the results from these various studies strengthens the hypothesis that sappan wood is a source of phenolic compounds with the potential to increase total phenols in various types of processed products [21].

Total Flavonoids

Based on the results of the ANOVA test, the combination of soursop leaves and sappan wood in steeping products has a significant effect on total flavonoids ($P<0.05$). The average total flavonoids of the soursop leaf and sappan wood combination are presented in Table 2.

Table 2. Total Flavonoids of the Soursop Leaf and Sappan Wood Beverage

Formulation	Mean Flavonoids (mgQE/100gr)
A1B1	1347.32[a]
A2B2	1632.80[b]
A3B3	1978.52[c]
A4B4	1990.32[d]
A5B5	1999.87[e]
A6B6	2093.64[f]
A7B7	2180.35[g*]

Description:
1. Different notations indicate that the interaction between treatments has a significant effect ($P< 0.05$).
2. The value of total flavonoids is the mean value of 3 replicates.
3. * sign indicates the highest antioxidant activity

Based on Table 2, the highest average total flavonoid content was found in treatment A7B7 (70% soursop leaves: 30% sappan wood), at 2180.35 mg QE/100 g. Conversely, the lowest value (1347.32 mg QE/100 g) was found in the control treatment (100% soursop leaves). This pattern indicates that increasing the proportion of sappan wood contributes to increased flavonoid levels in the brew. This increase is thought to originate from the bioactive compounds in sappan wood, which are naturally rich in flavonoids and other polyphenol-derived compounds.

This finding is consistent with research reporting that the addition of sappan wood (*Caesalpinia sappan*) to herbal drink formulations has been reported to increase total flavonoid levels. A 4:1:1 ratio of roselle, ginger, and sappanwood formulation increased total flavonoid levels from 24.81 mg QE/g to 28.81 mg QE/g [22]. A similar study also reported that a herbal drink formulation supplemented with 10% sappanwood resulted in a flavonoid content of 18.42 mg QE/g, an increase from 12.77 mg QE/g in the control without sappanwood—equivalent to a 44.2% increase [23].

Antioxidant Activity (IC50)

Based on the results of the ANOVA test, the combination of soursop leaves and sappan wood in steeping products has a significant effect on the IC50 value ($P< 0.05$). The average antioxidant activity of soursop leaf and Sappan wood combination is presented in Table 3

Table 3. Antioxidant Activity Test Results of the Soursop Leaf and Sappan Wood Beverage

Formulation	Antioxidant Activity (ppm)
A1B1	68.9819[d]
A2B2	66.9301[c]
A3B3	65.8588[b]
A4B4	62.2971[a]
A5B5	62.2855[a]
A6B6	62.0401[a]
A7B7	62.0169[a*]

Description:
1. Different notations indicate that the interaction between treatments has a significant effect ($P< 0.05$).
2. The value of antioxidant activity is the mean value of 3 replicates.
3. * sign indicates the highest antioxidant activity

Based on Table 3, the highest average antioxidant activity value was obtained in treatment A7B7 (70% soursop leaves: 30% sappan wood), which was 62.0169 ppm. Conversely, the lowest value was found in treatment A1B1 (100% soursop leaves), which was 68.9819 ppm. This pattern suggests that the addition of sappan wood enhanced the antioxidant activity of the infusion. Based on the following classification, compounds with IC_{50} values of less than 50 ppm are classified as very strong antioxidants, 50–100 ppm as strong, 100–150 ppm as moderate, and 150–200 ppm as weak. Thus, the antioxidant activity in all treatments falls into the strong category [24].

The high antioxidant activity of the combination of soursop leaves and sappan wood is in line with the increase in total phenol and flavonoid levels, as shown in Tables 1 and 2. Phenolic and flavonoid compounds are known to act as hydrogen donors or free radical scavengers, thus directly reducing the IC_{50} value. Polyphenols, including flavonoids, have antioxidant activity because their structure allows for the stabilization of free radicals through a resonance mechanism. The higher the content of phenolic compounds

in a material, the greater its contribution to antioxidant activity [25].

Therefore, the high antioxidant activity in the A7B7 treatment is not only due to the presence of sappan wood, but also due to the increase in total phenol and flavonoid levels that accompany it. This finding is supported by research indicating that Caesalpinia sappan is rich in phenolic compounds, which directly contribute to increasing the antioxidant capacity of functional food and beverage products [26]. In addition, other studies have reported that sappan wood extract has a high total phenolic and flavonoid content, as well as DPPH radical scavenging activity, which increases with the concentration of the extract used, thereby strengthening its antioxidant potential in beverage formulations [27].

The increased antioxidant activity of formulations containing sappan wood is due to the presence of its main bioactive compounds, namely brazilin, brazilein, flavonoids, and other polyphenols. Brazilin and brazilein are the main components of sappan wood, which have a fire ring with a phenolic hydroxyl group, enabling them to donate hydrogen atoms and neutralize free radicals through an oxidation-inhibiting mechanism. Furthermore, the flavonoids and other polyphenols in sappan wood contribute to inhibiting free radical reactions through resonance stabilization [28].

Brazilin, the main compound in sappanwood, exhibits potent antioxidant activity through several interdependent mechanisms. Recent studies have shown that brazilin is capable of directly scavenging free radicals by donating hydrogen atoms from its phenolic hydroxyl group. This hydrogen donation neutralizes reactive radicals that can trigger oxidative stress and biomolecular damage. Furthermore, brazilin's aromatic structure allows for radical stabilization through resonance, preventing the neutralized radicals from becoming reactive again. This dual mechanism, hydrogen donation and resonance stabilization, makes brazilin effective in inhibiting free radical propagation and reducing oxidative potential in food and biological systems [29]. Therefore, increasing the concentration of sappanwood in beverage formulations can directly enhance antioxidant capacity through the contribution of brazilin compounds.

Organoleptic Test

The results of the ANOVA test showed that the formulation of soursop leaves and sappan wood was significantly different ($P < 0.05$) in terms of color attribute, while in terms of aroma, texture, and taste attributes, the formulation showed no significant difference ($P > 0.05$). The sensory results of the combination of soursop leaves and sappan wood are presented in Table 4.

Based on Table 4, the highest average color assessment score was obtained for treatment A7B7 (70% soursop leaves: 30% sappan wood), at 4.333. Conversely, the lowest score was for treatment A1B1 (100% soursop leaves), at 1.667. These scores indicate that panellists disliked the color of the product in the treatment without the addition of sappan wood, while treatment A7B7 was considered the most preferred. The addition of a higher proportion enhanced the color intensity of the brew, making it more visually appealing to panellists. This increase in color intensity is related to the natural pigment content of sappan wood. Sappan wood is known to contain brazilin, which oxidizes to brazilein when dissolved in water, producing a bright red color [30]. This distinctive color provides greater visual appeal than using soursop leaves alone. Furthermore, the combination of sappan wood pigments and soursop leaf color components results in a more appealing beverage organoleptically. This finding aligns with research by Widyaningsih et al [31], which reported that increasing the concentration of sappan wood in functional beverage formulations increased the intensity of the red to purplish-red color, which consumers preferred.

Table 4. Organoleptic Test of the Soursop Leaf and Sappan Wood Beverage

Code	Color	Aroma	Texture	Flavor	Overall
A1B1	1.66^{c}	2.33^{c}	3.20^{a}	2.26^{b}	2.26^{c}
A2B2	1.73^{c}	2.60^{bc}	3.20^{a}	2.73^{ab}	3.00^{b}
A3B3	2.86^{b}	2.86^{b}	3.46^{a}	2.93^{ab}	3.20^{b}
A4B4	3.26^{ab}	3.06^{abc}	3.73^{a}	2.93^{ab}	3.33^{ab}
A5B5	3.46^{b}	3.13^{ab}	3.80^{a}	3.00^{ab}	3.33^{ab}
A6B6	3.80^{ab}	3.33^{ab}	3.80^{a}	3.20^{a}	3.40^{ab}
A7B7	4.33^{a}	3.40^{a}	3.80^{a}	3.33^{a}	3.93^{a}

Description:
1. Different notations indicate that the interaction between treatments has a significant effect ($P< 0.05$).
2. The value of the organoleptic test is the mean value of 3 replicates.

Table 4 shows that the highest average value for aroma parameters was found in treatment A7B7 (soursop leaves 70%: sappan wood 30%) at 3.40, while the lowest value was found in treatment A1B1 (soursop leaves 100%) at 2.33. The aroma test results did not show a statistically significant difference ($P > 0.05$) between treatments. This is likely because the addition of sappan wood was still relatively low, at only 30%, so the aroma was not strong enough to produce a significant difference. Furthermore, the dominant aroma of soursop leaves likely masked the distinctive aroma of sappan wood. This finding is in line with research reporting that increasing the concentration of sappanwood in functional beverage formulations increases the intensity of the red color to a purplish red that consumers prefer [32].

Table 4 shows that the highest average texture value was found in treatment A7B7 (70% soursop leaves: 30% sappan wood), which was 3.80, indicating that the product texture in this treatment was quite favored by the panelists. The product's texture remained unchanged because all treatments used a liquid form without the addition of thickeners. Consequently, there was no significant difference in viscosity, leading the panelists to give relatively similar scores.

Table 4 shows that the highest average value for taste parameters was found in treatment A7B7 (70% soursop leaves: 30% sappan wood), which was 3.33. The highest score indicates that the taste of this treatment was most preferred by the panelists. Although there was variation in the average values between treatments, the statistical test results showed that the difference was not significant ($P > 0.05$). Thus, the panelists did not perceive a clear difference in taste between treatments. This condition is likely due to the addition of sappan wood in this amount not being able to produce a significant change in taste, so the product's taste

remained relatively similar. Increasing the proportion of sappan wood can generally strengthen the bitter and astringent taste due to the saponin and tannin content [33]. This finding is consistent with research reporting that the addition of herbal ingredients in small amounts often does not produce a taste difference that is easily recognized by panelists [34].

Based on Table 4, the highest overall average score was obtained for treatment A7B7 (70% soursop leaves: 30% sappan wood), which was 3.93. This value indicates that the product in this treatment was the most preferred by the panelists. Preference here is a comprehensive assessment that includes taste, color, aroma, and texture. Treatment A7B7 was chosen as the most preferred because it produced a brighter color, balanced taste, and neutral aroma, thus providing an overall better sensory perception for the panelists.

Conclusion

The soursop leaves and sappan wood formulation had a significant effect ($P<0.05$) on total phenol and flavonoid parameters. The highest total phenol and total flavonoid values, at 3335.97 and 2180.35, respectively, were found in treatment A7B7. The soursop leaf and sappan wood formulation had a significant effect ($P<0.05$) on antioxidant activity, with an IC50 value of 68.98 ppm (strong antioxidant) in treatment A7B7. Sensory testing showed that the formulation had a significant effect ($P<0.05$) on color parameters, while taste, aroma, texture, and preference showed no significant differences ($P>0.05$). Treatment A7B7 was the best formulation in the overall assessment.

Author's Contribution

K. F. Eno: performed the experiments, collected the data, and drafted the manuscript. P. R. Sintyadewi: validated the results, and revised the manuscript. I. A. P. A. Widnyani: contributed to data analysis and interpretation. N. D. A. W. Putra: assisted with organoleptic testing and statistical analysis. I. G. A. Y. Rabani RS: contributed to the ldata interpretation.

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