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ANALYSIS OF SECONDARY METABOLITE LEVELS AND ANTIOXIDANT ACTIVITY OF THE COMBINED EXTRACT OF RAJA BANANA PEEL AND PONTIANAK SWEET ORANGE PEEL

Analisis Kadar Metabolit Sekunder dan Aktivitas Antioksidan Kombinasi Ekstrak Kulit Pisang Raja dan Kulit Jeruk Manis Pontianak

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ABSTRAK

Ekstrak kulit pisang raja dan ekstrak kulit jeruk manis Pontianak mengandung senyawa bioaktif, seperti flavonoid dan antosianin, yang berpotensi sebagai sumber senyawa bioaktif dengan aktivitas antioksidan. Kombinasi ekstrak kedua bahan ini belum pernah dianalisis sebelumnya. Penelitian ini bertujuan untuk menganalisis kadar total flavonoid, antosianin, aktivitas antioksidan, fenol dan tanin pada ekstrak kombinasi kulit pisang raja dan kulit jeruk manis. Metode penelitian ini menggunakan pendekatan eksperimen, yang dilakukan di Laboratorium Setia Budi pada bulan Juli tahun 2024, dengan menggunakan pelarut etanol 80 % dengan perbandingan 1:10 melalui remaserasi sebanyak lima kali untuk meningkatkan rendemen dan kualitas senyawa yang dihasilkan. Formulasi ekstrak yang digunakan terdiri dari F1 (15:85%), F2 (25:75%), F3 (50:50%), F4 (75:25%). Kadar flavonoid dan tanin dianalisis dengan spektrofotometri, antosianin dengan metode pH diferensial, serta aktivitas antioksidan dan fenol dengan metode DPPH. Hasil penelitian menunjukkan bahwa formula F1 memiliki kadar flavonoid tertinggi (59,53 mgQE/g), F4 menunjukkan aktivitas antioksidan terbaik (91,47% inhibisi), dan F1 mengandung antosianin (361,45 ppm), fenol (1,80%), dan tanin (0,84%) tertinggi. Penambahan kulit jeruk manis meningkatkan kadar antosianin, fenol, dan tanin dalam ekstrak, sedangkan penambahan ekstrak kulit pisang raja meningkatkan aktivitas antioksidan. Penelitian lebih lanjut diperlukan untuk mengeksplorasi senyawa fitokimia lainnya dan potensi kesehatan dari kombinasi ekstrak ini.

Kata kunci : *aktivitas antioksidan, antosianin, flavonoid, kombinasi ekstrak, metabolik sekunder*

ABSTRACT

Raja banana peel and Pontianak sweet orange peel extracts contain bioactive compounds, such as flavonoids and anthocyanins, which have the potential to be sources of bioactive compounds with antioxidant activity. The combination of these extracts has not been previously analyzed. This study aimed to explore the total levels of flavonoids, anthocyanins, antioxidant activity, phenols, and tannins in the combined extract of Raja banana peel and sweet orange peel. This experimental study was conducted at Setia Budi Laboratory in July 2024. The extraction process used 80% ethanol as a solvent at a 1:10 ratio through maceration, repeated five times to enhance yield and improve the quality of the extracted compounds. The extract formulations consisted of F1 (15:85%), F2 (25:75%), F3 (50:50%), and F4 (75:25%). Flavonoid and tannin levels were analyzed using spectrophotometry, anthocyanins using the pH differential method, and antioxidant activity and phenols using the DPPH method. The

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results showed that F1 had the highest flavonoid content (59.53 mgQE/g), F4 exhibited the best antioxidant activity (91.47% inhibition), and F1 contained the highest levels of anthocyanins (361.45 ppm), phenols (1.80%), and tannins (0.84%). The addition of sweet orange peel increased anthocyanin, phenol, and tannin levels in the extract, while the addition of Raja banana peel extract enhanced antioxidant activity. Further studies are needed to explore other phytochemical compounds and the potential health benefits of this extract combination.

Keywords: antioxidant activity, anthocyanins, flavonoids, extract combination, secondary metabolites

INTRODUCTION

Sweet oranges and bananas are tropical fruits. According to Bureau Statistics Indonesia, the production of sweet oranges in Indonesia increased by 17.09% from 2021 to 2023, while banana production grew by 6.79% over the same period. One well-known variety of sweet orange in Indonesia is the *Citrus sinensis* L, commonly called the "Pontianak orange"[1]. Pontianak oranges are renowned for their sweet yet slightly sour taste, thin skin, and ease of peeling. The peel is typically bright yellow, while the flesh is orange and juicy. Despite the peel often being discarded as waste, it contains numerous bioactive compounds with significant health potential [2]. These include fiber, flavonoids, vitamin C, phenolic compounds, terpenoids, steroids, alkanes, and ethyl esters [2]. These components make orange peels beneficial for health by supporting metabolism, making orange peel a valuable addition to diets and health products [3].

Plantain (*Musa acuminata x M. balbisiana*) is a widely sold and consumed type of banana in Indonesia [4]. Indonesia produces significant fruit peel waste, such as banana peels. Banana peels contain abundant bioactive compounds that offer significant health benefits. However, their potential is not fully optimized, as they are mainly utilized for animal feed, fertilizer, or simply discarded. Because they are used only as animal feed and fertilizer and are thrown away. These compounds can help deal with oxidative stress in the body, which is associated with various chronic diseases [5]. Banana peels contain polyphenols, flavonoids, and other bioactive compounds that can provide anti-inflammatory and antimicrobial effects. In addition, banana peels contain soluble and insoluble fibers that help control appetite and increase satiety, making them beneficial in weight management [6].

In research conducted by Batubara [7], 100 grams of Berastagi orange peel extract contains 3.41% fat, 3.23% protein, 68.88% carbohydrates, and 1556.6 mg of vitamin C. The study also noted that the Berastagi oranges used contained fewer flavonoids than other varieties, leading to the selection of Pontianak sweet oranges for this research. In the study by Devina [8], 100 grams of plantain peel contain 4.44% fat, 3.46% protein, 58.08% carbohydrates, and 0.11% crude fiber. Combining the potent antioxidant compounds in Pontianak sweet orange peel with the fiber found in plantain peel is expected to create a more effective synergistic effect. Flavonoids in sweet orange peels function more effectively in a fiber-rich environment, such as that of plantain peel, which enhances the duration of antioxidant exposure in the body. This study aims to evaluate the bioactive potential of the combination of sweet orange peel (*Citrus sinensis* L.) and plantain peel *(Musa acuminata x M. balbisiana)* extracts based on the measurement of antioxidant activity, as well as flavonoid, anthocyanin, phenolic, and tannin contents. This research is essential as the combination of these ingredients may contain superior bioactive compounds that provide health benefits, particularly in combating free radicals and supporting metabolic regulation. It should be conducted because it may contain bioactive compounds that are better and more beneficial to health. So far, there has not been much research related to the combination of sweet orange peel and banana peel, so this study provides empirical evidence related to the content of bioactive compounds

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in the combination of sweet orange peel and banana peel extracts to see their health benefits.

METHODS

This study employed an experimental approach and was carried out in three main stages: extraction of banana peel and sweet orange peel, phytochemical screening, and proximate analysis. The extraction process took place at the Phytochemistry Laboratory of Setia Budi University Surakarta in July 2024, whereas phytochemical screening and proximate analysis were performed at the Food Technology Laboratory, Faculty of Agriculture, Sebelas Maret University Surakarta in September 2024. The extraction utilized 80% ethanol as the solvent. This study used ripe and yellow plantain and sweet orange peels. The plantain peels were sourced from Klaten City, while the sweet orange peels were obtained from Sambas, Pontianak, with direct shipment from their place of origin.

The plantain peels *(Musa acuminata x M. balbisiana*) and sweet orange peels (*Citrus sinensis L.)* used in this study were obtained directly from their places of origin, namely Klaten, Central Java, and Sambas, Pontianak. The samples were chosen through a purposive sampling approach, considering the optimal ripeness level and physical condition for this research. The inclusion criteria for sample selection included fully ripe banana and orange peels (yellow), free from fermentation, decay, or fungal contamination, and obtained directly from known sources without additional chemical treatment. Meanwhile, the exclusion criteria included fruit peels that showed physical damage such as rot, mold growth, or dryness, as well as those that had undergone chemical preservation or natural fermentation. To ensure result reliability, each analysis was conducted in triplicate. The banana peel pieces were soaked in $Na_2S_2HAl_3$ to inhibit the oxidation process, and then the banana and orange peel pieces were dried using a cabinet dryer at 80HaiC. For eight hours, the dried banana and orange peels were finely milled into powder [9]. The extract was prepared using a modified maceration method based on the previous [8]. In a closed container, 600 g of banana peel flour and sweet oranges were soaked in 10 liters of 80% ethanol (1:10) for three days.

The solution of banana and orange peels was filtered using filter paper to separate the filtrate from the residue. The remaining solid was re-extracted with 80% ethanol at a 1:10 ratio for 48 hours, followed by filtration to collect the filtrate. This extraction was repeated sequentially using 80% ethanol at decreasing ratios of 1:8, 1:6, 1:4, and 1:2, each for 48 hours, with filtration after each step to obtain the corresponding filtrates. All filtrates from the five extraction cycles were then combined and concentrated using a rotary evaporator at 80 rpm and 80°C. The resulting extract was further dried in an oven at 45–50°C until a thick extract was obtained [10]. Phytochemical screening was conducted using spectrophotometry to analyze vitamin C, flavonoids, and tannins. Phenolic compounds were identified through the Folin–Ciocalteu method, anthocyanins were assessed using the pH differential method, and antioxidant activity was determined by the DPPH assay. Crude fiber content was quantified using the acid-base hydrolysis method to ensure effective extraction. Protein content was analyzed following the Kjeldahl method, while fat content was determined using the Soxhlet extraction technique. Moisture content was measured by the thermogravimetric method, and mineral content was assessed using the ashing technique. Carbohydrate content was calculated by the difference method, subtracting 100% from the total protein, fat, water, and mineral content. This analysis method was carried out with a repetition of 2 times to find the average of the best analysis results with the following formulation comparison. Determination of total flavonoid levels using the UV-Vis spectrophotometric method was carried out by weighing 1-2 g of the combined extract sample and then dissolving it in 2 ml of 80% ethanol. Then, 1 ml of the solution was taken, and 5 ml of $FeCl_3$ solution was

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added until a red color was formed. 80% ethanol was added to the solution to make the final volume 10 ml. Absorbance was read with a spectrophotometer at a wavelength of 520 nm [11].

Determination of total anthocyanin levels using the differential pH method was done by diluting a combination of plantain peel extract and sweet orange peel with KCl buffer pH one until an absorbance of less than 1.2 was obtained with a wavelength of 510 nm. After that, two types of samples were made: the first using KCl buffer pH one and the second using Na-acetate buffer pH 4.5. Both samples were read at a wavelength of 510–750 nm [12]. Antioxidant activity in this study was measured using the DPPH (2,2-*diphenyl-1-picrylhydrazyl*) method, where the extra is mixed with a DPPH solution, which produces a purple color as an indicator of free radicals. The reaction between the antioxidants in the sample and DPPH radicals reduced the purple color, which was measured using a spectrophotometer at a wavelength of 515 nm after incubation for 30 minutes in the dark. The higher the purple color reduction, the stronger the antioxidant activity of the sample. The IC50 value, which indicates the concentration required to inhibit 50% free radicals, is calculated for each formulatio [13]. The formulation of Banana peel and sweet orange peel in various ratio was designed to evaluate their total anthocyanin content and antioxidant activity. Table 1 presents the different formulations, detailing the proportion of each extract used in the study.

Table 1. Comparative Formulation of Plantain Peel Extract and Sweet Orange Peel Extract

Ingredients	Banana Peel Extract (gram)	Sweet Orange Peel Extract (gram)
F1	15	85
F2	25	75
F3	50	50
F4	75	25

RESULTS

Yield of Banana Peel Extract and Sweet Orange Peel Extract

The extraction yield of banana peel and sweet orange peel was evaluated using the maceration method, which is both cost-effective and efficient for extracting bioactive compounds. The extraction solvent used was 80% ethanol, and the maceration process was repeated five times to ensure the maximum extraction of active compounds. After extraction,the filtrate underwent concentration through solvent evaporation using a rotary evaporator [14][15]. The extract yield was then calculated and presented in Table 2. The results showed that the yield of orange peel extract was higher (49.5%) than that of banana peel extract at 34.1%. The higher yield obtained from orange sweet peel is attributed to the prolonged extraction duration, which facilitates the breakdown of plant cell walls and enhances the solubilization of bioactive compounds [12].

Table 2. Yield of Banana Peel and Sweet Orange Peel Extract

Sample	Simplisia (gram)	Extract (gram)	Yield (%)	Concentration
Banana peel (Ethanol 80 %)	600	205	34,1	Pasta
Sweet orange peel (Ethanol 80 %)	600	297	49,5	Pasta

The nutritional composition of banana peel and sweet orange peel extract formulations was analyzed to determine variations in macronutrients, vitamin C, water, minerals and clude fiber. Table 3 presents the nutritional content across different formulations.

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Table 3. Nutritional Composition of Different Extract Formulation

Nutrients	Ingredients			
	F1	F2	F3	F4
Carbohydrates (%)	66,17	56,35	59,85	64,33
Protein (%)	4.63	3,40	4,55	4,40
Fat (%)	1.61	4,91	3,11	1,66
Vitamin C (mm/100g)	1175,77	1013,20	1020,95	1116,70
Water (%)	23,73	22,13	21,94	23,43
Minerals (%)	3,86	13,21	20,55	6,18
Crude Fiber (%)	0,21	0,44	0,47	0,32

Carbohydrate content is highest in F1 (66.17%) and lowest in F2 (56.35%), indicating differences in ingredient composition. Protein levels vary slightly, ranging from 3.40% (F2) to 4.63% (F1), while fat content is highest in F2 (4.91%) and lowest in F1 (1.61%). Vitamin C concentration is notably high across all formulations, with F1 having the highest amount (1175.77 mm/100g) and F2 the lowest (1013.20 mm/100g). Water content remains relatively stable, mineral content varies significantly, with F3 containing the highest amount (20.55%) and F1 the lowest (3.86%). Crude fibre levels are generally low, with F3 showing the highest value (0.47%) and F1 the lowest (0.21%). These differences highlight the impact of formulation composition on the nutritional properties of the extracts.

Secondary Metabolite Content and Antioxidant Activity

The combination of banana peel and Pontianak sweet orange peel extracts was analyzed to determine the total flavonoid, anthocyanin, phenol, and tannin content, as well as antioxidant activity. The measurement results of various extract combination formulations are presented in Table 4.

Table 4 Total Flavonoid Combination Extracts

Extracts	Flavonoid mgQE/g	Anthocyanin ppm*	Antioxidant %	Phenol %	Tannin %
F1	59,53	361,45	83,44	2,10	0,84
F2	44,50	355,32	83,68	1,80	0,81
F3	42,31	180,65	76,55	1,52	0,81
F4	50,99	109,75	91,47	1,24	0,55

Table 4 presents the total flavonoid, anthocyanin, antioxidant, phenol, and tannin content in different formulations of banana peel and sweet orange peel extracts. Flavonoids were found in the highest amount in the F1 formulation (59.53 mg QE/g), indicating that the dominance of sweet orange peel in the combination increases the flavonoid content. Anthocyanins also showed the highest levels in F1 (361.45 ppm), which was higher than other formulations. The highest antioxidant activity was found in the F4 formulation (91.47% inhibition), which contains more plantain peels than other formulations. This shows that plantain peel extract plays a dominant role in increasing the antioxidant effect. The highest phenol content was found in F1 (2.10%), which shows a correlation with higher flavonoid and anthocyanin content. Tannin also showed a similar trend, where the highest levels were found in F1 (0.84%). Overall, the results of this study indicate that formulations dominated by sweet orange peel (F1) tend to increase flavonoid, anthocyanin, phenol, and tannin levels, while formulations dominated by banana peel (F4) provide the highest antioxidant activity. Further research is needed

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to explore the mechanism more deeply through in vivo tests and a more complete spectrum analysis of bioactive compounds.

DISCUSSION

This study showed that the maceration method using 80% ethanol as a solvent for 2 x 24 hours resulted in a yield of 49.5%. This yield is higher compared to the study by Yerizam [16], which obtained a yield of 39%. Furthermore, an extract yield is considered good if it is below 50% [17], making the yield in this study categorized as good. The differences in yield could be attributed to variations in the type of orange peel used, extraction methods, and the solvents applied. Maceration with 80% ethanol is known to be an efficient method for extracting bioactive compounds from fruit peels, including orange peel, as the solvent can dissolve both polar and semi-polar compounds effectively [3]. The high yield obtained in this study aligns with previous research, which demonstrated that maceration is an effective method for extracting bioactive compounds from fruit peels [18]. This method's efficiency can be attributed to its ability to break down plant cell walls during extended soaking periods, enhancing the dissolution of active compounds. Variations in yield between studies can be influenced by factors such as the type of fruit peel used, extraction time, and solvent composition [19]. Total flavonoid analysis showed that the extract combination (F1) had the highest content at 59.53 mg QE/g. Flavonoids are well known for their potent antioxidant properties and roles in regulating lipid and glucose metabolism [20]. These compounds contribute to reducing oxidative stress and inflammation, which are key factors in preventing metabolic disorders. This is in line with Devina [8], who reported that sweet orange peel extract contains significant amounts of flavonoids. A study by Liu also reported that flavonoids in banana peel exhibit significant antioxidant activity, supporting our findings [21].

The analysis results showed variations in anthocyanin content in various extracts, with the highest concentration found in the F1 extract, namely 361.45 ppm. Anthocyanins, as bioactive compounds, have significant potential health benefits, including improved lipid profiles and weight loss through antioxidant and anti-inflammatory mechanisms. This compound can help fight free radicals and reduce oxidative stress, which plays a role in preventing cell damage and inflammation, both of which are associated with the development of cardiovascular disease and obesity [22]. Several studies have shown that anthocyanins can improve lipid metabolism, reduce fat absorption, and increase satiety, ultimately supporting weight loss. Although the F1 extract exhibited the highest anthocyanin content, further studies, particularly in vivo experiments, are necessary to confirm its effects on lipid profile and body weight. Parameters such as LDL cholesterol, HDL, and triglyceride levels need to be measured for a more comprehensive understanding. In addition, other factors influencing lipid profiles and body weight, such as calorie intake, physical activity, and genetic factors, must also be considered. Batubara[7], also identified the presence of anthocyanins in plantain peels using the TLC method, although without quantifying specific levels. These results indicate that combinations of extracts with a higher percentage of sweet orange peel significantly increase anthocyanin levels [23].

Previous studies by Devina also highlighted that banana peel extract possesses antioxidant potential, although no quantitative data was provided [8]. The results of the antioxidant activity test showed that the F4 extract exhibited the highest percentage of inhibition, 91.47%, indicating its strong potential in combating free radicals. The correlation between phenolic compounds and flavonoids with antioxidant activity is well-documented in the literature, with phenolic compounds playing a significant role in contributing to antioxidant properties [21][3]**.** The high antioxidant capacity of the F5 extract further suggests its potential in reducing oxidative damage associated with chronic diseases. Additionally, the elevated antioxidant content in the F4 extract

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indicates potential health benefits, such as reducing oxidative stress and inflammation, both of which are major risk factors for cardiovascular disease and obesity [24]. Several previous studies have also demonstrated that antioxidants can boost metabolism, reduce fat absorption, and increase satiety, ultimately contributing to weight loss and a healthier lipid profile [25]. Therefore, F4 extract may be a promising candidate for mitigating the risk of metabolism-related diseases.

Analysis of total phenolics revealed that the F2 extract had the highest content, namely 2.10%, which is consistent with its potent antioxidant activity. Phenolic compounds, including flavonoids and phenolic acids, are known to protect cells by reducing oxidative stress and inflammation [19]. Banana peels are a rich source of phenolic compounds, which contribute significantly to antioxidant activity. The higher phenol levels in the F2 extract indicate its greater potential health benefits, particularly in combating oxidative damage and inflammation, which are key factors in the development of chronic diseases [26]. While these results are promising, the bioactivity of phenolic compounds can be influenced by various factors, such as the type and amount of specific phenolic compounds, the food matrix, and the digestive process. Therefore, further research is needed to identify and quantify the specific phenolic compounds in these extracts and assess their biological effects in vivo. This will provide a deeper understanding of the therapeutic potential of the F2 extract and support the scientific basis for its claimed health benefits [22]. Phenol plays a critical role in protecting against oxidative stress and exhibits significant anti-inflammatory effects, further emphasizing the potential of F2 extract in addressing chronic disease risk factors. Phenol plays a critical role in protecting against oxidative stress and exhibits significant anti-inflammatory effects, further emphasizing the potential of F2 extract in addressing chronic disease risk factors.

Tannin is known for its astringent properties and its role in regulating lipid metabolism by inhibiting fat absorption in the intestine. The highest tannin content was found in the F2 extract, indicating its potential role in appetite regulation and fat metabolism. Tannins, with their astringent properties, can slow digestion, increase satiety, and contribute to weight management [27]. Additionally, tannins may help reduce chronic inflammation associated with obesity, offering broader health benefits. This compound also has the potential to influence fat metabolism and reduce fat absorption in the intestine, thus supporting weight loss [28]. While specific research on tannins in banana peels and sweet orange peels remains limited, the metabolic health benefits of tannins have been widely documented in the literature [3]. However, this study has several limitations. The extraction method used was maceration with 80% ethanol, without a comparison to other techniques, such as ultrasonication or Soxhlet extraction, which might yield higher efficiency. Additionally, this study only analyzed total flavonoid, phenolic, anthocyanin, and tannin contents without identifying specific active compounds using chromatography or mass spectrometry. While the results indicate potential bioactivity, no in vivo testing was performed to confirm the physiological effects of the extracts. Furthermore, the raw materials were sourced from specific regions (Klaten and Sambas), which could lead to variations in bioactive compound content due to environmental factors. Future research should focus on testing different extraction techniques, conducting in vivo studies, and evaluating samples from diverse geographical locations to enhance the generalizability and applicability of these findings.

CONCLUSION

This study provides significant insights into the bioactive potential of combining sweet orange peel (*Citrus sinensis L.*) and plantain peel (*Musa acuminata x M.balbisiana*) extracts, focusing on their antioxidant activity, flavonoid, anthocyanin, phenolic, and tannin contents. The extraction using the maceration method with 80% ethanol resulted

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in a higher yield from sweet orange peel compared to plantain peel. The combination extracts exhibited enhanced bioactive profiles, with one extract showing the highest flavonoid and anthocyanin content, which are well known for their antioxidant and anti-inflammatory properties. Another extract demonstrated the most potent antioxidant capacity, highlighting its potential in combating oxidative stress, a key factor in metabolic disorders such as obesity and cardiovascular disease. Additionally, an extract with the highest total phenolic and tannin content showed promising effects in modulating inflammation and supporting weight management. These findings suggest that the combination of sweet orange peel and plantain peel extracts provides a synergistic effect, enhancing antioxidant defense and supporting metabolic regulation. This research underscores the potential of these fruit peels as functional ingredients for health-promoting products aimed at managing metabolic disorders and related chronic diseases. Further studies, including in vivo trials, are recommended to evaluate the therapeutic efficacy of these extracts in clinical settings and their broader implications for human health. The results contribute to the growing body of evidence supporting fruit peel waste as a valuable source of bioactive compounds, offering sustainable alternatives for developing nutraceuticals and functional foods.

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