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Potential Combination of Red Onion and Bean Sprouts Extract as Natural Regulatory Substance (PGR) through Total Phenolic Analysis and Antioxidant Activity

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Abstract: Organic farming is now increasingly gaining attention as a solution to the challenges of environmental damage and the need for healthy food in the modern era. One important aspect of organic farming practices is the use of natural Plant Growth Regulators (PGRs) to increase crop yields and quality. This study aims to examine the potential combination of red onion and bean sprout extracts as natural PGRs through total phenolic analysis and antioxidant activity as a reference basis for further application to potato plants. Extracts from both materials were obtained through the maceration method, then formulated in six different ratio comparisons and analyzed for their total phenolic content using the Folin–Ciocalteu method and their antioxidant activity through the DPPH test. The results showed that variation B (8 g/L red onion + 2 g/L bean sprouts) had the highest total phenolic content of 14.77 mg GAE/g and the strongest antioxidant activity with an IC_{50} value of 173.7 ppm. The results were supported by the DMRT test, which showed significant differences between the combination variations. The content of natural hormones such as auxin, gibberellin, and cytokinin in both materials works synergistically in stimulating the biosynthesis of secondary metabolites such as phenolics, which contribute to antioxidant activity and its effectiveness as a PGR. Thus, variation B has the highest potential to be developed as an environmentally friendly and sustainable natural PGR to support the growth and resistance of potato plants. This formulation shows promising potential for further development in the field of organic farming and nature-based agricultural innovation.

Keywords: Antioxidants; Bean Sprouts; PGR; Phenolics; Red Onion.

Introduction

In recent years, the issue of sustainability in the agricultural sector has become a major concern as awareness of the importance of environmentally friendly practices increases. As a form of sustainable agriculture, organic farming aims to avoid the use of artificial chemicals that risk polluting the environment and endangering human health. In the organic farming system, the use of natural materials as a source of nutrients and stimulation of plant growth is highly recommended. This is considered more environmentally friendly and sustainable [1].

In an effort to encourage optimal plant growth, organic agriculture requires alternative strategies, one of which is through the use of natural growth regulators (PGR). PGR plays a crucial role in controlling various physiological activities of plants, such as germination, cell division and elongation, root and shoot formation, and flowering and fertilization [2]. Organic farming is closely related to the use of natural PGR as one of the efforts to improve crop productivity and quality. In conventional agriculture, the use of synthetic PGRs such as auxin, gibberellin, and cytokines has been widely used. However, prolonged and uncontrolled use can have negative impacts, such as decreased soil fertility, the presence of chemical residues in plants and dependence on chemical industry-based products [3]. So in organic farming systems, natural sources of PGR are needed that are not only safe for the environment, but also contain bioactive compounds that support the growth of plants efficiently. Thus, the application of natural PGR is one of the important efforts in supporting the success of organic farming, both in terms of productivity, yield quality, and environmental conservation.

Several studies state that natural materials available around the community can be used as an alternative to natural sources of PGR that are easy and affordable, making it an economical and potential option for households looking to implement organic farming on a yard scale. Natural ingredients such as onions and bean sprouts contain a number of phytohormones, including auxin, gibberellic acid, and cytokinins. Red onion specifically contains natural phytohormones, with auxin content of 251.76 ppm and gibberellin of 594.12 ppm. The content of the hormone gibberellin in red onion is higher than in other plants, such as moringa leaves, at only 371.56 ppm and banana humps, at 104.12 ppm [4]. In addition to red onion, bean sprouts can also be used as a natural source of PGR. In the research of [4] it was stated that mung bean sprout extract (bean sprouts) contained auxin hormone 227.37 ppm, gibberellin 371.56 ppm, cytokinin in the form of kinetin 125 ppm and zeatine 95.45 ppm. Auxin and gibberellin play a role in spurring cell lengthening and tissue division. Meanwhile, cytokinin is an important hormone in stimulating cell division and bud growth [5].

Furthermore, the combination of onion and bean sprout extract as natural PGR is estimated to be able to produce a stronger synergistic effect than a single use. The interaction of auxine, gibberellin and cytokinin hormones

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naturally contained in onions and bean sprouts works synergistically in regulating plant growth and can play a role in stimulating the production of secondary metabolites, including phenolic compounds and antioxidants [6]. The interaction of these three hormones can trigger an increase in total phenolic content that not only acts as plant defence compounds against stress but also contributes to an overall increase in antioxidant activity. This is important because phenolics and antioxidants are not only involved in the physiological regulation of plants but also strengthen the resistance of cells to oxidative damage and improve the metabolic efficiency of plants [7]. Thus, total phenolic content and antioxidant activity can be used as important parameters to evaluate the effectiveness of an ingredient as a natural PGR.

Potatoes (*Solanum tuberosum L.*) are an important commodity in the horticultural sector that requires PGR to accelerate and strengthen the growth process, especially in the early stages. The application of natural PGR on potato seedling tubers is expected to improve the speed and quality of sprout growth and strengthen the root system, which contributes directly to plant productivity. With the increasing interest in organic potato cultivation, the availability of effective and environmentally friendly natural PGR is important to develop.

The use of natural plant growth regulators (PGRs) derived from plant-based ingredients such as red onion and bean sprouts shows great potential in supporting organic farming. The active compounds, such as phenolics, flavonoids, and natural growth hormone compounds, in these ingredients can stimulate plant growth and development without the need for synthetic substances. This aligns with the principles of organic farming, which emphasize sustainability, environmental safety, and the reduction of chemical residues [8]. By utilizing natural PGRs as agricultural inputs, not only can crop production efficiency be increased, but also the quality of the harvest can be maintained within the framework of an organic farming system [9]. Therefore, the development and utilization of PGRs based on natural ingredients has the potential to become an integral part of environmentally friendly biotechnology innovation in the agricultural sector.

Based on this, the purpose of this study is to examine the potential combination of onion and bean sprout extract as natural PGR through testing its total phenolic content and antioxidant activity. This study is a strategic initial stage to determine the bioactive effectiveness of the combination as the basis for the development of natural PGR products. Currently, there are no studies related to the synergistic use of a combination of red onion extract and bean sprouts as a natural PGR, so this research was conducted as a novelty. The results of the research are expected to be the scientific basis for further research in the form of the application of the combination extract to potato plants, both in the initial growth phase and in order to increase overall crop yields. Thus, this research not only supports the development of natural biostimulants but also contributes to sustainable and science-based organic farming practices.

Research Methods

Manufacture of PGR Red onion and Bean Sprout Extract

The process of making the extract begins by washing 10 kg of red onion and bean sprouts to remove the inherent impurities. Red onions are peeled, then thinly sliced to a thickness of approximately 1 mm, then dried in the sun for 5 to 7 days until completely dry. After that, the onion and bean sprouts are mashed and filtered using a 100-mesh sieve. The resulting powder is stored in a tightly sealed container to maintain its quality. The extraction process is carried out using the maceration method, which involves soaking the powder from each ingredient in a 96% ethanol solvent in a ratio of 1:5 (b/v) for 72 hours. After immersion, the mixture is filtered with the help of a vacuum pump to separate the filtrate and the residue. The obtained filtrate is then evaporated using a rotary evaporator with low pressure at a temperature of 40–60°C to produce a viscous extract [8]. The thick extract is stored in a dark glass bottle to be used as a preparation for making variations of PGR combinations. The thick extract is stored in dark glass bottles to be used as a supply for making various plant growth regulator (PGR) combinations. One of the main reasons for storing the extract in dark glass bottles is to protect the phenolic compounds, flavonoids, and natural growth hormones such as auxins, gibberellins, and cytokinins from damage caused by light exposure, particularly ultraviolet and visible light [11]. These compounds are highly sensitive to photodegradation, which can reduce their effectiveness as natural plant growth regulators (PGRs). Furthermore, storage conditions must be strictly controlled. Storage at cool temperatures, ideally in a refrigerator at around 4°C, is recommended to slow the chemical degradation process and suppress the growth of contaminating microorganisms [12]. Under these conditions, the thick extract generally remains stable for up to 14–30 days. However, if stored at room temperature (25–30°C), the shelf life is very limited, and it is not recommended to exceed 3–5 days because the risk of fermentation and microbial contamination increases significantly [13].

Combination of PGR from Red onion and Bean Sprouts Extract

In this study, 6 variations of PGR combination of onion extract and bean sprout extract were used. Variations were made by combining PGR supplies of red onion condensed extract and condensed bean sprout extract into several comparisons, as shown in Table 1. Each variation with several ratios was diluted with distilled water into a 1000 mL measuring flask to the meniscus limit mark.

Table 1. Variations of the combination of onion and bean sprout extract.

Variations	Red onion (g/L)	Bean Sprouts (g/L)
A	10	0
B	8	2
C	6	4
D	4	6
E	2	8
F	0	10

Determination of Total Phenolics Content

Determination of total phenolic content was carried out based on a method adapted from [8] with several modifications. The analysis was carried out using a UV-Vis

spectrophotometer. The procedure begins by weighing 10 mg of gallic acid and dissolving it in 10 mL of ethanol to obtain a standard solution of 1000 ppm. Furthermore, 2.5 mL of the solution was diluted in 25 mL of 96% ethanol to obtain a solution of 100 ppm, which was then used to make standard solutions with concentrations of 10, 20, 30, 40, and 50 ppm. Each variation of the sample combination was weighed at 10 mg, then dissolved in 10 mL of ethanol to obtain a solution of 1000 ppm concentration. Then, 1 mL of the sample solution was mixed with 2 mL of 10% Folin–Ciocalteu reagent, homogenized and left for 5 minutes. After that, 2 mL of 7.5% Na_2CO_3 solution was added, vortexed and incubated for 30 minutes at room temperature. A blank solution was made by replacing the sample with ethanol. Absorbance was measured in triplicate using a UV-Vis spectrophotometer at a wavelength of 767.5 nm. Total phenolic content was determined based on the linear regression equation of the gallic acid standard curve ($y = bx + a$), where y is the absorbance value and x is the concentration in ppm. The concentration value obtained was then converted into units of mg GAE/g sample with the following formula:

$$\text{Total phenolics} = \frac{C \times V \times fP}{g}$$

Information:
C = phenolic compound concentration of standard curve (mg/mL)
V = sample volume (mL)
fp = dilution factor
g = sample weight (grams)

Determination of Antioxidant Activity

Antioxidant activity analysis was carried out using the DPPH method adapted from [8] with several adjustments. The initial step involves making a 40 ppm DPPH solution, namely by dissolving 4 mg of DPPH powder in 100 mL of ethanol. For the sample, each combination variation was weighed 10 mg and dissolved in ethanol until it reached a volume of 10 mL, so that a 1000 ppm concentrated stock solution was obtained. This solution was then diluted into several concentrations: 50, 100, 150, 200, and 250 ppm. Each concentration solution was taken as much as 4 mL and mixed with 1 mL of 40 ppm DPPH solution, then shaken using a vortex for 1 minute and incubated for 30 minutes in the dark. After the incubation process, the absorbance was measured at a wavelength of 517 nm using a UV-Vis spectrophotometer, and was carried out in triplicate. The absorbance data obtained were then used to calculate the percentage of inhibition (% inhibition) using the appropriate formula:

$$\% \text{ inhibition} = \times 100\% \frac{(\text{control absorbance} - \text{sample absorbance})}{\text{control absorbance}}$$

Then a linearity curve was made between % inhibition and sample concentration so that the linear equation $y = ax + b$ was obtained, which was used to determine the IC50 value in each treatment.

Data Analysis Techniques

The data obtained were processed using the IBM SPSS 25 statistical program, one-way ANOVA test at a significance level of 5% to determine whether there was a significant effect between treatments. If the ANOVA results showed a significant difference ($p < 0.05$), then a further test was carried out using DMRT to determine which treatment had a significantly different effect on each treatment in each observation parameter.

Results and Discussion

Manufacture of PGR Red onion and Bean Sprout Extract

In this study, red onion and bean sprouts were selected as natural sources of plant growth regulators (PGRs) based on their phytohormone content. This selection refers to the results of research conducted by [4], which showed that both ingredients contain key phytohormones such as auxin, gibberellin, and cytokinin in high and balanced levels, thus potentially supporting optimal plant growth and development. According to research conducted by [4], it was recorded that red onion bulbs had the highest gibberellin content among the five organic materials tested, namely 594.12 ppm. In addition, red onion bulbs also contained a fairly high amount of auxin, namely 251.76 ppm. The auxin content in red onion was higher when compared to banana corms, which were only 94.20 ppm, and sweet corn kernels at 62.20 ppm. Meanwhile, bean sprouts or mung bean sprouts have an auxin content of 227.37 ppm, with additional cytokinin content in the form of kinetin of 125 ppm and zeatin of 95.45 ppm. Compared with other natural ingredients analyzed, such as moringa leaves, banana stems, and sweet corn kernels, the phytohormone combination of red onion and bean sprouts is more balanced. The high phytohormone content in red onion and bean sprouts not only supports physiological plant growth but also increases phenolic compounds and antioxidant activity, so this extract combination has the potential to provide a dual effect: acting as a natural growth regulator (PGR) and protecting plants from environmental stress through antioxidant mechanisms.

The process of making PGR from red onion and bean sprouts consists of several stages designed to produce extracts with optimal bioactive compound content. The initial stage includes washing the red onion and bean sprouts to remove dirt or foreign particles that can cause microbial contamination or interference during the extraction process. Once clean, the material is dried in the sun to reduce the water content, thereby preventing rotting and fungal growth. After the drying process is complete, the material is then ground using a blender to reduce the particle size to facilitate the next extraction process. This grinding increases the surface area of the material so that the active compounds in it can be more easily extracted. The refined powder is then filtered using a 100 mesh sieve to obtain particles with a finer and more uniform size. The fine powder is then extracted using a maceration technique, namely by soaking it in 96% ethanol at a ratio of 1:5 (w/v) for 72 hours at room temperature. The maceration method was chosen because it does not involve excessive heating, so that active compounds that are thermolabile, such as hormones and antioxidants, are maintained. After the maceration period is complete, the mixture of materials and solvents is filtered using a vacuum pump to separate the extract solution (filtrate) from the remaining material dregs. The filtrate is then evaporated using a rotary vacuum evaporator to remove the solvent

(ethanol) under low pressure and low temperature, so that the bioactive compounds are not damaged and a thick extract is obtained from each material. The thick evaporated extract is stored in a clean, tightly closed dark glass bottle as stock for making variations of PGR combinations.

The next step is to formulate a combination of extracts from red onion and bean sprouts consisting of six types, as shown in Table 1. The combination process is carried out by mixing stock solutions of red onion and bean sprout extracts in certain ratios, then each mixture is diluted with distilled water in a 1000 mL measuring flask until it reaches the meniscus line. This formulation aims to evaluate the synergistic or dominant potential of each extract as a natural PGR. The use of a combination of these two ingredients is based on the content of complementary natural hormones as well as the potential of antioxidants and phenolic compounds that contribute to the effectiveness of the extract in supporting plant growth organically.

Figure 1. Results of Variations in The Combination of Red Onion and Bean Sprout Extracts

Figure 1 shows six clear glass bottles, each labelled with variations A to F, representing six combinations of red onion and bean sprout extracts with a total fixed concentration of 10 g/L, but with different composition ratios. Visually, it can be seen that all variations produce a relatively uniform pale-yellow solution. This indicates that there is no precipitation or significant visual difference between each variation, indicating that the active compound is well distributed in the solvent.

Determination of Total Phenolics Content

Analysis of total phenolic content in PGR from the combination of red onion and bean sprout extracts was carried out using the Folin–Ciocalteu reagent, then measured with a UV-Vis spectrophotometer. The Folin–Ciocalteu method is based on the principle of colorimetric redox reactions to detect all phenolic compound content in samples [9]. The Folin–Ciocalteu reagent itself is a complex mixture of polymeric ions formed from phosphomolybdic acid and phosphotungstic acid. In this reaction, the reagent acts as an oxidizer against the phenolic hydroxyl group (in the form of an alkali salt) and causes the reduction of heteropoly acids into blue molybdenum–tungsten complex compounds [10]. The intensity of the blue color formed is proportional to the amount of phenolic ions produced, which means that the higher the phenolic compound content in the sample, the greater the phenolic ions that are able to reduce heteropoly acids, so that the blue color that appears will be more concentrated [11].

In the analysis of total phenolic content using the spectrophotometric method, a reference standard is needed to determine the total number of phenolic hydroxyl groups in the extract. Gallic acid is used as a standard solution because it is a natural phenolic compound that has high stability, good purity, and relatively low cost compared to other phenolic compounds. This compound is a derivative of hydroxybenzoic acid and belongs to the group of simple phenolic acids. When reacting with the Folin-Ciocalteu reagent, gallic acid will form a yellow color as an indicator of the presence of phenolic compounds. Furthermore, the addition of Na_2CO_3 solution to create basic conditions will change the color of the solution to blue [12].

The standard curve of gallic acid was prepared using solutions with graded concentrations, namely 10, 20, 30, 40, and 50 ppm. From the standard curve of gallic acid, a linear regression equation was obtained, which was then used to calculate the total phenolic content in the PGR resulting from the combination of red onion and bean sprout extracts. Before the measurement, a wavelength scan of the standard gallic acid solution was carried out in the range of 400–800 nm using a UV-Vis spectrophotometer to determine the maximum wavelength, which was then obtained at 767.5 nm. This value shows a slight difference compared to the theoretical maximum wavelength, which is 760 nm [18]. However, based on the provisions stated in the Indonesian Pharmacopoeia, edition IV, the tolerance limit for wavelength shifts is still allowed within a range of ±10 nm from the reference value [19]. The wavelength in this study was taken based on the results of determining the actual λmax in the sample, so the method refers to specific measurement conditions and may differ from the reference. A wavelength of 767.5 nm can still be used for valid measurements, as long as measurements are carried out consistently on all samples and standard solutions. Wavelength shifts are caused by chemical interactions with reagents and matrix conditions, resulting in changes in the spectral properties of phenolic compounds in the extract [20].

After that, the absorbance of the standard solution at various concentrations was measured at that wavelength. The absorbance data were then used to prepare a calibration curve that describes the relationship between concentration (C) and absorbance (A), where a linear line equation was obtained. The following is the standard curve of gallic acid produced.

From the results of measuring the standard gallic acid solution, a calibration curve was obtained with a linear regression equation of $y = 0.0119x + 0.0912$ and a coefficient of determination (R^2) of 0.9894 (Figure 2). This equation is used as the basis for calculating the total phenolic compounds in the sample. Measurement of phenolic content was carried out by observing the absorbance value of the extract using a UV-Vis spectrophotometer, where each sample was analyzed three times (triple) to ensure the accuracy of the results. Samples of the combination of red onion and bean sprout extract PGR solution were prepared at a concentration of 1000 ppm. The results of measuring the total phenolic content of the combination of extracts are presented in Table 2.

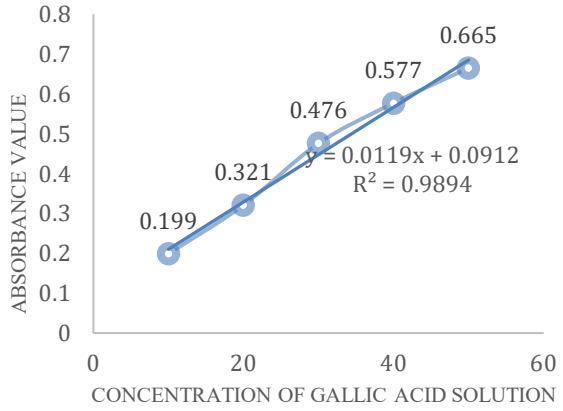

Figure 2. Gallic Acid Standard Curve

Table 2. Data from Total Phenolic Analysis

Sample	Total Phenolics (mg GAE/g extract)
Variation A (10 g/L red onion)	6.7 ± 0.08[a]
Variation B (8 g/L red onion + 2 g/L bean sprouts)	14.77 ± 0.08[b]
Variation C (6 g/L red onion + 4 g/L bean sprouts)	5.33 ± 0.127[c]
Variation D (4 g/L red onion + 6 g/L bean sprouts)	12.1 ± 0.12[d]
Variation E (2 g/L red onion + 8 g/L bean sprouts)	5.1 ± 0.12[c]
Variation F (10 g/L bean sprouts)	6.4 ± 0.08[e]

Note: Numbers with the same letter in the same column indicate insignificant differences based on the DMRT test at the 5% level.

The results of the analysis of total phenolic content showed significant differences between the variations in extract combinations. The highest value was obtained in variation B (a combination of 8 g/L red onion and 2 g/L bean sprouts) of 14.77 ± 0.08 mg GAE/g, followed by variation D (4 g/L red onion + 6 g/L bean sprouts) with a content of 12.1 ± 0.12 mg GAE/g. Conversely, the lowest phenolic content was found in variation E (2 g/L red onion + 8 g/L bean sprouts), which was 5.1 ± 0.12 mg GAE/g, followed by variations C (5.33 ± 0.127 mg GAE/g) and F (6.4 ± 0.08 mg GAE/g). Based on references to several studies, total phenolic content can be categorized as low (<10 mg GAE/g), medium (10-70 mg GAE/g) and high (>70 mg GAE/g) [13].

The total phenolic value of variation B is included in the medium category. This makes variation B the most potential formulation to be developed as a natural PGR, because it is not only high in hormonal content but also supported by high total phenolic content. The total phenolic value of variation B is in the moderate category. This makes variation B the most potential formulation for development as a natural growth regulator (PGR), as it not only has a high hormonal content but also a high total phenolic content. This indicates that certain combinations of red onion and bean sprout extracts can more effectively induce phenolic compound biosynthesis. This increase is strongly suspected to be related to the presence of active compounds in the extract that have hormonal activity, such as auxin, gibberellin, and cytokinin. Auxin can stimulate the activity of the enzyme phenylalanine ammonia lyase (PAL), a key enzyme in the phenolic compound biosynthesis pathway. Increased PAL levels contribute to the accumulation of flavonoids, tannins, and phenolic acids, which are known to have strong antioxidant properties. PAL activation increases the conversion of the amino acid phenylalanine to phenolic compounds. Auxin not only triggers cell division and elongation but also acts as a physiological regulator or inducer of the phenolic metabolic pathway [22]. Cytokinins act as regulators of secondary metabolism by increasing gene expression and enzyme activity in the phenylpropanoid pathway, thereby supporting the accumulation of phenolic compounds [23]. On the other side, gibberellins act as elicitors that trigger the formation of secondary metabolites by inducing a self-protective response when applied to plant cell tissue, thereby triggering increased production of phenolic and flavonoid compounds as a defense mechanism [24]. The above statement is supported by research by [25] that exogenous hormone content can act as a signal that stimulates the formation of secondary metabolites through inducing a response in plants, resulting in higher total phenolic levels compared to the control.

Variation D is in second place which is also included in the medium category. Although dominated by bean sprouts, the presence of red onion in sufficient proportions is still able to contribute auxin and gibberellin needed in phenolic biosynthesis, and is supported by cytokinins from bean sprouts. In contrast, variations C, E, and F are each in the low category. This low content occurs because the comparison is not balanced, so that hormonal synergy is not optimal in supporting phenolic formation. Variation E (2 g/L red onion + 8 g/L bean sprouts), for example, is too dominated by bean sprouts so that it lacks auxin and gibberellin which are important in the formation of secondary metabolites. While variation F (only contains bean sprouts) although it has a high cytokinin content, does not show a significant increase in phenol content due to the lack of support from other hormones. Variation A (pure red onion) is also included in the low category, allegedly due to the absence of cytokinin contribution from bean sprouts which is needed to optimize phenolic metabolism. The high content of phenolic compounds in the extract indicate that the sample has antioxidant potential [14].

In the context of its use as a PGR, phenolic compounds are able to support plant growth and development while increasing resistance to environmental stress [15]. Phenolic compounds are also known to indirectly affect plant growth hormone activity. Several studies have shown that phenolics can modulate auxin activity by inhibiting the oxidase enzyme that damages auxin, thereby increasing the effectiveness of the hormone in stimulating cell elongation and root formation [16]. In addition, the combination of phenolic compounds with hormones such as auxin, gibberellin, and cytokinin contained naturally in red onion and bean sprout extracts will strengthen its role as a natural PGR. This combination not only accelerates physiological processes such as germination and vegetative growth, but also stimulates the biosynthesis of secondary metabolites that strengthen plant resistance as a whole. Therefore, the high phenolic content in certain extract combinations, such as in variation B, is an important indicator in selecting the most potential PGR formulation to be applied to organic and sustainable potato cultivation.

Determination of Antioxidant Activity

The antioxidant activity of the combination of red onion extract and bean sprout extract PGR was determined by the DPPH (2,2-diphenyl-2-picrylhydrazyl) method. This method is based on the ability of antioxidant compounds to release hydrogen atoms that will react with free radicals, thereby changing DPPH (diphenylpicrylhydrazyl), an organic compound containing nitrogen, which is a free radical and purple in color, into a non-radical form, namely diphenylpicrylhydrazine, which is yellow [17]. A decrease in the intensity of the purple color to pale yellow indicates a decrease in the number of DPPH radicals that react with the sample, indicating antioxidant activity [18].

The parameter for interpreting the test results using the DPPH method is IC_{50} (inhibition concentration). The IC_{50} value is the concentration of antioxidant compounds needed to reduce free radical activity by 50% [19]. The IC_{50} value represents the level of effectiveness of an antioxidant compound in neutralizing free radicals. There is an inverse relationship between the IC_{50} value and antioxidant activity, meaning that the smaller the IC_{50} value, the stronger the compound's ability to act as an antioxidant. Conversely, the greater the IC_{50} value, the weaker the antioxidant activity. [18]. In this study, PGR from a combination of red onion and bean sprout extracts was tested at concentrations of 50, 100, 150, 200, and 250 ppm. Each concentration was then mixed with DPPH solution, homogenized, and incubated for 30 minutes in the dark at 37°C. After the incubation process was completed, the absorbance of each solution was measured using a UV-Vis spectrophotometer at a maximum wavelength of 517 nm. Measurements were carried out three times for each concentration. The data from the antioxidant activity test of the combination of extracts are presented in Table 3.

Table 3. Data from Antioxidant Activity

Sample	IC_{50} (ppm)
Variation A (10 g/L red onion)	243.2 ± 1.89^{a}
Variation B (8 g/L red onion + 2 g/L bean sprouts)	173.7 ± 0.82^{b}
Variation C (6 g/L red onion + 4 g/L bean sprouts)	304.99 ± 7.85^{c}
Variation D (4 g/L red onion + 6 g/L bean sprouts)	199.09 ± 1.03^{d}
Variation E (2 g/L red onion + 8 g/L bean sprouts)	334.44 ± 6.0^{e}
Variation F (10 g/L bean sprouts)	301.44 ± 5.9^{c}

Note: Numbers with the same letter in the same column indicate insignificant differences based on the DMRT test at the 5% level.

The results of the antioxidant activity analysis showed that variation B was the only sample with the strongest antioxidant activity and was significantly different compared to all other variations. Variation B (a combination of 8 g/L red onion and 2 g/L bean sprouts) had the best antioxidant activity, indicated by the lowest IC_{50} value (173.7 ± 0.82b ppm). In contrast, variation E (2 g/L red onion and 8 g/L bean sprouts) had the lowest antioxidant activity, with a significantly higher IC50 value of 334.44 ± 6.0 ppm. Based on the general classification, the strength of antioxidant activity can be categorized as very strong (IC50 <50 ppm), strong (IC50 50-100 ppm), moderate (IC¬50 100-250 ppm), or weak (IC50 > 250 ppm). A low IC_{50} is a sign that the compound has high antioxidant capacity [20].

Based on the antioxidant activity test data (IC_{50}), it was found that variation B was in the moderate category, but the best among all variations. Followed by variations D and A, which were also classified as moderate, while Variations C, E, and F were in the weak category. These results are in line with the total phenolic content data, where variation B also had the highest phenolic content (14.77 ± 0.08 mg GAE/g), which was also followed by variation D (12.1 ± 0.12 mg GAE/g). This strengthens the hypothesis that the greater the amount of phenolic compounds in the extract, the higher the antioxidant potential.

The relationship between total phenolics and antioxidant activity appears consistent and mutually supportive. Antioxidant activity plays a crucial role in maintaining cellular redox balance by neutralizing free radicals or Reactive Oxygen Species (ROS), which typically increase dramatically when plants experience abiotic stress conditions, such as drought, salinity, extreme temperatures, or exposure to heavy metals [32]. Excessive accumulation of ROS can damage the structure of cell membranes, proteins, and DNA, leading to metabolic disorders and even cell death [33]. In this regard, phenolic compounds such as flavonoids, phenolic acids (e.g., ferulic acid, chlorogenic acid, and p-coumaric acid), and tannins act as effective ROS scavengers, thereby reducing oxidative damage and increasing plant tolerance to various types of abiotic stress. Phenolic compounds can stabilize cell membranes and maintain intracellular redox balance, thus helping plants continue to grow and develop even under stressful conditions [11]. In terms of disease resistance, phenolic compounds play a role as part of the plant defence system. Some phenolics have natural antimicrobial properties that can inhibit the growth of pathogens, such as fungi and bacteria [34]. In the context of growth physiology, phenolic compounds are known to modulate the activity of enzymes and growth hormones, such as auxins and cytokinins, which play a crucial role in cell division and elongation. The combination of phenolic and antioxidant activity ultimately increases plant vigor, accelerates root and shoot growth, and increases resistance to unfavorable environmental conditions [35].

Combinations with higher phenolic content tend to show higher content of antioxidant activity, as shown in variations B and D. This is in line with the basic principle that phenolic compounds, especially flavonoids and tannins, are able to act as antioxidants through the mechanism of providing hydrogen atoms to neutralize reactive free radicals, thereby stopping the chain oxidative reaction that is detrimental to plant tissue [21]. The combination of red onion and bean sprout extracts shows a synergistic effect, especially when the proportion of red onion is more dominant. Red onions naturally contain auxins and gibberellins, as well as phenolics such as quercetin and anthocyanins, which support the production of secondary metabolites, including antioxidants [5]. On the other hand, cytokinins found in bean sprouts have a crucial role in stimulating cell division and in the process of forming bioactive compounds in plants [22]. When combined in optimal proportions (as in variation B), these hormones can work synergistically to increase phenolic content, which in turn also increases antioxidant activity.

Thus, variation B proved to be the most potential as a natural PGR. This formulation not only contains the highest total phenolic content and the strongest antioxidant activity among other variations, but also reflects a hormonal combination that supports plant growth and increases resistance to abiotic and biotic stress. This potential is very relevant in the context of organic farming, where the use of natural ingredients rich in bioactive compounds and growth hormones is highly recommended. Meanwhile, variations such as E and F, which are dominated by bean sprouts, although containing cytokinins, do not show high phenolic content and antioxidant activity, indicating that the hormonal imbalance in the formulation is not effective in stimulating the production of secondary metabolites that are beneficial for plant growth. This reinforces the importance of the right ratio composition in designing PGR formulations from natural ingredients.

Further research is needed to validate the bioactivity of phenolic compounds and antioxidant activity in red onion and bean sprout extracts directly in plants through bioassays and more measurable physiological observations. This validation can be conducted through in vivo testing on model plants, such as potatoes, mung beans, and spinach, with parameters including germination rate, root and shoot length, leaf number, leaf area, vigor index, and dry and fresh biomass. Further testing can also include microscopic observation of tissue structure to determine the effects on root or shoot meristems, as well as enzymatic analysis (e.g., peroxidase or catalase activity) that can indicate the stimulation of plant metabolism due to the application of natural PGRs. Robust statistical analysis is also important to ensure that the bioactivity effects can be significantly differentiated from the control and other treatments. Furthermore, long-term testing in fields or semi-field conditions is also needed to determine the impact of extract application on the entire plant life cycle, including the generative phase (flowering and fruiting). With this approach, bioactivity validation is not only based on chemical content, but also proven through real and measurable physiological effects on plant growth and development, which is an important basis for recommending this natural PGR as an effective and sustainable agricultural input.

Conclusion

The results showed that the combination of red onion and bean sprout extracts has the potential to be used as a natural PGR to support organic farming practices, especially in potato cultivation. Based on the analysis of total phenolic content and antioxidant activity, variation B (with a composition of 8 g/L red onion and 2 g/L bean sprouts) was identified as the most effective formulation. This variation provided the highest total phenolic value of 14.77 mg GAE/g and the best antioxidant activity with an IC_{50} value of 173.7 ppm, indicating a strong correlation between high phenolic compound content and antioxidant capacity. The content of natural hormones such as auxin, gibberellin, and cytokinin from both ingredients work synergistically in stimulating the production of secondary metabolites that support plant growth and resistance to stress. Thus, variation B can be recommended as the most potential combination formula to be further developed as a natural PGR. Further research could focus on testing directly applied to potato plants to confirm the effectiveness of this B variety of PGR, by examining its effects on plant growth parameters, secondary metabolite yields, and other aspects. Meanwhile, this research could also be integrated into lessons on phenolic compounds, antioxidants, bioactive compound extraction, and quantitative and qualitative analysis methods. This provides students with the opportunity to apply chemistry concepts contextually in the real world, strengthening critical and investigative thinking skills.

Author's Contribution

Cintania Putri Aristya: Collecting research data and writing articles; and Mirwa Adiprahara Anggarani: responsible person and article compiler.

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