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Development and formulation of rosella (hibiscus sabdariffa) tea bags combined with pandan (pandanus amaryllifolius) aroma ('rosedan') and its antioxidant activity

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Abstract*:* This study aimed to develop and evaluate a rosella (*Hibiscus sabdariffa L.*) and pandan (*Pandanus amaryllifolius Roxb.*) herbal tea bag formulation ("Rosedan") with antioxidant properties and consumer acceptance. A Research and Development (R&D) approach using the 4D model was applied, involving formulation design, antioxidant testing, and organoleptic evaluation. Three formulations were developed with different ratios: 70:30, 80:20, and 100% rosella (control). Antioxidant activity was assessed using the DPPH method, while organoleptic testing (color, aroma, taste, and overall liking) was conducted on 50 respondents. The instrument for sensory evaluation was validated by three experts and categorized as very valid (mean score = 4.00). The results showed that Formulation 1 (70:30) had the strongest antioxidant activity (IC_{50} = 0.400 mg/mL), indicating a synergistic effect between rosella and pandan compounds. Statistical analysis revealed that the data were not normally distributed; thus, nonparametric tests were applied. The Friedman test showed a significant difference in overall preference among formulations ($p < 0.05$), with Formulation 1 ranked highest. Further analysis using the Wilcoxon Signed Rank Test confirmed that Formulation 1 was significantly more preferred than the others. In conclusion, the 70:30 rosella–pandan formulation is the most optimal in terms of antioxidant activity and consumer acceptance, making it a promising functional herbal beverage for further development
Keywords: rosella, pandan, herbal tea, antioxidant, organoleptic test.

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INTRODUCTION

The 2023 Indonesian Health Survey stated that the prevalence of health problems in Indonesia is currently dominated by an increase in non-communicable diseases (NCDs) such as stroke, diabetes mellitus, cardiovascular disease, cancer, and metabolic syndrome, reaching 8.3 per 1,000 population based on . Most of these conditions are closely related to oxidative stress, namely an imbalance between the production of free radicals and the ability of the body's antioxidant system to neutralize them. Oxidative stress plays a role in damage to cell membranes, proteins, lipids, and DNA, ultimately accelerating chronic inflammation and tissue degeneration. Therefore, an antioxidant-based approach is becoming increasingly relevant in promotive, preventive, and complementary therapy efforts to address public health issues in Indonesia (Kemenkes, 2024a). Antioxidants are essential for the body to prevent cell damage caused by free radicals. These free radicals can come from exposure to sunlight, radiation from digital devices (such as gadgets, laptops, computers), ozone, cigarette smoke, vehicle exhaust, and from foods that undergo extensive processing such as instant noodles, junk food, canned meat, and so on. Antioxidants play an important role in protecting cells from free radical damage. Free radicals are byproducts of the body's metabolism that, if not neutralized, can trigger various chronic diseases and accelerate the aging process (Lobo et al., 2010).

In the Indonesian context, modern diets, exposure to pollution, smoking, stress, and low consumption of phytonutrient-rich foods contribute to the burden of oxidative stress in the population. This situation makes exploring natural antioxidant sources, particularly from the biodiversity of Indonesian medicinal plants, a strategic opportunity to support national health resilience (Kemenkes, 2024b). Therefore, it is important for individuals with active lifestyles to meet their daily antioxidant needs through natural foods. One such solution is modifying the antioxidant-rich rosella herb into tea bags, which is a promising solution because it is practical and, through appropriate formulation, maintains its high antioxidant content.

METHODS

This study used a Research and Development (R&D) approach with the 4D model (Define, Design, Develop, Disseminate) to produce a herbal tea bag product based on roselle and pandan. The subjects in this study were residents of Keceme Hamlet, Caturharjo Village, Sleman, aged 30–60 years. Respondents were selected using a purposive sampling technique, considering predetermined inclusion and exclusion criteria. The sample size of 20 individuals was determined using the Slovin formula for a limited population. Inclusion criteria included respondents who had a permanent domicile, were able to communicate well, and were willing to participate in the study. Exclusion criteria included those with health conditions that prevented them from participating, pregnant or breastfeeding women, and respondents who did not complete the study. The use of human subjects in organoleptic testing aims to evaluate consumer acceptance of the product's sensory attributes, which is a crucial factor in the development of functional foods (Stone & Sidel, 2004).

The tea bag formulation was developed by combining dried roselle flower petals (*Hibiscus sabdariffa L.*) and pandan leaves (*Pandanus amaryllifolius Roxb.*). The formulation was based on the principle of balancing biological activity (antioxidants) and sensory acceptability. similar research formulation conducted by Pratiwi, et all (2022) by eliminating cinnamon to maintain relevance to the topic being studied. The ratio of formulation 1 with a ratio of rosella: pandan leaves of 70:30, formulation 2 80: 20 and formulation 3 100% rosella, size of the brew, and type of packaging that complies with food safety standards. Three formulation variations were used in this study: F1: Roselle 70%: Pandan 30%; F2: Roselle 80%: Pandan 20%; F3: Roselle 100% (control). The formulation process included sorting the ingredients, drying the herbs, grinding them, mixing them according to the ratio, and packaging them in food-grade tea bags. Theoretically, herbal product formulations must consider the stability of active compounds such as flavonoids and anthocyanins, as well as interactions between ingredients that can affect antioxidant activity and organoleptic quality (Fadhilah et al., 2024). In addition, the combination of herbal ingredients also aims to create a synergistic effect between health functions and increased palatability (Tamba et al., 2023). Antioxidant activity testing was performed using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method, a spectrophotometric method for measuring a compound's ability to scavenge free radicals. The testing procedure was conducted at the Gadjah Mada University Pharmacy Laboratory and included:

a) Preparing a DPPH solution in methanol
b) Mixing the sample with the DPPH solution
c) Incubating for 30 minutes in the dark
d) Measuring absorbance at a wavelength of ±517 nm using a UV-Vis spectrophotometer

Antioxidant activity is calculated as the percent inhibition and IC50 value. The lower the IC50 value, the higher the antioxidant activity of a sample (Gulcin, 2020). The DPPH method is widely used due to its high sensitivity, simple procedure, and ability to provide a rapid overview of a material's antioxidant capacity (Blois, 1958; Shahidi & Zhong, 2015). The best formulation data will be known through the results of the lab tests carried out.

Organoleptic testing is conducted to assess consumer acceptance of a product based on the following attributes:

a) Color
b) Aroma
c) Taste

d) Overall liking

Assessments are made using a hedonic scale of 1–5 (dislike very much to like very much). This test involves untrained panelists from the local community. Organoleptic testing is an important method in food product development because it determines a product's success in the market, in addition to its functional aspects (Lawless & Heymann, 2010). The data obtained were analyzed using descriptive and inferential statistical approaches. Descriptive statistics to see preference trends ANOVA or Kruskal-Walli's test to compare preference levels between formulations

RESULT AND DISCUSSION

The validation of the organoleptic test instrument in this study was conducted by three expert judges, to assess the aroma, taste, and level of liking of the herbal tea product. Based on the assessment using a 4-point Likert scale, all validators gave a maximum score for each indicator with an average of 4.00, indicating that the instrument is categorized as very valid. These results confirm that the instrument has met content validity, both in terms of indicator clarity, language suitability, and relevance to organoleptic quality measurements, so it is suitable for use without revision in testing consumer acceptance of the rosella-pandan herbal tea formula.

The initial stage was an antioxidant test using the DPPH method, which was carried out on 3 formulas with the following results.

Table 1. Results of the DPPH Antioxidant Test Method

Herbal Formulation	Method	DPPH Free Radical Scavenging Activity (IC_{50})
Formulation 1 (Rosella 70% : Pandan 30%)	IKU/7.2/TF-UV-01 (UV-Vis Spectrophotometry)	0.400 mg/mL
Formulation 2 (Rosella 80% : Pandan 20%)	IKU/7.2/TF-UV-01 (UV-Vis Spectrophotometry)	0.625 mg/mL
Formulation 3 Control (Rosella 100%)	IKU/7.2/TF-UV-01 (UV-Vis Spectrophotometry)	0.493 mg/mL

In the DPPH assay, a lower IC_{50} value indicates stronger antioxidant activity; therefore, the rosella–pandan formulation at a 70:30 ratio showed the most optimal activity compared to the control and other ratios. Although rosella is rich in anthocyanins and flavonoids, the addition of 30% pandan appears to enhance antioxidant activity through a possible synergistic interaction between phenolic and aromatic compounds, while the 80:20 ratio did not achieve this effect. These finding highlights that the bioactivity of herbal formulations is not always linear but is influenced by complex interactions among secondary metabolites. The organoleptic test was conducted through a survey of 50 residents of Dusun Keceme, and the data were analyzed using classical assumption tests to ensure validity and reliability. Normality testing was first performed to determine data distribution and guide the selection of statistical methods. If the data were normally distributed, a parametric test was applied; otherwise, a nonparametric alternative was used for the independent difference test, ensuring accurate and appropriate analysis.

Based on the results of normality tests using the Kolmogorov-Smirnov and Shapiro-Wilk methods, all variables in each formulation demonstrated a significance value (Sig.) of 0.000, or <0.05. This applied to all parameters tested, including color, aroma, taste, texture, and the overall assessment for Formulations 1, 2, and 3. Therefore, based on the decision-making criteria (Sig. <0.05), it can be concluded that the data are not normally distributed. This classical assumption test demonstrates a rejection of the normality assumption. Therefore, the basic assumption for subsequent hypothesis testing uses non-parametric tests.

Based on the results of the previous normality test, which indicated that all data were not normally distributed (Sig. < 0.05), the analysis could not be continued using parametric tests. Therefore, a more appropriate nonparametric approach, the Friedman test, was used, considering that the data came from three treatments (formulations) tested on the same respondents.

Table 2. Friedmen Test

Ranks	
Formulation 2 Overall	2.43
Formulation 1 Overall	2.57
Formulation 3 Overall	1.00

Test Statistics[a]	
N	50
Chi-Square	85.299
df	2
Asymp. Sig.	.000
a. Friedman Test	

The Friedman test results showed a Chi-Square value of 85.299 (df = 2) with a significance level of 0.000 (< 0.05), indicating a statistically significant difference in overall preference among the three formulations. Based on mean rank values, Formulation 1 ranked highest (2.57), followed by Formulation 2 (2.43), while Formulation 3 had the lowest acceptance (1.00). These findings confirm that the formulations significantly influenced respondents' preferences, with Formulation 1 being the most preferred. Based on the results of the Friedman test conducted previously, a statistically significant difference ($p < 0.05$) was found in the level of preference of the three 'Rosedan' tea bag formulations. To determine which formulation pairs had significant differences, a further test (Post Hoc Test) was conducted using the Wilcoxon Signed Rank Test.

Tabel 3. Wilcoxon Signed Rank Test

Ranks				
		N	Mean Rank	Sum of Ranks
Formulation 2 Overall - Formulation 1 Overall	Negative Ranks	17[a]	16.41	279.00
	Positive Ranks	10[b]	9.90	99.00
	Ties	23[c]		
	Total	50		
Formulation 3 Overall Formulation 1 Overall	Negative Ranks	50[d]	25.50	1275.00
	Positive Ranks	0[e]	.00	.00
	Ties	0[f]		
	Total	50		

a. Formulation 2 Overall < Formulation 1 Overall
b. Formulation 2 Overall > Formulation 1 Overall
c. Formulation 2 Overall = Formulation 1 Overall
d. Formulation 3 Overall< Formulation 1 Overall
e. Formulation 3 Overall> Formulation 1 Overall
f. Formulation 3 Overall= Formulation 1 Overall

Test Statistics[a]		
	Formulation 2 Overall Formulation 1 Overall	Formulation 3 Overall - Formulation 1 Overall
Z	-2.180[b]	-6.198[b]
Asymp. Sig. (2-tailed)	.029	.000
a. Wilcoxon Signed Ranks Test		
b. Based on positive ranks.		

Based on the results of the Wilcoxon Signed Rank Test on the overall parameters, Formulation 1 was found to have a significant advantage over Formulation 2 (Z = -2.180; p = 0.029). This is supported by the ranking data, where most panelists gave Formulation 1 a higher score. A more striking difference was seen in the comparison of Formulation 3, where all respondents (50 panelists) gave a significantly lower rating than Formulation 1 (Z = -6.198; p = 0.000). Thus, it can be concluded that Formulation 1 is the most effective product.

CONCLUSION

The results of this study indicate that the organoleptic test instrument used to evaluate aroma, taste, and overall liking was highly valid, as confirmed by three expert judges who assigned the maximum score (mean = 4.00). This demonstrates that the instrument met content validity requirements and was appropriate for assessing consumer acceptance without revision. In addition, antioxidant testing using the DPPH method showed that the rosella–pandan formulation at a 70:30 ratio had the strongest activity (IC_{50} = 0.400 mg/mL), outperforming both the control and other formulations. This finding suggests a synergistic interaction between rosella and pandan compounds, indicating that optimal bioactivity depends on formulation balance rather than a single dominant ingredient.

Furthermore, organoleptic evaluation involving 50 respondents revealed that the data were not normally distributed, leading to the use of nonparametric statistical analysis. The Friedman test demonstrated a significant difference in overall preference among the three formulations (Chi-Square = 85.299; $p < 0.05$). Based on mean rank values, Formulation 1 was the most preferred, followed by Formulation 2, while Formulation 3 showed the lowest level of acceptance. These results confirm that variations in formulation significantly influence consumer perception and preference.

The Wilcoxon Signed Rank Test further strengthened these findings by showing that Formulation 1 was significantly more preferred than both Formulation 2 ($p = 0.029$) and Formulation 3 ($p = 0.000$). Notably, all respondents rated Formulation 3 lower than Formulation 1, highlighting a clear difference in acceptance. Overall, it can be concluded that the 70:30 rosella–pandan formulation is the most effective and optimal product, both in terms of antioxidant activity and consumer preference, making it the best candidate for further development and application.

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