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Original Article

Quantification of Phenolics, Flavonoids, and In Vitro Antioxidant Activity in Rosella and Breadfruit Leaf Extracts

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ABSTRACT

Indonesia is a country rich in biodiversity, with numerous plant species traditionally used in medicine. One such plant is breadfruit (*Artocarpus altilis*), whose leaves contain bioactive compounds such as flavonoids, saponins, tannins, quercetin, artocarpanone, and artoindonesianin. Another plant with high medicinal value is roselle (*Hibiscus sabdariffa L.*), a tropical plant from the Malvaceae family known for its rich phenolic content. Both plants are widely found across Indonesia and have potential as natural antioxidants. In this study, the dry extracts were prepared using the decoction method followed by spray drying. Phytochemical screening, total phenolic and flavonoid content analysis, and antioxidant activity tests were performed using standard in vitro methods. The DE2C extract (a combination of breadfruit leaves and roselle flowers) exhibited high total phenol content at 0.953 ± 0.005 g GAE/100 g and flavonoid content at 136.97 ± 5.050 µg QE/100 g. Meanwhile, the DE3C extract showed an IC_{50} value of 540.55 ppm in the DPPH assay. Pearson correlation analysis showed a strong positive correlation between total phenolic content and antioxidant activity ($R = 0.956$, $p < 0.05$), while flavonoids also showed a moderate correlation ($R = 0.502$, $p < 0.05$). These results confirm that phenolic compounds play a key role in the antioxidant potential of the extract.

Keyword: Decoction, Spray drying, Antoxidant activity, Breadfruit leaves, Rosella.

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INTRODUCTION

Flavonoids are the largest secondary metabolite compounds in the plant world and are one of the largest classes of phenolic compounds. Flavonoids are found in almost all parts of plants including fruit, roots, leaves and the outer skin of stems. Flavonoids have a potential function as antioxidants, and have anti-bacterial, anti-inflammatory, anti-allergic, anti-thrombotic activity and can inhibit the growth of cancer cells[1].

Breadfruit (*Artocarpus altilis)* is a plant belonging to the Moraceae family. Breadfruit leaves have many active compounds including quercetin and artoindonesianin which are a group of flavonoids[2]. Breadfruit leaves have different types of flavonoids depending on the type of breadfruit leaf, the highest content of flavonoid compounds is found in old breadfruit leaves, namely 100.68 mg/g, young leaves 87.03 mg/g and old fallen leaves 42.89 mg / g[3]. The results of research by[4] which compared the flavonoid levels in various breadf *et al,* ruit leaves, it was found that the highest flavonoid levels were in fallen yellow leaves, namely 9.20 g QE/100g, sticky yellow, fresh green and the lowest flavonoid levels were found in green breadfruit leaves. fermentation, analysis of the increase in high levels of flavonoids in fallen yellow breadfruit leaves could be caused by changes in the biosynthesis process. It is possible that fallen yellow breadfruit leaves have a very high total phenol content, considering that flavonoids are compounds in the phenol group. Therefore, further research is needed to specifically determine the total phenol content contained in fallen yellow breadfruit leaves.

A part from the type of breadfruit leaves, flavonoid levels can also have different levels based on the solvent and method used in extraction, where the highest flavonoid levels are found in the ethanol extract of breadfruit leaves using the reflux method, namely 5.05 mg quercetin equivalent/g extract[5]. According to[3], the chemical compounds contained in breadfruit leaves include flavonoids, saponins, tannins, quercetin, artocarpanone, and artoindonesianin, where almost all of these compounds belong to the group of phenolic compounds. Phenolic compounds are secondary metabolite compounds found in plants which are characterized by having an aromatic ring containing one or more hydroxy groups (OH)[6]. The importance of the function of phenolic compounds as antioxidants is their ability to stabilize free radicals, namely by providing hydrogen atoms quickly to free radicals, while the radicals originating from these phenolic compound antioxidants will be more stable than free radicals[7].

Apart from breadfruit leaves, the plant which contains flavonoids and phenols is rosella (*Hibiscus sabdariffa* L.), which is a plant belonging to the Malvaceae family, roselle flowers contain an important content found in rosella flower petals, namely anthocyanins which are part of the flavonoids which act as antioxidants. The flavonoids of roselle flower petals consist of flavonols and anthocyanin pigments, apart from that, roselle flowers also contain chemical compounds in the terpenoid group, namely beta carotene[8]. According to research conducted by[9], the ethanol extract of rosella flowers has an IC_{50} value of 30.158 µg/ml.

Based on these two plants, a combination of breadfruit leaves (*Artocarpus altilis* (Parkinson) Fosberg) and rosella flowers (*Hibiscus sabdarifa* L.) was carried out for the reason that previously fallen

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yellow breadfruit leaves contained a high flavonoid content, one of which is anthocyanin. has a working mechanism, namely reducing cholesterol levels, the HMG-CoA reductase enzyme which causes a decrease in cholesterol synthesis, this compound is a compound that is included in the group of phenolic compounds so that when the two plants are combined with the compounds already mentioned it will be able to provide greater and better results to her womb.

Extraction needs to be carried out to extract secondary metabolites from the simplicia matrix, namely the phenol and flavonoid groups. The solvent used is water because phenolic compounds tend to dissolve easily in water because they generally bind to sugar as glycosides. The type and quality of solvent used determines the success of the extraction process. The extraction process with solvents is based on the polarity of the substances in the solvent during extraction. The extraction method used is the decoctation method because the solvent used is water where water has a high boiling point so the decoctation method can be used and is polar so it is more effective at attracting very polar compounds. The extract drying process is carried out using a spray dryer, so that the secondary metabolites in the extract are more stable and are not grown by microorganisms.

Based on the description above, this is what underlies the need to carry out research which is intended to determine the total levels of phenols, flavonoids and whether these compounds have a correlation with the antioxidant activity of water extracts of breadfruit leaves, rosella leaves, and a combination of DE1C, DE2C, and DE3C, which is obtained using the decoctation method.

MATERIALS AND METHODS

Plant collection

The plant used is the yellow fall variety of breadfruit (*Artocarpus altilis* (Parkinson) Fosberg) obtained from Cisalak Village, Kec. Cisarua, Sumedang Regency and Rosella flowers (*Hibiscus sabdariffa* L.) obtained from Kebon Jeruk, Kec. Andir, Bandung City. Rosella flowers were obtained from the Manoko medicinal plant plantation, Cikahuripan, Lembang District, West Bandung Regency. The determination was carried out at the Plant Taxonomy Herbarium Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA) Padjadjaran University, Jatinangor-Bandung.

Preparation of simplicia and dry extract

The breadfruit leaves and roselle flowers that are obtained are wet sorted to select the parts of the plant that will be used, washed with running water to clean the remaining dirt, then chopped to speed up the drying process, after the plants are dry then the process of making simplicia powder in sizes is carried out 40 mesh powder, Simplicia powder was characterized including specific and non-specific parameters[10] 5.150 grams of breadfruit leaf simplicia powder was extracted using the decoctation method using 37 liters of distilled water as a solvent. 2.000 grams of rosella flowers with 20 liters of distilled water using an extractor at a temperature of 90°C (the extraction process starts for 30 minutes accompanied by a stirring process), filter the extract using an American drill cloth. The liquid extract obtained from breadfruit leaves was 12.62 liters and rosella flowers were 22.092 liters. The extract was dried using spray drying type LPG-5 with a temperature of 90-100 °Celsius

Phytochemical screening

All samples were analyzed using amyl alcohol reagent to detect flavonoid compounds[11], $FeCl_3$ 10% for detecting phenolic compounds[12], gelatin reagent for tannin detection[13] Dragendorf and Mayer reagent for alkaloid detection[14], and secondary metabolites. The quinone group can be identified using 5% KOH reagent[15]. Steroid and triterpenoid secondary metabolites can be identified using Lieberman-Buchard reagent, while volatile compounds in the monoterpene and sesquiterpenoid subgroups can be identified using vanillin sulfate reagent (10% vanillin in H_2SO_4)[16]. Additionally, saponins, another type of secondary metabolite, were determined by observing continuous foaming after shaking the aqueous extracts for 10 minutes[17].

Qualitative analysis with Thin Layer Chromatography (TLC)

TLC was used to find out the secondary metabolite compounds in breadfruit leaves and roselle flowers. The mobile phase used is n-hexane: ethyl acetate: acetic acid (3:7:1 drops) where the mobile phase has been optimized first. Silica gel plate GF_{254} was used as the stationary phase, and $FeCl_3$ was used to detect phenolic compounds, ammonia vapor, $AlCl_3$ and citroborat to detect flavonoids, and 0.2% DPPH in methanol to detect antioxidant activity qualitatively[18].

Determination of the free-radical scavenging activity

The free-radical scavenging activity was measured by the 2,2 diphenyl-1-picrylhydrazyl (DPPH) method described by Moon and Terao, with some modification[19]. Different amounts of each extract and methanol were added to a solution of 0.3 mg/mL methanolic solution of DPPH to make up a total volume of 3.0 mL. After standing for 15 min at room temperature, the absorbance was measured at 517 nm using UV–Vis spectrophotometer (UNICO 2100: USA). High absorbance of the reaction mixture indicated low free radical scavenging activity. Butylated hydroxytoluene (BHT) was used as positive control. Inhibition of free radical by DPPH was calculated as follows: Antiradical activity (%)=(Acontrol – A sample)/A control × 100. The IC50 value, defined as the amount of antioxidant necessary to decrease the initial DPPH concentration by 50% and was calculated based on linear regression of plots of the percentage antiradical activity against the concentration of the tested compounds[20]. The experiment was carried out in triplicate and the results are average values.

Determination of total phenolic content

The total phenolic content was determined using Folin-Ciocalteu method. Briefly, 0.1 ml of each of the diluted samples was added to 0.5 ml of 10% (v/v) Folin-Ciocalteu reagent. After 3-8 min, 0.4 ml of 7.5% (w/v) sodium carbonate solution was added to the mixture. After being kept in total darkness for 30 min, the absorbance of the reaction mixture was measured at 765 nm using a UV–Vis spectrophotometer (UNICO 2100: USA). Amounts of total phenolic were calculated using a gallic acid calibration curve. The results were expressed as gallic acid equivalents (GAE) g/g of dry plant matter[21].

Determination of total flavonol content

The total flavonol content was measured following a previously reported method . Briefly, 0.5 ml of each diluted plant material was independently mixed with 1.5 ml of methanol, 0.1 ml of 10% (w/v) aluminum chloride, 0.1 ml of 1M potassium acetate, and 2.8 ml of distilled water. After incubation at room temperature for two and a half h absorbance of the reaction mixture was read at 440 nm using a UV–Vis spectrophotometer (UNICO 2100: USA). The results were expressed in mg of rutin equivalents of dry plant matter by comparison with the standard curve, which was made in the same condition. All measurements were done in triplicate and statistical analysis was done by statistical software using one-way analysis of variance (ANOVA)[22].

RESULT AND DISCUSSION

Plant Determination

The initial stage carried out in this research was plant determination. The plants used in this research were yellow breadfruit leaves

(*Artocarpus altilis* (Parkinson ex F.A. Zorn) Fosberg) and rosella flowers (*Hibiscus sabdariffa* L.). The aim of plant determination is to identify and find out the correctness of the test material to be used, so that errors can be avoided in collecting the material to be studied. Plant determination is based on the plant classification system. Based on the results of plant determination carried out at the Plant Taxonomy Laboratory, Biology Department, FMIPA UNPAD No.43/HB/01/2024 and No.44/HB/01/2024, it shows that the plant used was the rosella flower plant with the Malvaceae family and the yellow breadfruit plant with the family moraceae.

Characterization of Simplicia

Simplicia characterization is an essential step to ensure the quality and uniformity of herbal raw materials so that they can meet the requirements stated in the[23]. The characterization process involves analysis of water content, water soluble essence content, ethanol soluble essence content, drying loss, and ash content. This research evaluates the simplicia of breadfruit leaves and rosella flowers with characterization results presented in Table 1.

The water content of simplicia plays an important role in controlling the growth of microorganisms that can damage the bioactive content. Analysis shows that the water content of breadfruit leaves and rosella flowers is 15.33% and 14.66% respectively, exceeding the maximum limit of 10% set by[24]. The cause is thought to be a less than optimal drying process, both in terms of time and temperature. Because the water content in simplicia does not meet standards, the extract drying process will be carried out using the spray drying method, with the aim of the resulting extract being more stable during storage and the testing process.

Determination of extract content provides information about secondary metabolite compounds that can be extracted in certain solvents. The results of the research showed that the water soluble essence content of breadfruit leaves was 16.17% and that of rosella flowers was 35.50%, while the ethanol soluble essence content was 12.43% and 33.27% respectively. These findings indicate that most of the bioactive compounds in both simplicia are polar and more easily soluble in water than ethanol[25].

Characterization of breadfruit leaf simplicia and rosella flowers showed that the water content, drying loss and total ash content did not meet the standards of[25], while the water and ethanol soluble essence content parameters showed suitable results. Improvements in post-harvest processes, such as mechanical drying with controlled temperatures and hygiene management, are needed to improve the quality of simplicia.

Table 1. Results of Characterization of Simplisia Breadfruit Leaves and Rosella Flowers.

Characterization	Result			
	Breadfruit leave	Literature (23)	Rosella flower	Literature (24)
Water content	15,33%	<10%	14,66%	<10%
Total Ash Content	19,93%	<5,6%	6,55%	<5,6%
Drying Shrinkage	15,97%	<10%	14,80%	<10%
Water Soluble Content	16,17%	>5,3%	35,50%	>15,0%
Ethanol Soluble Content	12,43%	>8,6%	33,27%	>16,3%

Table 2. Total Phenolic Content

Extract	Total phenol content in the extract (g GAE/100g)
Breadfruit leaves	0.546 ± 0.006^{a}
Rosella flower	0.823 ± 0.019^{b}
DE1C	0.798 ± 0.005^{c}
DE2C	0.953 ± 0.005^{d}
DE3C	0.800 ± 0.016^{e}

Making Dry Extracts

Extract yield is measured as a percentage of the weight of the starting material after the extraction process is complete. In this study, Rosella showed a much higher extract yield (28.9745%) compared to Breadfruit Leaves (8.9306%). These differences can be explained by several factors, including secondary metabolite content, extraction methods, solvents used, and the physicochemical characteristics of the materials.

Rosella is known to be rich in secondary metabolites, especially phenolic compounds such as anthocyanins, flavonoids and organic acids, which are well soluble in polar solvents1. The content of the compounds delphinidin-3-sambubioside, cyanidin-3-sambubioside, cyanidin-3-glucoside and delphinidin-3-glucoside contributed to the high yield of Rosella extract[26]. On the other hand, Breadfruit Leaves may have a lower content of active compounds or different solubility properties, which affects extraction efficiency[27].

The extraction method used also greatly influences the yield results. If you use an extraction method with a polar solvent such as ethanol or water, Rosella is more likely to give high yields because most of its compounds are polar[28].

Phytochemical screening

Phytochemical screening aims to ensure the chemical compound content in simplicia and verify that the extraction and concentration process does not damage the active compounds[29]. The results of phytochemical screening on simplicia and extracts of breadfruit leaves and rosella flowers showed the presence of flavonoids, saponins, tannins, phenolics and triterpenoids.

Alkaloid testing using Dragendorff, Bouchardat, and Mayer reagents begins with the addition of 2N HCl to form alkaloid salts, followed by heating and cooling. The results showed that no orange (Dragendorff), brownish red (Bouchardat), or white (Mayer) precipitate was formed[30], The flavonoid test was carried out with concentrated Mg powder and HCl, producing an orange color on the amyl alcohol layer as a positive indication. This shows that the flavonoids in simplicia and extracts of breadfruit leaves and rosella flowers were successfully identified through the glycoside bond reduction process[31].

Saponin testing involves adding hot water, cooling, and shaking. A positive result is indicated by the formation of stable foam of more than 1 cm for 10 minutes, indicating the presence of saponin in both samples. This process shows the hydrolysis of glycosides into glucose which plays a role in foam formation[32]. The phenolic test with the $FeCl_3$ reagent produced a blackish green color change, indicating the presence of phenolic compounds in the simplicia and extracts of both plants. Further testing with gelatin showed a slight precipitate, which confirmed the presence of tannin, as tannin is known to precipitate proteins such as gelatin[33].

The quinone test using benzene and 2N NaOH showed negative results, because there was no yellow to red color change. This indicates that there is no reaction to deprotonate the phenol group on the quinone in the sample[34]. Identification of triterpenoids and steroids using the Lieberman-Burchard method showed positive results for triterpenoids in breadfruit leaves with a purple color change. However, the results were negative for rosella flowers because there was no brownish or bluish green color change, so it was stated that they did not contain steroids or terpenoids[35].

Qualitative testing of phenolic compounds using thin layer chromatography

The confirmation test for the identification of phenolic compound groups in the water extract of breadfruit leaves and rosella flowers was carried out using thin layer chromatography (TLC). Separation in TLC occurs due to the interaction between the stationary phase and the mobile phase to bind the components contained in the sample to be separated. The stationary phase used was F_{254} silica gel plates, and the mobile phase used was chloroform:methanol (8:2). The selection of the mobile phase is based on its ability to elute the compounds in the extract. A good eluent is an eluent that can separate large amounts of compounds as indicated by the appearance of stains.

Next, the separation results were observed under 254 nm and 366 nm UV light to see the separation that occurred and the spots that formed as a result of the separation. Apart from that, TLC analysis in this research was visualized by spraying visible spots of H_2SO_4 and $FeCl_3$ which will produce green, red, purple, blue, yellow or black on the spots which indicates a positive result for phenolic compounds[36].

Based on the results in Figure 1, the identification results of breadfruit leaf extract, rosella flowers and their combination show that there is blue attenuation at UV 254, blue fluorescence at UV 366, there is a brown spot on the TLC plate that has been sprayed with H_2SO_4 and shows a black spot. after being sprayed with $FeCl_3$, so these results can be concluded that the extracts of breadfruit leaves, rosella flowers and their combination show the presence of phenol compounds. Calculation of the spots that appear with low and high Rf (Retention factor) values indicates that the analyte compounds tend to be polar or nonpolar. The Rf values obtained indicate differences in compound properties and can be used for compound identification. Compounds with higher rf values have low polarity, while compounds with lower Rf values have high polarity. Compounds that are more polar will be retained longer in the stationary phase, resulting in lower Rf values[37].

Qualitative Testing of Flavonoid Compounds Using Thin Layer Chromatography

Identification of chemical compound groups from breadfruit leaf and rosella flower extracts by TLC using silica gel GF_{254} and using mobile phase N-hexane:ethyl acetate:acetic acid (3 :7:1 drops). In addition, to clarify the results obtained, the plate was sprayed and evaporated to reveal spots. The spot displays used were H_2SO_4(Figure 2), citroborate(Figure 3), $AlCl_3$(Figure 4), and ammonia vapor(Figure 5).

Spraying the H_2SO_4 spot visible is carried out because it is known to be a spot visible that is commonly used to see primary metabolite compounds or secondary metabolite compounds[38], spraying the spot visible citroborate will give greenish yellow spots, whereas when seen under 366 nm UV light gives blue spots to flavonoid compounds[39]. Spraying the $AlCl_3$ spot enhancer gives a yellow color to the spots and shows a bright blue color under 366 nm UV light for flavonoid compounds[40], and the spot display using ammonia vapor gives a light blue color for flavonoid compounds under 366 nm UV light[38,41]. Identification of flavonoid compounds on the plate was observed using UV 254 and UV 366 light.

The sample after spraying with H_2SO_4 gave a dark red color that was more visible in samples no. 5 and 6 with Rf 0.56. This change was caused by an electrophilic substitution reaction that occurred when H_2SO_4 was sprayed on the sample[42].

Spraying of cytroborate spots shows blue and yellowish fluorescent spots under a 366 nm UV lamp with an Rf value of 0.56 on rosella flowers, 0.76 on breadfruit leaves and 0.8 in a ratio of 1:1, 1:2 and 2:1. Spraying the cytroborate spot will give greenish yellow spots, and when viewed under 366 nm UV light it will give blue spots on flavonoid compounds[43], spraying shows $AlCl_3$ spots showing blue fluorescent spots under a 366 nm UV lamp with Rf values obtained 0.26 and 0.56 on rosella flowers, 0.86 on breadfruit leaves, 0.88 and 0.56 in a DE1C and DE2C and Rf 0.86 at a DE3C. Spraying $AlCl_3$ spots gives a yellow

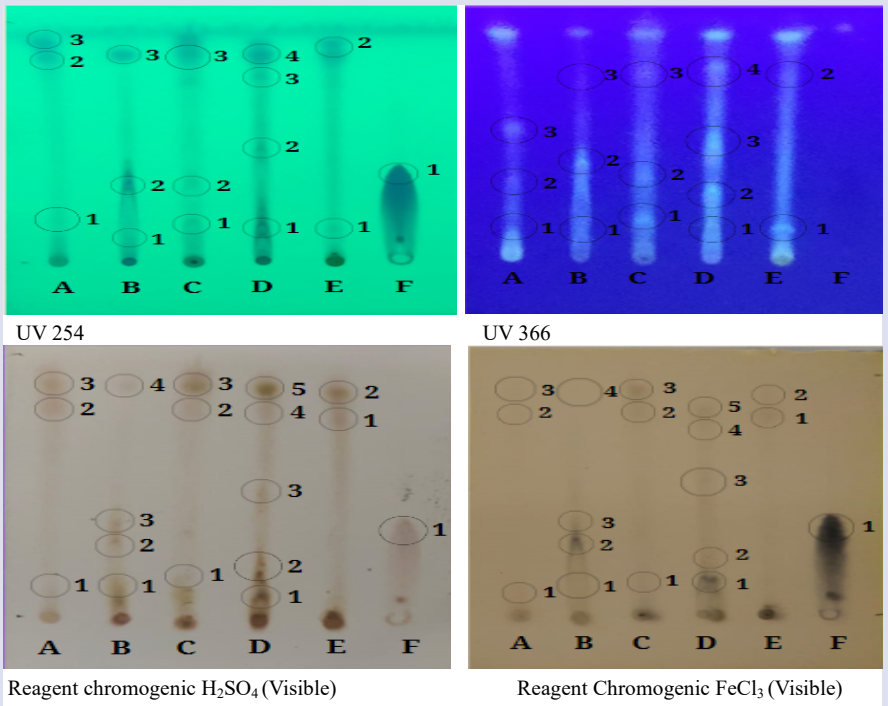

Figure 1. Results of identification of phenolic chemical compound groups in breadfruit leaf extract and rosella flower extract using TLC. (A) breadfruit leaf extract, (B) rosella flower extract, (C) DE1C, (D) DE2C, (E) DE3C, (F) gallic acid standard.

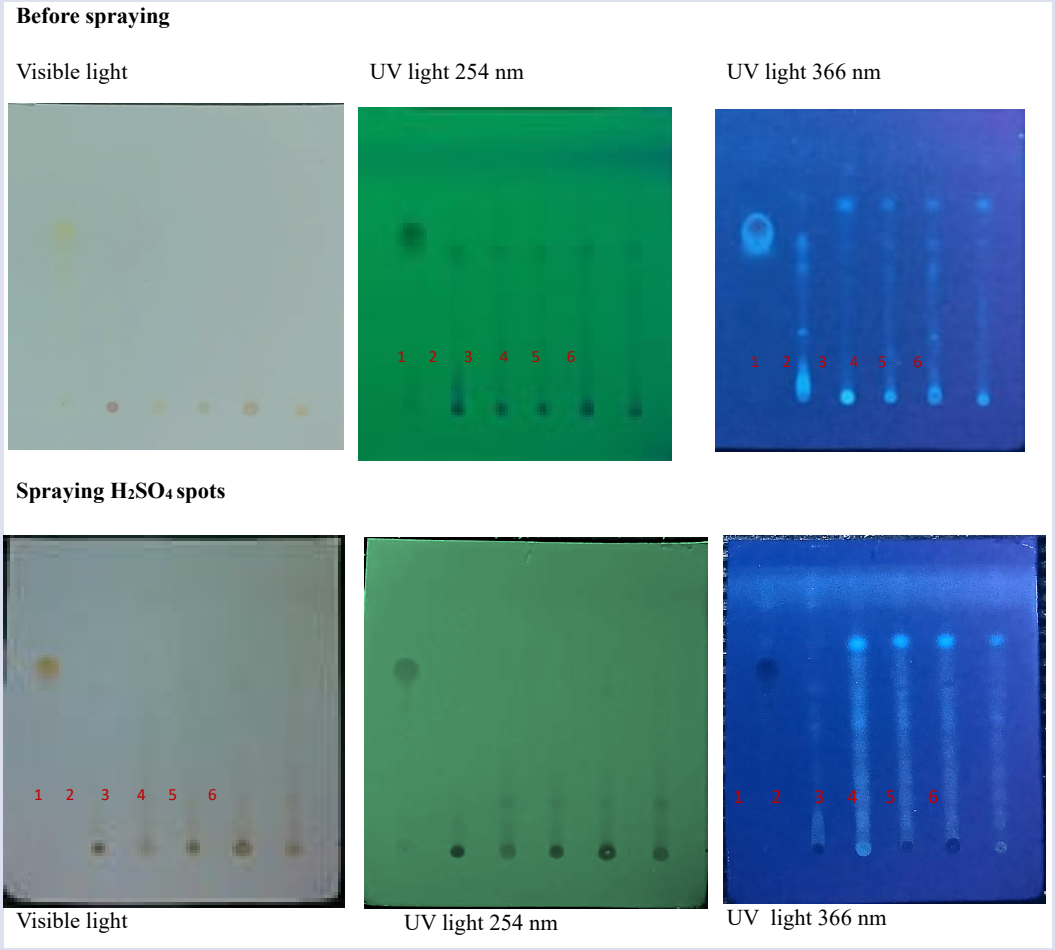

Figure 2. Chromatogram pattern with TLC showing H_2SO_4 spots

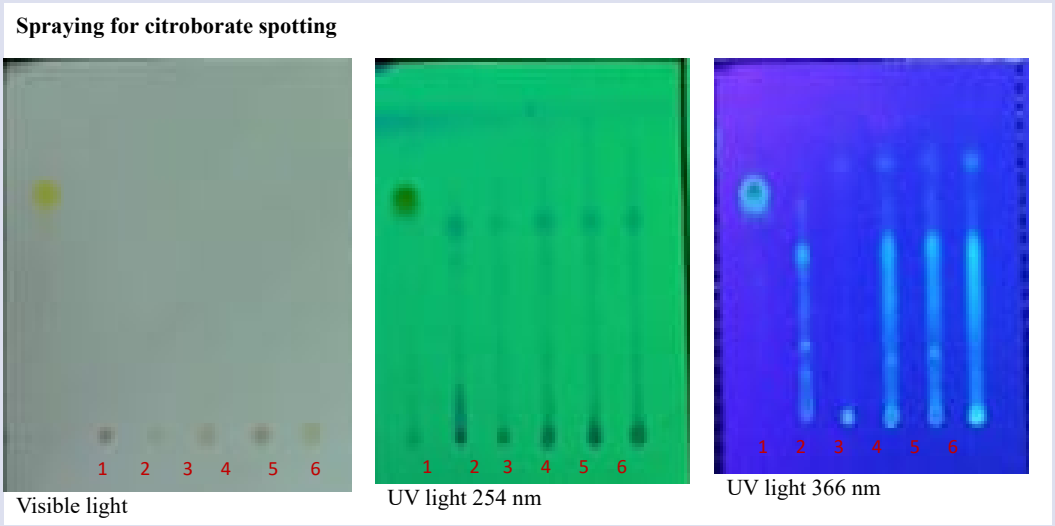

Figure 3. Chromatogram pattern with TLC showing citroborate spots

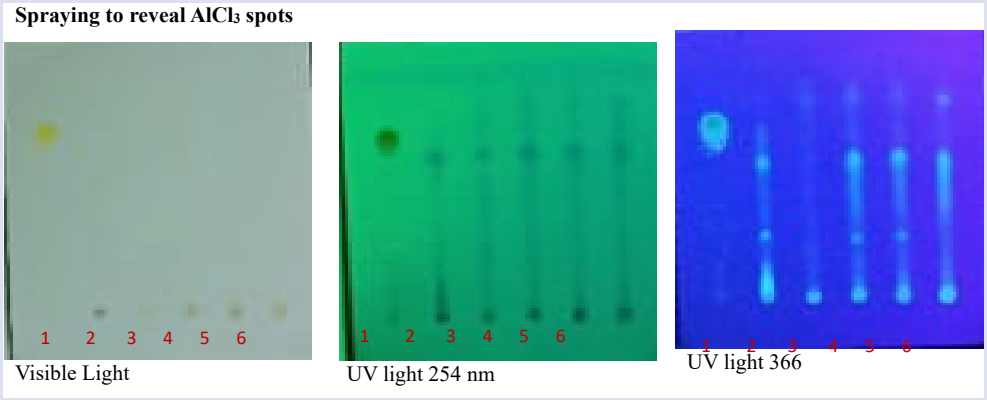

Figure 4. Chromatogram pattern with TLC showing $AlCl_3$ spots

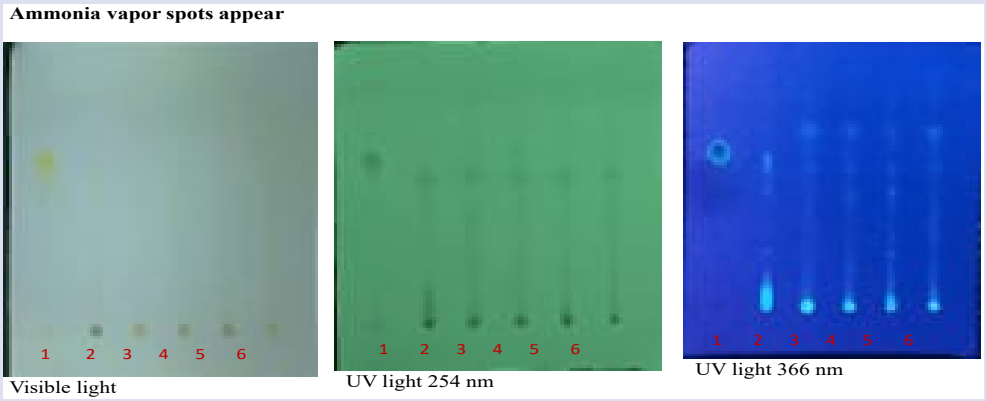

Figure 5. Chromatogram pattern using TLC showing spots of ammonia vapor

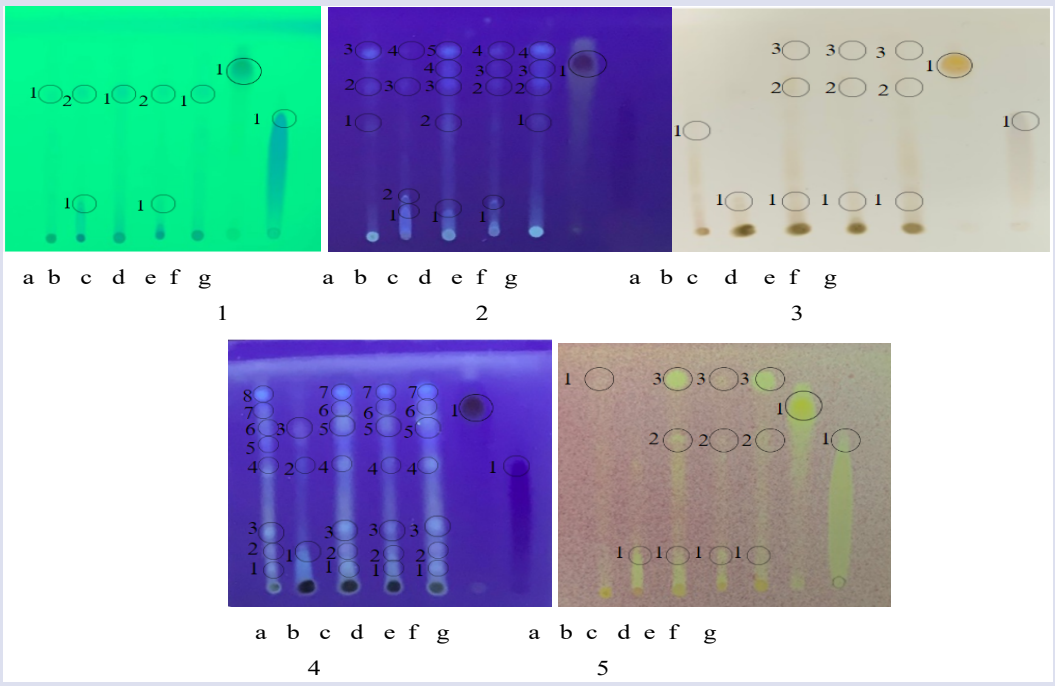

Figure 6. Chromatogram pattern using TLC showing spots of DPPH 0.2%

Information: (a) breadfruit, (b) rosella, (c) DE1C, (d) DE2C (e) DE3C, (f) quercetin, (g) gallic acid. (1) UV λ 254 nm, (2) UV λ 366 nm, (3) H_2SO_4 spot appearance and visible heating, (4) H_2SO_4 spot appearance and UV λ 366 nm heating, (5) 0.2% DPPH spot appearance (visible).

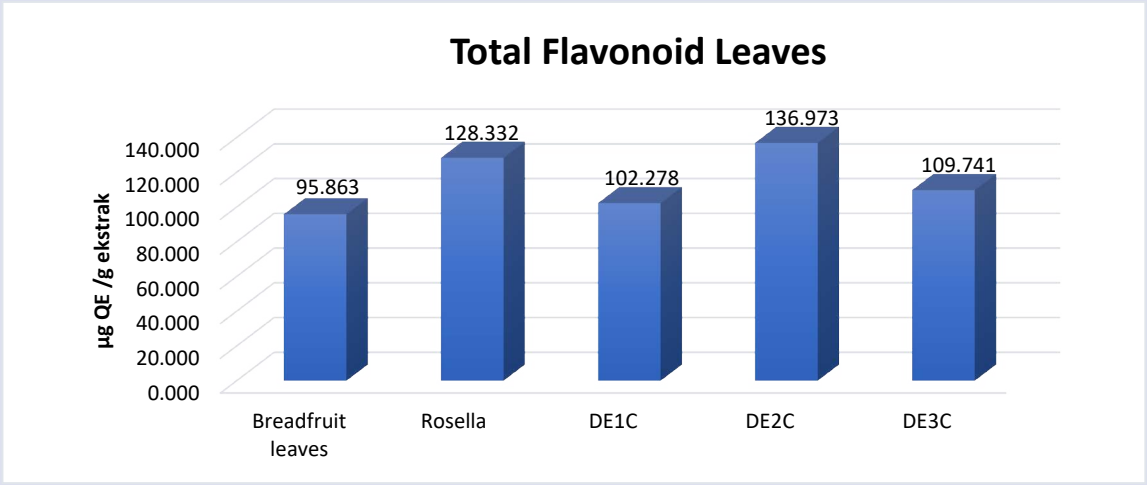

Figure 7. Graph of Total Flavonoid Levels

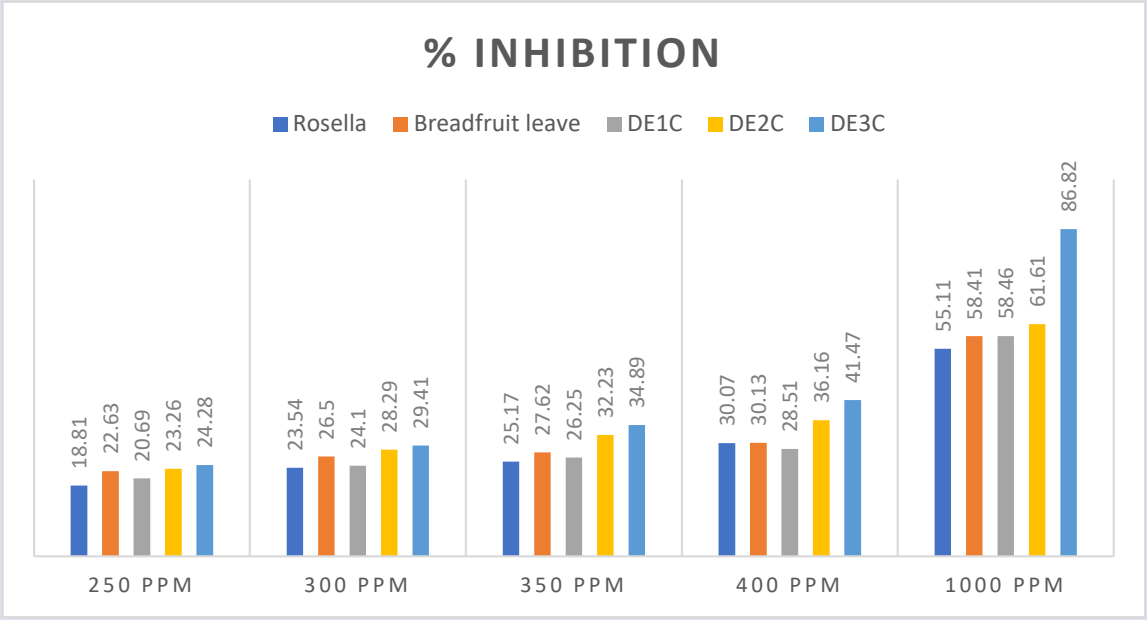

Figure 8. Graph of percentage of antioxidant inhibition

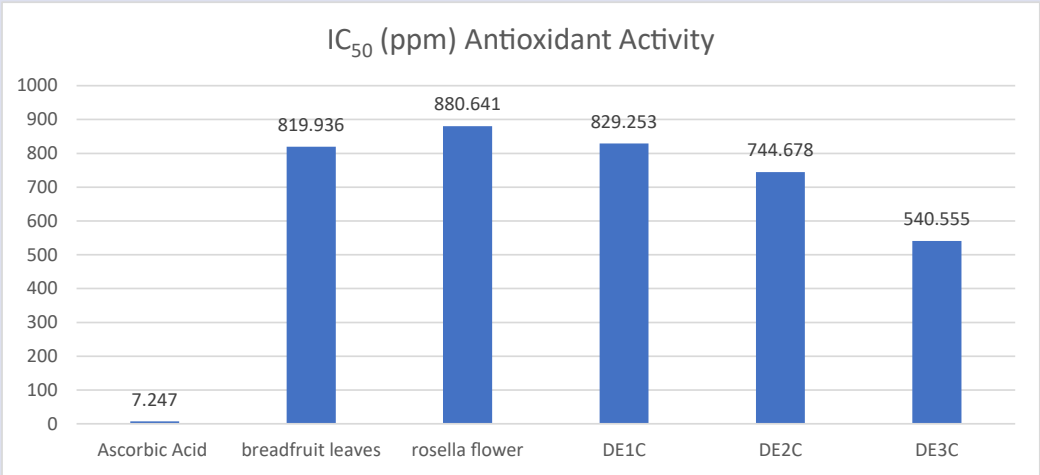

Figure 9. IC_{50} Antioxidant Activity Graph

color to the spots and shows a bright blue color under a 366 nm UV lamp for flavonoid compounds. The appearance of ammonia vapor spots is a blue fluorescent spot under a 366 nm UV lamp with an Rf value of 0.6 on rosella flowers, 0.72 on breadfruit leaves, 0.78 present in all combinations.

Qualitative Antioxidant Test Using Thin Layer Chromatography

Observations using UV light at a wavelength of 254 nm utilize the principle that at this wavelength, the thin layer chromatography (TLC) plate has the characteristic that the sample will appear dark. The dark color on the TLC plate indicates an interaction between UV light and the fluorescent indicator on the plate. This fluorescence is caused by the excitation of electrons from a basic energy level to a higher energy level, which then returns to the basic energy level while releasing energy in the form of light[43].

At a wavelength of 366 nm, the compound separation spot on TLC is visible more clearly. This is caused by the interaction of UV light with the chromophore group attached to the auxochrome in the compound which produces visible light emissions[44]. Sulfuric acid is used because of its ability to destroy the chromophore group, thus clarifying the stain on the TLC plate and making it easier to observe. Then, spraying with 0.2% DPPH spotting was carried out to detect antioxidant compounds. The yellow spots that appear on a purple background indicate the presence of compounds in the sample that can donate hydrogen atoms, react with reduced DPPH (1,1-diphenyl-2-picrylhydrazine), and cause a color change in the TLC stationary phase. This process is an effective analytical method for detecting and visualizing compounds with antioxidant activity in sample extracts (Figure 6).

The sample sprayed with 0.2% DPPH on the single extract sample of breadfruit leaves contained spots of compounds that gave positive antioxidant test results by producing yellow spots on a purple background, namely with an Rf value of 0.94. In a single extract sample of rosella flowers, compound spots were obtained that gave positive antioxidant test results by producing yellow spots on a purple background, namely with an Rf value of 0.18.

The TLC results of the combination extracts DE1C, DE2C, DE3C showed that there were spots of compounds that gave positive antioxidant test results, indicated by the appearance of yellow spots on a purple background after being sprayed with DPPH 0.2% . The Rf value obtained was 0.18; 0.74; and 0.94, which shows variations in the polarity of the compounds contained in the extract.

The differences in the stain spots produced can be interpreted as an indication that samples with more numerous, larger and more concentrated spots have stronger antioxidant activity in warding off free radicals, because they are able to produce more significant reactions with DPPH.

The free radical inhibitory activity observed through the TLC method with DPPH visualization indicated the presence of antioxidant compounds in the five samples. These antioxidant compounds play an important role in warding off free radicals, which is generally measured based on the ability of a sample to change the purple color from DPPH to yellow, indicating hydrogen atom donation activity by the compound in the sample.

Quantitative Analysis of Phenolic Compounds

Determination of total phenol content in breadfruit leaf and rosella flower extracts was carried out using the Folin-Ciocalteu reagent using a UV-Vis spectrophotometer. The Folin-Ciocalteu method is used because phenolic compounds can react with Folin-Ciocalteau to form a solution whose absorbance can be measured. This Folin-Ciocalteau reagent can oxidize the hydroxyl group of phenol group compounds to form a blue complex. This reaction runs slowly in an acidic environment, so in the test sodium carbonate was added to form an alkaline environment and the reaction could run more quickly[45]. This method can be measured using Uv-Vis spectrophotometry and is considered simpler, faster and more accurate because the absorbance obtained is at a high wavelength, namely around 745 - 765 nm[46]. Statistical analysis of total phenol levels was carried out using the Kruskal-wallis test, this test is a non-parametric statistical test that can be used to test whether there is a significant difference between groups of independent variables and the dependent variable. Because to see significant differences between groups, this test is clearly used to see comparisons of more than 2 groups. The results of total phenolic levels can be seen in Table 2.

The results of determining the total phenol content from breadfruit leaf and rosella flower extracts showed that rosella flower extract had higher phenol levels than fallen yellow breadfruit leaves. This may be caused by the higher phenol content in rosella flowers compared to breadfruit leaves. Based on research by[47,48] the phenolic content in rosella flowers is flavonoids, polyphenols, ascorbic acid, quercetin and anthocyanins, where these anthocyanins are known to have strong antioxidant activity. Apart from that, breadfruit leaves also contain phenolic compounds such as flavonoids, tannins and saponins but the content contained in breadfruit leaves is in lower amounts compared to rosella flowers. Therefore, the total phenol content obtained from rosella flowers is higher.

When breadfruit leaves and rosella flowers are combined. A combination DE2C gives the highest total phenol content. This shows that rosella has a higher phenol content and contributes significantly to the combination. The increase in total phenol content in the combination of breadfruit and rosella shows the synergistic potential between these two plants in increasing phenol content, which can be related to antioxidant activity. This often happens because phenolic compounds can interact and work together to overcome free radicals, resulting in a stronger antioxidant effect[49]. Synergistic potential occurs because the DE2C combination allows the more dominant phenolic compounds contained in rosella flowers to complement the compounds from breadfruit leaves, resulting in a higher increase in total phenol levels. Thus, this combination produces a balanced phenolic profile, which can increase the stability of the combined extract and maintain its effectiveness for longer. In addition, the DE2C combination allows for better interactions between phenolic compounds, which not only increases the total phenol content but also increases the antioxidant effectiveness of the extract. Thus, these results emphasize the importance of combination ratios in plant extract research, where appropriate combinations can maximize the benefits of each extract, resulting in better potency compared to the use of single extracts.

Determination of Flavonoid Levels

Determination of flavonoid levels uses a standard standard, namely curcetin, quercetin is used because it is the largest flavonoid compound. When measuring flavonoid levels, 10% $AlCl_3$ was added to form a complex, resulting in a shift in wavelength towards the visible, which was indicated by the solution producing a yellower color. The addition of sodium acetate to determine flavonoid levels functions to maintain wavelengths in the visible area. Incubation treatment for 30 minutes before measurement is intended so that the reaction runs perfectly, so as to provide maximum color intensity. Absorbance measurement using a UV-Vis spectrophotometer with a wavelength of 415nm (22). Making a standard quercetin solution of 500 ppm, then varying the concentration of the quercetin standard solution to 15, 30, 45, 55, 65, and 75 ppm. The linear regression equation obtained in making the standard curve is $y= 0.1273x + 0.0036$; $R^2 = 0.9968$ This equation is

used to calculate the flavonoid content of breadfruit leaves, rosella flowers, as well as the ratio between breadfruit leaves and rosella flowers (Figure 7).

The levels obtained show the differences between variations in the average comparison. The highest flavonoid content results were obtained in extract DE2C with a content of 136,973 µgQE/gram extract, compared to breadfruit leaf extract of 95,863 µg QE/gram extract, rosella flower 128,332 µgQE/gram extract, DE1C 102,278 µgQE/gram extract and DE3C 109,741 µgQE/gram of extract, the average flavonoid content test is then visualized in the graph below.

Breadfruit extract showed flavonoid levels of 95.863 ± 3.564 µg QE/g extract. Rosella extract has higher flavonoid levels, namely 128,332 ± 6,761 µg QE/g extract. This is in accordance with previous research which shows that rosella (*Hibiscus sabdariffa*) is rich in flavonoids, especially anthocyanins, which play a role in high antioxidant activity[50]. The combination DE1C produced flavonoid levels of 102,278 ± 1,416 µg QE/g extract. This value is higher than breadfruit alone but lower than rosella alone. This combination increases flavonoid levels compared to breadfruit alone, although it is still lower than rosella. This combination provides an additional (additive) effect but is not optimal for increasing flavonoid levels significantly[51].

The combination DE2C had the highest flavonoid content, namely 136,973 ± 5,050 µg QE/g extract. The addition of the amount of rosella increases the contribution of flavonoids in the mixture, which is in accordance with research results that the dominance of flavonoid-rich ingredients in the mixture can increase its bioactive activity. The combination DE3C showed flavonoid levels of 109,741 ± 2,186 µg QE/g extract. This reduction compared DE2C shows that reducing the proportion of rosella has a significant effect on total flavonoid levels. The combination of extracts can create a synergistic effect in flavonoid content, especially at an optimal DE2C. This synergistic effect is important in increasing the potential of antioxidant activity. This is also supported by literature which states that combinations of herbal plants are often more effective than single extracts because various active compounds can work synergistically[52].

Data shows that the combination of breadfruit leaf and rosella flower extracts provides varying levels of flavonoids, depending on the ratio used. The DE2C combination produced the highest flavonoid levels, indicating that a higher proportion of rosella in the mixture contributed dominant flavonoids. Combinations of plant extracts often exhibit synergistic effects[51], where the active compounds in both ingredients work together to increase total flavonoid levels. In this case, anthocyanins from rosella are likely to be the dominant factor that increases flavonoid levels in a DE2C combination. Flavonoids are phenolic compounds that have high antioxidant, anti-inflammatory and anti-cancer activity. In herbal or functional product formulations, this combination can increase pharmacological effectiveness compared to single extracts. Flavonoids are widely recognized for their significant antioxidant activity, which plays a crucial role in pharmacological applications. This activity is largely influenced by the structural arrangement of functional groups within the flavonoid backbone. Key factors such as molecular conformation, the type and position of substituents, and the total number of hydroxyl (–OH) groups contribute substantially to their mechanisms of action, including metal ion chelation and free radical scavenging[53].

Antioxidant Activity Test

DPPH is a purple molecule that in a radical state can change to a stable yellow color by reaction with antioxidants by donating one hydrogen atom to DPPH so that DPPH free radicals are reduced. This change in the intensity of the purple color occurs due to the dampening of free radicals produced by the reaction of the DPPH molecule with the hydrogen atoms released by the sample compound molecule, resulting in the formation of the diphenyl picrylhydrazine compound and causing the DPPH color to decay from purple to yellow. This color change causes the absorbance value to decrease with each increase in concentration and there is an increase in the % inhibition value in the sample being tested.

In this study, antioxidant tests using the DPPH method were carried out on several variations in concentration of the comparison and samples. The comparison compound used is ascorbic acid and the test uses a DE1C ratio, which means 2 ml of DPPH is mixed with 2 ml of sample solution. After the sample solution and comparison solution were mixed with DPPH reagent, the test solution was left for 30 minutes before the absorbance was measured. This aims to ensure that the sample solution which has the potential to act as an antioxidant and the comparison solution react to reduce DPPH free radicals until the color changes in the sample solution and comparison solution from purple to yellow, then the absorbance is measured at a wavelength of 517 nm using a UV-Vis spectrophotometer and determined its inhibition. % Inhibition graph in Figure 8.

Based on the data measuring absorbance values, it can be analyzed that the higher the concentration of the sample or comparison used is followed by an increase in the antioxidant activity. This is because at high concentrations, the ability of antioxidants to capture free radicals is greater. The remaining DPPH concentration becomes smaller so that the resulting absorbance value decreases. So the increase in activity is proportional to the increase in sample or comparison concentration.

The IC_{50} obtained for breadfruit leaf extract showed a higher value compared to rosella flowers and other extract combinations. This means that breadfruit leaf extract requires a higher concentration to provide antioxidant effects equivalent to those provided by other samples. This shows that although breadfruit leaves contain flavonoids, their antioxidant activity is lower, which could be caused by other active components or the interaction of compounds in the extract. Rosella flowers showed the lowest IC_{50} value, which means that rosella has the strongest antioxidant activity among the samples tested. This is consistent with the higher content of flavonoids, especially anthocyanins, which are known to have strong antioxidant activity. Rosella has been proven in various studies to have potential as a significant antioxidant agent[54]. Combinations DE1C, DE2C, DE3C showed lower IC_{50} values compared to breadfruit leaf extract alone. The lowest IC_{50} value was found in a DE2C, which also recorded the highest flavonoid levels in previous data. This shows the synergistic potential between the two extracts, where rosella makes a dominant contribution to increasing antioxidant activity in the mixture. The combination DE3C also showed good antioxidant activity, but it was higher than DE2C, indicating that a higher proportion of rosella contributed more to increasing antioxidant activity (Figure 9).

From the research results, there is a positive correlation between flavonoid levels and antioxidant activity (as measured by IC_{50}). Previous data shows that rosella flowers have the highest flavonoid levels, followed by combinations DE2C,DE1C and finally breadfruit leaves with the lowest flavonoid levels. This reflects the importance of flavonoids in antioxidant activity, where rosella flowers, which contain the most flavonoids, show the lowest IC_{50}, or highest antioxidant activity. The combination of extracts with a DE2C which produced the highest flavonoid levels, also showed the lowest IC_{50} value, indicating that this combination produced better antioxidant activity. This reflects the principle of synergistic effect where the contribution of flavonoids from rosella, which is dominant in this mixture, plays a very important role in increasing antioxidant activity. Although breadfruit leaves contain significant amounts of flavonoids, their antioxidant activity (based on higher IC_{50} values) is lower than that of rosella. This could be caused by variations in the composition of other active compounds

which may have lower or less antioxidant activity in breadfruit leaves compared to rosella flowers.

CONCLUSION

Breadfruit leaf and rosella flower extracts contain various bioactive compounds, namely flavonoids, saponins, tannins, phenolics and triterpenoids. Qualitative analysis using thin layer chromatography (TLC) showed the presence of phenol compounds with the $FeCl_3$ reagent which produced a black color on the spot. Flavonoids were detected by spraying $AlCl_3$ reagent which gave yellow fluorescence, citroborate produced yellowish blue fluorescence, and ammonia vapor produced blue fluorescence under a 366 nm UV lamp. Qualitative antioxidant tests with DPPH reagent showed antioxidant activity in breadfruit leaf extract with an Rf value of 0.94, in rosella extract with an Rf of 0.18, as well as in a combination of extracts which produced an Rf of 0.18, 0.74, and 0.94. Quantitatively, the highest levels of total phenols and flavonoids were found in a combination of breadfruit and rosella leaf extracts with a DE2C namely 0.953 ± 0.005 g GAE/100g and 136.973 ± 5.050 µg QE/g, respectively. However, the highest antioxidant activity, shown by an IC_{50} value of 540.55 ppm, was found in a combination of breadfruit and rosella leaf extracts DE3C. This mismatch between total phenol and flavonoid levels and antioxidant activity is likely caused by the contribution of other compounds, such as saponins, tannins and triterpenoids, which also provide antioxidant activity.

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