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Antioxidant Activity of a Combination of *Pegagan* (*Centella asiatica*) and *Temulawak* (*Curcuma xanthorrhiza*) Rhizomes Using the DPPH Method

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Abstract

Pegagan (*Centella asiatica*) and *temulawak* (*Curcuma xanthorrhiza*) rhizomes are plants commonly recommended for enhancing the body's immune system. This study aimed to evaluate the antioxidant activity of extracts from *pegagan*, *temulawak*, and their combination to determine if a synergistic effect exists between the two extracts. Extraction was performed using the maceration method with 70% ethanol as the solvent. The yield of the *pegagan* herb extract was 20.96%, while the *temulawak* rhizome extract yielded 10.88%. For preliminary testing, thin-layer chromatography (TLC) was used to analyze the samples in various mobile phases, including the polar chloroform:methanol:water (7:2:0.15), non-polar n-hexane:ethyl acetate (6:5), and semi-polar chloroform:methanol (9:1). The results indicated the presence of flavonoids, phenols, and 0.2% DPPH. Antioxidant activity was measured using UV-Vis spectrophotometry at a wavelength of 516 nm with the DPPH method. The antioxidant activity results showed that the *pegagan* extract alone had an IC_{50} of 96.109 µg/mL, the *temulawak* extract alone had 94.151 µg/mL, and the combinations had IC_{50} values of 41.884 µg/mL (1:1), 71.074 µg/mL (1:2), and 26.335 µg/mL (2:1). Based on these results, both single and combined extracts exhibited antioxidant activity, with the combination showing higher activity and a synergistic effect.

Keywords: Antioxidant Activity, *Pegagan* (*Centella asiatica*), *Temulawak* (*Curcuma xanthorrhiza*), DPPH Method, Synergistic Effect

1. Introduction

Free radicals are reactive compounds with unpaired electrons in their outer shell and are known to contribute to degenerative diseases such as cancer, rheumatoid arthritis, coronary heart disease, and cataracts (Ifeanyi, 2018). Antioxidants are chemical compounds that protect biological components such as lipids, proteins, vitamins, and DNA from oxidative damage or discoloration (Gulcin, 2020). Both synthetic and natural antioxidants serve as valuable sources of protection (Uzombah, 2022). Many medicinal plants contain antioxidant compounds, including *Pegagan* (*Centella asiatica*) and *Temulawak* (*Curcuma xanthorrhiza*).

One method to evaluate antioxidant activity is by using the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay. *Pegagan* (*Centella asiatica* (L.) Urb) is a plant native to tropical and subtropical regions, commonly found in Indonesia. It has long been used in traditional medicine for its various health benefits. This herb is often found growing in gardens, plantations, and along roadsides (Susanti et al., 2021). Previous studies have reported that *Pegagan* possesses a wide range of beneficial properties, including antibacterial, antioxidant, wound-healing, anti-inflammatory, and anticancer activities (Gallego et al., 2014). It contains bioactive compounds such as polyphenols, flavonoids, carotenoids, tannins, vitamin C, and triterpenoids, which contribute to its antioxidant activity, with Asiaticoside being the main marker compound (Kusuma et al., 2024).

Temulawak (*Curcuma xanthorrhiza* Roxb) is a medicinal plant from the Zingiberaceae family, widely found in Indonesia and commonly used as a raw material for traditional medicines. The rhizome of *Temulawak* has been empirically used both as a standalone remedy and in combination with other herbs (Surya et al., 2024). There is substantial empirical evidence, including in vitro, preclinical, and clinical studies, supporting the antioxidant properties of *Temulawak*. The active curcuminoid compounds in *Temulawak* are known to contribute to its antioxidant effects (Nurrulhidayah et al., 2020).

The primary objectives of this research were to evaluate the antioxidant activity of *Pegagan* (*Centella asiatica*) and *Temulawak* (*Curcuma xanthorrhiza*) extracts, both individually and in combination. Additionally, the study aimed to determine whether a synergistic effect existed between the combined extracts, enhancing their antioxidant activity. By assessing both the individual and combined effects of these two herbal extracts, the research sought to provide a deeper understanding of their potential therapeutic benefits when used together.

Given this, the present study will focus on investigating the antioxidant activity of *Pegagan* and *Temulawak* extracts and their combination, using the DPPH assay to gain insights into their potential synergistic effects.

2. Methods

Equipment

The equipment used in this study included an oven, blender, analytical balance, Thin Layer Chromatography (TLC) plates, rotary evaporator, UV-Vis spectrophotometer, chamber, graduated cylinders, Erlenmeyer flasks, watch glasses, test tubes, volumetric flasks, crucibles, dropper pipettes, volumetric pipettes, spray bottles, vials, stirring rods, glass funnels, furnace, crucible tongs, porcelain crucibles, parchment paper, filter paper, and cuvettes.

Materials

The materials used in this study included the following: *Pegagan* herb (*Centella asiatica* (L.) Urb), *Temulawak* rhizomes (*Curcuma xanthorrhiza* Roxb) from Manoko Lembang, 70% ethanol, distilled water, hydrochloric acid (HCl), magnesium powder, toluene, chloroform, Mayer's reagent, Dragendorff's reagent, Liebmann-Bouchardat reagent, sulfuric acid (H_2SO_4), 10% ferric chloride ($FeCl_3$), 10% aluminum chloride (AlCl3), amyl alcohol, DPPH powder, citroborate, vitamin C, and methanol p.a.

Plant Identification

To ensure the accuracy of the plant species used in the study, a plant identification process was carried out. The identification of *Pegagan* (*Centella asiatica*) and *Temulawak* (*Curcuma xanthorrhiza*) was performed at the Herbarium Laboratory of the Biology Department, FMIPA, Universitas Padjadjaran.

Sample Preparation

Both *Pegagan* herb and *Temulawak* rhizomes were processed through several stages: wet sorting, washing with clean water, draining, cutting into smaller pieces, and drying in an oven. After drying, the samples were sorted again, ground into a fine powder, and sieved to obtain particles smaller than 40 mesh.

Extraction

The extraction was carried out using the maceration method with 70% ethanol as the solvent. A total of 300 grams of the sample was macerated for 72 hours (3 days). The resulting extract was then concentrated using a rotary evaporator.

Characterization of Simplisia

The characterization of the simplisia (raw plant material) included macroscopic examination, moisture content determination, total ash content, water-soluble ash, acid-insoluble ash, water-soluble extractive, and ethanol-soluble extractive.

Determination of Total Ash Content

Total ash content was determined using the direct ash method. A sample of 2-3 grams of powdered simplisia was placed in a pre-ignited silica crucible. The sample was gradually heated until all organic material was burned, then cooled and weighed. The remaining ash was filtered through a filter paper and ignited in a furnace at 800 ± 25°C until a constant weight was achieved. The total ash content was calculated as a percentage of the initial weight of the sample.

Total ash (%) = [(Weight of crucible + ash) - Weight of empty crucible] / Weight of sample × 100%

Determination of Acid-Insoluble Ash

The ash obtained from the total ash determination was treated with 25 mL of 10% hydrochloric acid and boiled for 5 minutes. The insoluble ash was filtered through acid-free filter paper, washed with hot water, and then heated in a crucible at 800 ± 25°C until a constant weight was achieved. The percentage of acid-insoluble ash was calculated as a percentage of the initial sample weight .

Acid-insoluble ash (%) = [(Weight of crucible + acid-insoluble ash) - Weight of empty crucible] / Weight of sample × 100%

Determination of Moisture Content

Moisture content was determined using the Azeotropic Distillation method. A 2-gram sample was placed in a 200 mL flask, and the apparatus was set up. After toluene started boiling, the flask was heated carefully for 15 minutes. The distillation speed was then increased to 2 drops per second until most of the water was distilled off. The rate was further increased to 4 drops per second. After the distillation was complete, the water volume was measured, and the moisture content was calculated.

Moisture content (%) = [(Final volume - Initial volume) / Weight of sample] × 100%

Determination of Water-Soluble Extractive

Five grams of the powdered extract were placed in a stopper flask, and 100 mL of chloroform water was added. The mixture was shaken several times during the first 6 hours, then allowed to stand for 18 hours. The filtrate was quickly filtered, and 20 mL was evaporated until dry in a shallow, flat-bottomed dish that had been preheated to 105°C and weighed. The residue was further heated to a constant weight at 105°C. The water-soluble extractive was calculated as a percentage of the sample weight.

Water-soluble extractive (%) = [(Weight of dish + extract - Weight of empty dish) / Weight of sample] × (Solvent volume / Volume evaporated) × 100%

Determination of Ethanol-Soluble Extractive

Five grams of the powdered sample were placed in a stopper flask, and 100 mL of ethanol was added. The mixture was shaken several times for the first 6 hours and left to stand for 18 hours. The filtrate was filtered quickly, and 20 mL was evaporated until dry in a preheated shallow dish at 105°C. The

residue was heated to a constant weight at 105°C. The ethanol-soluble extractive was calculated as a percentage of the sample weight .

Ethanol-soluble extractive (%) = [(Weight of dish + extract - Weight of empty dish) / Weight of sample] × (Solvent volume / Volume evaporated) × 100%

Loss on Drying

One to two grams of the sample were placed in a preheated airtight bottle. The sample was spread evenly, and the bottle was shaken to form a 5-10 mm layer. The bottle was placed in a desiccator, opened, and dried at a specified temperature until the weight became constant. The loss on drying was calculated as a percentage of the sample weight.

Loss on drying (%) = [(Initial weight of sample + dish) - (Final weight of sample + dish)] / (Initial weight of sample + dish) × 100%

Alkaloid Test

To test for alkaloids, 2 grams of the sample were mixed with 25% ammonia (v/v) and 25 mL of chloroform, then ground together. The mixture was filtered to separate the filtrate. The resulting solution was extracted with 2 drops of 10% hydrochloric acid (v/v). The solution was then divided into three test tubes, and a few drops of Dragendorff's reagent, Mayer's reagent, and a blank solution were added to each. For the Mayer's reagent, a positive reaction was indicated by the formation of a white precipitate, while a positive result for Dragendorff's reagent was indicated by the appearance of an orange-red precipitate.

Saponin Test

For the saponin test, 10 mL of the sample solution in a test tube was shaken vertically for 10 seconds and allowed to stand. The formation of foam was observed. If, after adding 1 drop of 2N hydrochloric acid, a stable foam formed, it was considered a positive result for saponins.

Flavonoid Test

One gram of the powdered sample was mixed with 100 mL of hot water and allowed to stand for 5 minutes before being filtered. To 5 mL of the filtrate, magnesium powder and 2 mL of hydrochloric acid-ethanol solution (1:1) were added. Then, 10 mL of amyl alcohol was added. The formation of an orange, yellow, or red color in the amyl alcohol layer indicated a positive flavonoid reaction.

Tannin Test

One gram of the sample was added to 10 mL of hot water and boiled for 5 minutes, then filtered. To the filtrate, 1% gelatin solution was added. The formation of a white precipitate indicated the presence of tannins in the sample. In a separate test tube, a few drops of 0.1% $FeCl_3$ were added to the filtrate. The formation of a greenish-black color indicated a positive result for gallic tannins.

Quinone Test

Five milliliters of the sample solution were added to a few drops of 1N sodium hydroxide solution. The appearance of a red color indicated the presence of quinones.

Triterpenoid/Steroid Test

One gram of the sample was macerated with 10 mL of ether for 2 hours, then filtered. Five milliliters of the filtrate were evaporated in an evaporating dish until a residue remained. To the residue, 2 drops of Liebermann-Burchard reagent were added. The formation of a red-purple color indicated the presence of triterpenoids, while a greenish-blue color indicated the presence of steroids.

Antioxidant Evaluation

Preparation of DPPH Solution

To prepare the DPPH solution, 10 mg of DPPH was dissolved in methanol (p.a.) in a 10 mL volumetric flask to achieve a concentration of 1000 ppm. The DPPH solution was stored in a light-protected container, wrapped with aluminum foil.

Determining the Maximum Absorption Wavelength

A 70 ppm DPPH solution was used to determine its absorption spectrum using a UV-Vis spectrophotometer, scanning wavelengths from 400 nm to 800 nm. The absorption peak was measured at the wavelength range of 515–520 nm.

Preparation of Test Extract Solution

To prepare the test extract solution, a 1000 ppm stock solution was first made by dissolving 25 mL of the sample in 25 mL of methanol in a 50 mL volumetric flask. Dilutions were made according to the Lambert-Beer law, aiming for absorbance values in the range of 0.2–0.8.

Preparation of Vitamin C Standard Stock Solution

A 10 mg sample of vitamin C (ascorbic acid) was dissolved in 50 mL of methanol in a 50 mL volumetric flask, yielding a 1000 ppm solution. A series of dilutions were then made in the same volumetric flask by adding methanol to achieve final concentrations of 4, 5, 6, 7, 8, and 9 ppm.

Determination of DPPH Antioxidant Activity

The antioxidant activity was measured by adding 2 mL of DPPH solution to 2 mL of the test sample. The mixture was incubated at 37°C for 30 minutes in a dark container wrapped with aluminum foil. After incubation, the absorbance was measured at the maximum wavelength of 516 nm. The IC_{50} value was determined from a regression curve between the percentage inhibition and the concentration. The obtained IC_{50} value was then compared with that of vitamin C (ascorbic acid) to evaluate the antioxidant strength.

3. Result and discussion

Extraction Yield of *Pegagan* Herb (*Centella asiatica*) and *Temulawak* Rhizomes (*Curcuma xanthorrhiza*)

The extraction yields of *Pegagan* herb and *Temulawak* rhizomes were determined to assess the efficiency of the extraction process for each plant material. The results are shown in **Table 1**.

Table 1. Extraction Yields of *Pegagan* Herb and *Temulawak* Rhizomes

Plant Name	Initial Weight of Plant Material (grams)	Yield (%)
Pegagan Herb	300	20.96
Temulawak Rhizomes	300	10.88

The extraction yield for *Pegagan* herb was 20.96%, indicating a higher concentration of bioactive compounds in the extract compared to *Temulawak* rhizomes, which had a yield of 10.88%. These yields reflect the amount of extract obtained per unit weight of the plant material and provide insights into the potential effectiveness of each plant in the extraction process.

The higher yield from *Pegagan* herb suggests that it may contain a greater proportion of extractable compounds, which could contribute to its overall antioxidant activity. On the other hand, although *Temulawak* rhizomes had a lower yield, their unique phytochemical profile, as indicated by the presence of compounds like quinones and triterpenoids/steroids, may still offer significant antioxidant benefits when used in combination with *Pegagan* herb.

Overall, the extraction yields support the feasibility of using both *Pegagan* and *Temulawak* in herbal formulations aimed at enhancing antioxidant activity, with the yields serving as an important factor in determining the optimal amounts of plant material for further testing.

Source of *Pegagan* (*Centella asiatica*) and *Temulawak* (*Curcuma xanthorrhiza*) Rhizomes

This study utilized *Pegagan* (*Centella asiatica*) and *Temulawak* (*Curcuma xanthorrhiza*) rhizomes to assess antioxidant activity using the DPPH method. The plant samples (Fig. 1) were sourced from the Manoko farm in Lembang, West Java, a region renowned for its favorable climate and soil conditions that support the growth of high-quality medicinal plants.

The *Pegagan* (*Centella asiatica*) and *Temulawak* (*Curcuma xanthorrhiza*) were carefully selected due to their well-known antioxidant properties and their traditional use in herbal medicine. The Manoko farm's cultivation practices ensure the plants' quality and consistency, which are crucial for evaluating their bioactive potential.

Both *Pegagan* and *Temulawak* have been extensively studied for their health benefits, particularly their antioxidant and anti-inflammatory effects. These plants are known to contain various active compounds that contribute to their therapeutic properties, making them ideal candidates for the research conducted in this study.

a b

Figure 1. Visualization of *Pegagan* (*Centella asiatica*) and *Temulawak* (*Curcuma xanthorrhiza*) Rhizomes

Characteristics of *Pegagan* (*Centella asiatica*) Herb and *Temulawak* (*Curcuma xanthorrhiza*) Rhizomes

In this study, the characterization of *Pegagan* (*Centella asiatica*) herb and *Temulawak* (*Curcuma xanthorrhiza*) rhizomes was carried out to evaluate their physical and chemical properties (Table 2). The results of the characterization, as shown in Table 1, provided valuable insights into the composition of both plant materials and their potential for antioxidant activity.

Table 2. Characteristics of *Pegagan* Herb (*Centella asiatica*) and *Temulawak* Rhizomes (*Curcuma xanthorrhiza*)

Characterization Test	*Pegagan* Herb (%) (b/b)	*Temulawak* Rhizomes (%) (b/b)
Total Ash Content	10.5	3
Acid-Insoluble Ash Content	1.5	1
Moisture Content	3.5*	4.1*
Water-Soluble Extractive Content	33	15
Ethanol-Soluble Extractive Content	31.3	20
Loss on Drying	6.8	4.3

The percentages marked with an asterisk refer to volume/weight (% v/b).

The total ash content of the *Pegagan* herb was found to be 10.5%, which was higher than the 3% found in the *Temulawak* rhizomes. This difference may indicate variations in the mineral composition of the two plants. Higher ash content generally reflects the presence of more inorganic substances, which can contribute to the plants' overall nutritional value.

Regarding the acid-insoluble ash content, *Pegagan* had a value of 1.5%, while *Temulawak* showed a slightly lower value of 1%. This measure is important as it represents the residue left after acid digestion, indicating the presence of minerals that are not easily soluble in acids. These values suggest that both *Pegagan* and *Temulawak* contain a relatively small proportion of minerals that are resistant to acid dissolution.

The moisture content of the *Pegagan* herb was 3.5%, and *Temulawak* had a slightly higher moisture content of 4.1%. These values reflect the water content in the plant materials, which can influence the shelf life and stability of the extracts. The difference in moisture content might also affect the extraction efficiency during the preparation of plant extracts for antioxidant analysis.

In terms of water-soluble extractive content, *Pegagan* exhibited a significantly higher percentage of 33%, compared to the 15% in *Temulawak*. This suggests that *Pegagan* has a higher concentration of water-soluble compounds, which could include flavonoids, terpenoids, and other bioactive compounds that contribute to its medicinal properties.

The ethanol-soluble extractive content was also higher in *Pegagan* at 31.3%, compared to 20% in *Temulawak*. This further emphasizes that *Pegagan* may contain a greater variety of compounds that are soluble in ethanol, such as polyphenols and triterpenoids, which are known for their antioxidant and anti-inflammatory effects.

Finally, the drying loss (loss on drying) was 6.8% for *Pegagan* and 4.3% for *Temulawak*. This parameter reflects the amount of moisture lost during the drying process and can give an indication of the plant's stability and preservation during storage. *Pegagan* showed a higher drying loss, which could indicate higher volatile compound content compared to *Temulawak*.

In conclusion, the characterization results of both *Pegagan* and *Temulawak* highlight their distinct physical and chemical properties. These differences in their compositions could influence the antioxidant potential of their extracts, with *Pegagan* showing higher levels of water and ethanol-soluble compounds. The findings suggest that both plants possess valuable bioactive components, which are further investigated in this study for their antioxidant activity using the DPPH method.

Phytochemical Screening of *Pegagan* Herb (*Centella asiatica*) and *Temulawak* Rhizomes (*Curcuma xanthorrhiza*)

Phytochemical screening of *Pegagan* herb and *Temulawak* rhizomes was conducted to identify the presence of secondary metabolites, which are often associated with antioxidant activities. The results of the screening for both samples are shown in **Table 3**.

The phytochemical screening revealed several important findings. Both *Pegagan* herb and *Temulawak* rhizomes were found to contain flavonoids, tannins, and triterpenoids/steroids, compounds commonly associated with antioxidant properties. *Pegagan* herb was found to contain alkaloids, saponins, flavonoids, tannins, and triterpenoids/steroids, whereas *Temulawak* rhizomes contained flavonoids, tannins, quinones, and triterpenoids/steroids.

Interestingly, alkaloids and saponins were present only in *Pegagan* herb, while quinones were detected exclusively in *Temulawak* rhizomes. These findings suggest that each plant has its unique phytochemical profile, which could contribute differently to the overall antioxidant activity when combined.

Table 3. Results of Phytochemical Screening of *Pegagan* Herb and *Temulawak* Rhizomes

Compound Group	*Pegagan* Herb	*Temulawak* Rhizomes
Alkaloids	+	-
Saponins	+	-
Flavonoids	+	+
Tannins	+	+
Quinones	-	+
Triterpenoids/Steroids	+	+

(+) = Contains secondary metabolites
(-) = Does not contain secondary metabolites

The presence of these bioactive compounds in both *Pegagan* and *Temulawak* supports their potential use in herbal formulations aimed at combating oxidative stress, further justifying the need for studying their antioxidant activity using methods like the DPPH assay.

Antioxidant Activity of *Pegagan* (*Centella asiatica*) Herb and *Temulawak* (*Curcuma xanthorrhiza*) Rhizomes

Before conducting the antioxidant activity test, the maximum wavelength was first determined. Using a DPPH solution in the range of 400-800 nm, the optimal wavelength was found to be 516 nm. The best absorbance was obtained at 0.827 with a 70 ppm DPPH concentration. This wavelength was used for measuring antioxidant activity in the subsequent tests. Vitamin C was used as a reference material in this study, as it is known for its high antioxidant activity.

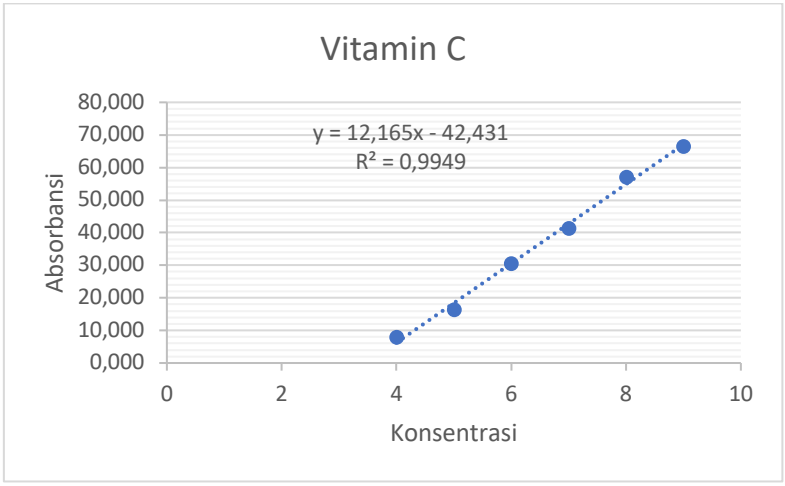

Figure 2. Vitamin C Standard Curve

Following the determination of the maximum wavelength, antioxidant activity testing was carried out on the samples to optimize the concentration. The goal was to find the conditions that would most effectively represent the interaction between antioxidants and the substrate. The optimization was conducted by adding the DPPH solution, which was then incubated for 30 minutes.

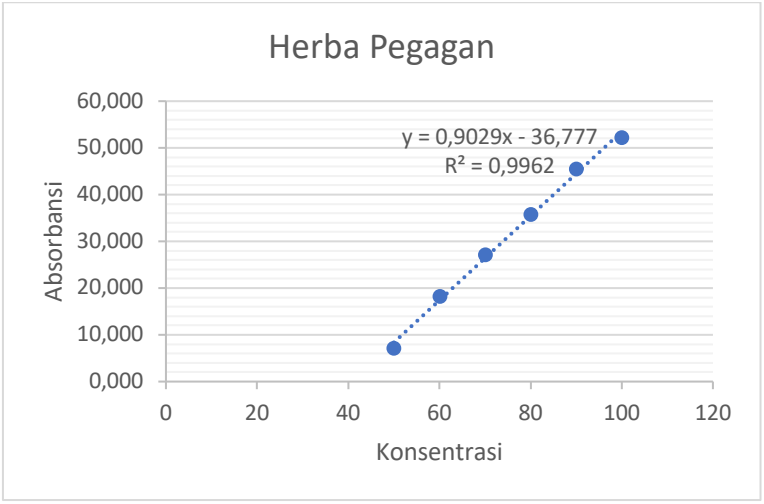

Figure 3. *Pegagan* Herb Extract Sample Curve

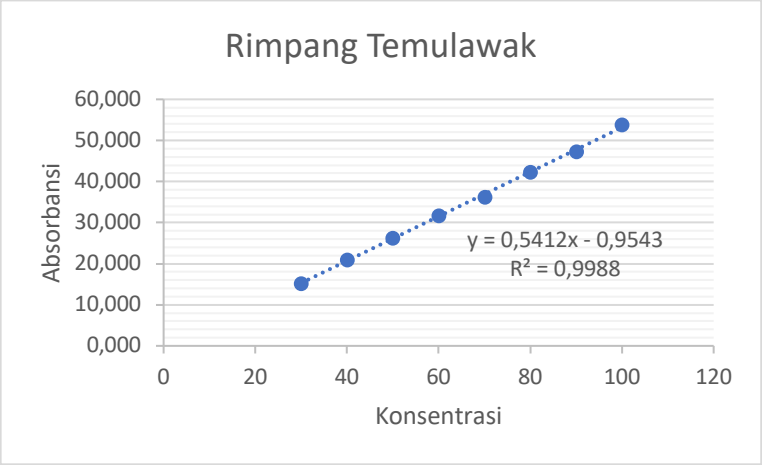

Figure 4. *Temulawak* Rhizome Extract Sample Curve

Table 4. Antioxidant Activity (IC50) of Individual Extracts

Sample	**IC_{50} (µg/mL)**
Pegagan Herb	96.109
Temulawak Rhizomes	94.151

Based on the data obtained, the IC_{50} of *Pegagan* herb extract was found to be 96.109 µg/mL, while the IC50 for *Temulawak* rhizome extract was 94.151 µg/mL. The antioxidant activity of *Pegagan* herb extract was slightly lower than that of *Temulawak* rhizome extract, as indicated by the higher IC50 value. A lower IC50 value typically suggests stronger antioxidant activity.

Both extracts, however, demonstrated strong antioxidant properties, indicating that both *Pegagan* and *Temulawak* possess potential for use in antioxidant-rich formulations. Following the single extract testing, combinations of *Pegagan* herb and *Temulawak* rhizome extracts at various ratios were tested to determine which combination showed the most potent antioxidant effect.

Table 5. Antioxidant Activity (IC50) of Extract Combinations

Sample Combination (b/b)	**IC50 (µg/mL)**
Pegagan + *Temulawak* (1:1)	41.884
Pegagan + *Temulawak* (1:2)	71.074
Pegagan + *Temulawak* (2:1)	26.335

The antioxidant activity of the combination extracts was evaluated by preparing stock solutions of each sample at a concentration of 400 ppm, considering the volume ratios of *Pegagan* and *Temulawak*. The combinations tested were *Pegagan* and *Temulawak* (1:1), (1:2), and (2:1), with each combination tested in triplicate.

The results showed significant differences in the percent inhibition, which could be attributed to the different components in each sample. These differences may be due to the varying interactions between compounds within each extract, as well as potential synergistic or antagonistic effects between the compounds.

The combination of *Pegagan* and *Temulawak* in a 2:1 ratio demonstrated the strongest antioxidant activity, with an IC_{50} value of 26.335 µg/mL. This was more potent than the other two combinations, which had IC_{50} values of 41.884 µg/mL (1:1) and 71.074 µg/mL (1:2). This suggests that the 2:1 combination of *Pegagan* and *Temulawak* provides the most effective antioxidant activity, likely due to the synergistic effects between the two plant extracts.

4. Conclusion

It has been presented related to the antioxidant activity of *Pegagan* (*Centella asiatica*) herb and *Temulawak* (*Curcuma xanthorrhiza*) rhizomes using the DPPH method that both plant extracts demonstrated promising potential as natural antioxidants. The findings from this study highlight the individual and combined antioxidant activities of these plants, shedding light on their potential applications in health and wellness. The individual extracts of *Pegagan* herb and *Temulawak* rhizomes exhibited notable antioxidant activity, with IC_{50} values of 96.109 µg/mL and 94.151 µg/mL, respectively. Both extracts demonstrated significant potential as antioxidants, although the *Temulawak* rhizome extract showed slightly stronger activity based on its lower IC_{50} value. The optimal wavelength for measuring antioxidant activity using the DPPH method was found to be 516 nm, based on the absorbance spectrum of a 70 ppm DPPH solution. This wavelength was used in subsequent measurements to assess the antioxidant potential of the plant extracts. Combining the *Pegagan* and *Temulawak* extracts, the 2:1 ratio (*Pegagan*:*Temulawak*) demonstrated the most potent antioxidant activity, with an IC_{50} value of 26.335 µg/mL. This combination showed stronger antioxidant activity compared to other ratios (1:1 and 1:2), indicating a synergistic effect between the two extracts that enhanced their antioxidant properties.

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