

Total Phenolics and Potential Antioxidant Activity in Natural Materials: Banana Stems and Bean Sprouts as Growth Regulators for Chilli Peppers (*Capsicum frutescens* L)

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Abstract: Indonesia has abundant horticultural commodities, including chilli plants, which have high economic value. However, their productivity remains low at 8.35 tons/ha, compared to the potential 20–40 tons/ha. To boost the productivity of red cayenne pepper, additional supplements such as phytohormones auxin, gibberellin, and cytokinin are needed. This study uses natural sources of phytohormones derived from banana stems and mung bean sprouts. The aim is to measure the antioxidant activity and total phenolic content of various combinations of these natural plant growth regulators (PGRs) to support plant productivity. An experimental design was used, analyzing antioxidant activity using the DPPH method with IC₅₀ values, and total phenolics using the Folin-Ciocalteu method. Six variations were tested: A (banana stem extract 10:0), B (banana stem:bean sprouts 8:2), C (6:4), D (4:6), E (2:8), and F (bean sprout extract 10:0). Antioxidant activity and total phenolics were measured using a UV-Vis spectrophotometer. The results showed that variation C (6:4) had the highest total phenolic content (8.8 mg GAE/g) and an IC₅₀ value of 210 ppm, indicating moderate antioxidant activity. These findings suggest that the combination of banana stem and bean sprout extracts in the right proportion can enhance the functional properties of natural PGRs, potentially supporting the growth and productivity of red cayenne pepper plants.

Keywords: Antioxidant Activity; Cayenne Pepper; Phytohormones; Plant Growth Regulator; Total phenolics.

Introduction

The sustainable development of a country is highly dependent on the contribution of the agricultural sector, which includes the provision of raw materials, employment, and the fulfilment of basic community needs[1]. In Indonesia, the agricultural sector has been a cultural heritage since the time of our ancestors, with the number of farmers reaching around 32 million people[2]. However, in practice, many farmers still apply traditional farming techniques with a high dependence on inorganic chemical fertilizers and pesticides in an uncontrolled manner[3]. This practice is contrary to the principles of sustainable development, which emphasize aspects of environmental sustainability and human health[4]. Therefore, organic farming becomes a relevant alternative solution because it prioritizes the use of natural materials such as banana stems, bean sprouts, shallots, and others as agricultural inputs. This approach not only supports environmental sustainability but is also able to meet food needs, especially in horticultural commodities [5].

Horticultural plants are plants that are cultivated to fulfil human needs, both in terms of food consumption, decoration, and environmental health[6]. In Indonesia, there are several types of horticultural plants that are widely planted, namely tomatoes, peas, long beans, and cayenne peppers[7]. Cayenne pepper in Indonesia has a fairly high demand, but is not supported by optimal production due to several factors, namely, low soil fertility, minimal nutrition, and extreme weather conditions[8]. Therefore, it is necessary to provide additional supplements from natural ingredients in the form of Plant Growth Regulators (PGRs) such as

banana stems and bean sprouts because they contain phytohormones and phenolic compounds[9].

Banana stump (*Musa spp*) is one of the natural ingredients that can be used as raw material for ZPT. Phytohormone compounds such as cytokinins and gibberellins in banana stumps function to trigger the regulation of the development phase and growth of plant shoots[10]. Then, the phytohormone cytokinin itself has the task of stimulating and encouraging cells to enlarge sideways, namely towards lateral dominance (side shoots) [11]. Then another natural ingredient used is bean sprouts (*Vigna radiata* L). Bean sprout extract has a dominant phytohormone in the form of auxin, which functions in plants as a stimulant for cell elongation by increasing the flexibility of cell walls, allowing cells to absorb water and enlarge [12]. The phytohormone levels found in bean sprouts were reported as auxin 1.25 mg/L, gibberellin 0.75 mg/L, and cytokinin 0.50 mg/L[13]. Phytohormones such as salicylic acid (SA) and indole-3-acetate (IAA) trigger increased biosynthesis of phenolic compounds in plant tissues, which further enhances antioxidant activity as a defence mechanism against oxidative stress. This suggests a reciprocal relationship between hormonal system regulation and secondary metabolite responses in maintaining plant cellular homeostasis[14].

Total phenolics are a group of secondary metabolites that play an important role in regulating plant growth through antioxidant mechanisms and physiological signals. Total phenolics are a group of secondary metabolites that play an important role in regulating plant growth through antioxidant mechanisms and physiological signals[15]. Application of

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plant growth regulators (PGRs) such as benzylaminopurine (BAP), kinetin, and gibberellic acid (GA_3) has been shown to increase total phenolic accumulation, which is then positively correlated with increases in biomass and crop yield[16]. For example, in lentil (*Lens culinaris*) plants, PGRs treatment increased total phenolic content along with increased shoot and seed dry weight, indicating that increased phenolics contribute to photosynthetic efficiency and vegetative growth[17]. Antioxidant activity is the ability of certain compounds to reduce the reaction of free radical compounds[18]. Antioxidant activity in ZPT compounds plays a crucial role in reducing oxidative stress, thereby supporting growth and tolerance to abiotic and biotic environmental stress[19].

Phenolic content is closely related to the ability of plants to protect themselves from biotic and abiotic disturbances, namely as an antioxidant agent[20]. Where the phenyl propanoid reaction works on the stem, it will result in a large total phenolic value of a plant, and its antioxidant activity will also be stronger[21]. The IC_{50} value is the concentration of the sample required to reduce free radicals. Thus, this study is a study that aims to examine the combination of ZPT, a combination of banana stem extract and bean sprouts, which can affect the quality of cayenne pepper plants (*Capsicum frutescens* L) through total phenolic parameters and antioxidant activity.

Research Methods

Materials

In this study, the materials used included ZPT variations of a combination of banana stem and bean sprout extracts, 96% ethanol, distilled water, gallic acid (Sigma-Aldrich, USA), 7.5% Na_2CO_3 solution, DPPH reagent (Merck, USA), and Folin-Ciocalteu reagent.

Tools

The tools used in this study were centrifugators (Eppendorf), vortex (Labnet), micropipettes and blue tips (Brand), test tubes, spatulas, stirring rods, jars, glass bottles, Erlenmeyers, desiccators, measuring cups, measuring flasks, volumetric pipettes, measuring pipettes, analytical balances (Denver SI-234, and UV-Vis spectrophotometers (Shimadzu UV -1800).

Making a combination extract of banana stem and bean sprouts

The sample preparation process begins with preparing 10 kg of 4-day-old bean sprouts and fresh banana stems after harvest, which are first cleaned of dirt. The material is then cut thinly with a thickness of about 1 mm, then dried on a tray under sunlight for approximately 7 days. After drying, the material is ground until smooth and sieved using a 100 mesh sieve to obtain a fine powder. Furthermore, the extraction process was carried out using the maceration method, namely by soaking the powder in 96% ethanol solvent with a ratio of 1: 5 (w / v) for 3 days (3×24 hours). This mixture was then filtered using a vacuum pump to separate the filtrate and residue. The filtrate obtained was then evaporated using a rotary vacuum evaporator at a

temperature of 40-60 ° C to produce a thick extract. The thick extract of banana stem and bean sprouts is then diluted into a combination of ZPT solutions of 6 variations of ZPT. Variations are made by combining the supply of ZPT from thick banana stem extract and thick bean sprout extract in several ratios and dissolving them in 1L of distilled water. The following is a comparison of the composition of the concentration of banana stem extract and bean sprout extract:

Table 1. Variation of combination treatments of banana stem and bean sprouts

Variation Name	Banana Stem Extract (gr)	Bean Sprout Extract (gr)
Variation A	10	0
Variation B	8	2
Variation C	6	4
Variation D	4	6
Variation E	2	8
Variation F	0	10

Preparation of Gallic Acid Standard Solution

Determination of phenolic content was carried out based on the absorbance curve of a standard solution of gallic acid. The standard solution was prepared by weighing 10 mg of gallic acid, which was then dissolved in 10 mL of distilled water, producing a stock solution with a concentration of 1000 ppm. Furthermore, 2.5 mL of the stock solution was diluted in a 25 mL volumetric flask to obtain a solution with a concentration of 100 ppm. This solution was then further diluted to produce standard solutions with graded concentrations, namely 10, 20, 30, 40, and 50 ppm[22].

Standard gallic acid solutions with concentrations of 10, 20, 30, 40, and 50 ppm were taken as much as 1 mL each. Each solution was then added with 2 mL of Folin-Ciocalteu reagent (with a dilution ratio of 1:10), then mixed using a vortex for approximately 1 minute and left for 10 minutes. After that, 2 mL of 7.5% Na_2CO_3 solution was added and vortexed again until homogeneous. The mixture was then incubated for 30 minutes, and absorbance measurements were carried out at a wavelength of 767.5 nm[23].

Measurement of Total Phenolics Sample

Variations of ZPT solutions, which are a combination of banana stem and bean sprout extracts, were prepared by weighing 10 mg and dissolving it in 10 mL of distilled water, so that a solution with a concentration of 1000 ppm was obtained. A total of 1 mL of this sample solution was taken, then 2 mL of Folin-Ciocalteu reagent was added (with a dilution of 1:10), then mixed using a vortex for approximately 1 minute and left for 10 minutes. After that, the solution was added with 2 mL of 7.5% Na_2CO_3 and vortexed again until evenly distributed, then incubated for 30 minutes. Absorbance measurements were carried out at a wavelength of 767.5 nm. Each sample was analyzed three times. The absorbance value obtained from the sample was compared with a standard solution of gallic acid. The total phenol content was expressed in units of mg GAE per gram of sample. The concentration of phenolic compounds in the sample was calculated using a linear regression equation from the standard curve. Furthermore, the total phenolic

content was calculated based on the dry weight of the sample using the following formula[24]:

$$TPC = \frac{C \cdot V \cdot Fp}{g}$$

Description:

TPC = Total Phenolic Content
C = Phenolic Concentration
V = Sample Volume (ml)
Fp = Dilution factor
g = Sample weight (gram)

Preparation of DPPH 40 ppm blank solution

A total of 10 mg of the combination of ZPT from banana stem and bean sprout extracts was weighed, then dissolved in ethanol until a volume of 10 mL was reached, so that a stock solution with a concentration of 1000 ppm was obtained. The solution was then diluted in a 10 mL measuring flask to produce five concentration variations, namely 250, 200, 150, 100, and 50 ppm. Each dilution solution was taken as much as 4 mL and put into a centrifuge tube, then 1 mL of 40 ppm DPPH solution was added. The mixture in the tube was then mixed using a vortex for 1 minute and stored in a dark place for 30 minutes before further analysis was carried out[22].

Measurement of antioxidant activity in samples

A total of 10 mg of the combination of ZPT from banana stem and bean sprout extracts was weighed, then dissolved in ethanol until a volume of 10 mL was reached, so that a stock solution with a concentration of 1000 ppm was obtained. The solution was then diluted in a 10 mL measuring flask to produce five concentration variations, namely 250, 200, 150, 100, and 50 ppm. Each dilution solution was taken as much as 4 mL and put into a centrifuge tube, then 1 mL of 40 ppm DPPH solution was added. The mixture in the tube was then mixed using a vortex for 1 minute and stored in a dark place for 30 minutes before further analysis. Then the absorbance was measured with UV-Vis at a wavelength of 517 nm [25]. Measurements were carried out in triplicate. The absorbance value obtained was used to calculate the % inhibition value with the following formula:

$$\%inhibition = \frac{\text{blank absorbance} - \text{sample absorbance}}{\text{blank absorbance}} \times 100\%$$

The antioxidant activity test method using DPPH reagent is based on the ability of antioxidant compounds to neutralize free radicals formed by DPPH. Measurements are carried out using a UV-Vis spectrophotometer at a certain wavelength to obtain the IC₅₀ (Inhibitory Concentration) value, which is the concentration of the compound needed to reduce free radicals by 50%[26].

Results and Discussion

Based on the measurement results of the standard solution of gallic acid using a UV-Vis spectrophotometer instrument, the resulting wavelength is 516 nm. The

absorption length of the standard solution is measured and the standard curve of gallic acid is obtained as in Figure 1.

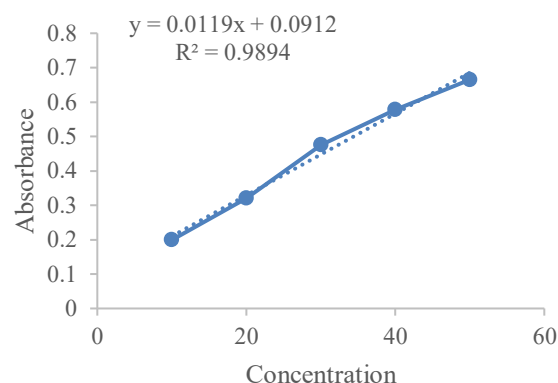


Figure 1. Curve of the relationship between concentration and absorbance of gallic acid standards

Based on the standard curve graph, a linear regression equation was obtained, namely $y = 0.0119x + 0.0912$, with a correlation coefficient value (r) of 0.9894. This value indicates a strong linear relationship between concentration and absorbance value; the higher the concentration of gallic acid, the higher the absorbance. The total phenolic value of each variation of the combination of ZPT extract between banana stem and bean sprout extracts was obtained, which is depicted in the form of a bar diagram as follows:

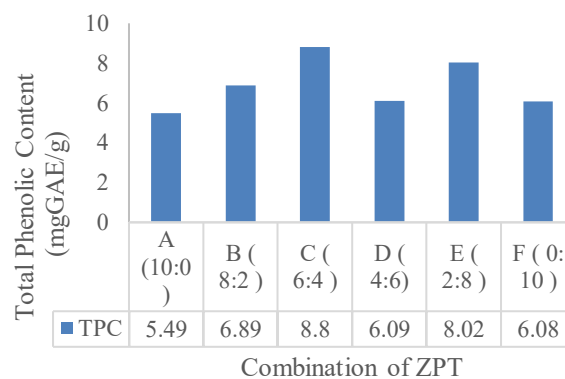


Figure 2. Variation of ZPT treatment vs Total phenolic ZPT Combination

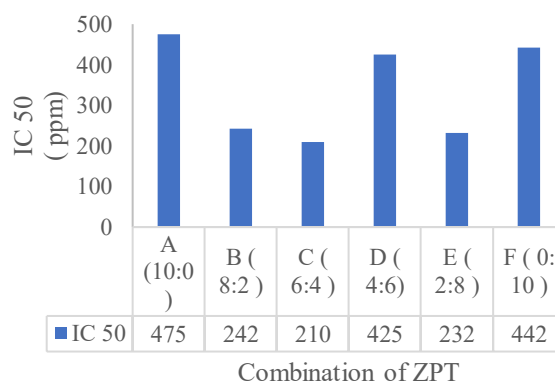


Figure 3. Variation of ZPT treatment vs IC 50 ZPT Combination

Based on the information listed in the 2 bars above, it can be seen that the total phenolic combination of plant growth regulators from banana stumps and bean sprouts obtained data in the order from lowest to highest, namely single banana stump PGR extract (10:0) of 5.49 mgGAE/g, single bean sprout PGR extract (10:0) of 6.08 mgGAE/g, banana stump and bean sprout combination PGR extract (4:6) of 6.09 mgGAE/g, banana stump and bean sprout combination PGR extract (8:2) of 6.89 mgGAE/g, banana stump and bean sprout combination PGR extract (2:8) of 8.02 mgGAE/g, and banana stump and bean sprout combination PGR extract (6:4) of 8.8 mgGAE/g. This difference indicates that bean sprout extract contains higher amounts of phenolic compounds compared to banana stem extract when used separately. During the germination process, phenolic compounds in mung bean sprouts are biosynthesized predominantly through the phenylpropanoid pathway, where phenylalanine is first deaminated by phenylalanine ammonia-lyase (PAL) into cinnamic acid, which subsequently undergoes hydroxylation and methylation reactions to form various phenolic acids and flavonoids[27]. The total phenolic content (TPC) in banana stems is relatively low, recorded at approximately 18.5 mg GAE/g, compared to other plant parts, such as young banana stems, which can reach around 32.7 mg GAE/g, a difference attributed to the lower abundance of secondary metabolite activating enzymes in the stem tissues[28]. The combination treatment represented by variance C produced a total phenolic content (TPC) of 6.09 mg GAE/g with an IC₅₀ value of 207 ppm, showing a distinctive response compared to other variances, which indicates a specific interaction between the natural phytohormones derived from banana stems and bean sprouts. These results suggest that variance C reflects a unique synergistic reaction of auxin and gibberellin contained in the natural ZPT sources, leading to a different metabolic modulation in phenolic biosynthesis and antioxidant activity relative to the other treatment combinations. Furthermore, differences in the proportion of combined natural ingredients can modulate the bioavailability and solubility of phenolic compounds, thereby reinforcing the observed synergistic interaction in variance C that influenced both TPC and IC₅₀ outcomes. [29].

Based on the information listed in Figure 3 bar graph above, it can be seen that the antioxidant activity or IC₅₀ combination of growth regulators from banana stumps and bean sprouts obtained data in the order from lowest to highest, namely single banana stump ZPT extract (10:0) of 475 ppm, single bean sprout ZPT extract (10:0) of 442 ppm, combination of banana stump and bean sprout ZPT extract (4:6) of 425 ppm, combination of banana stump and bean sprout ZPT extract (8:2) of 242 ppm, combination of banana stump and bean sprout ZPT extract (2:8) of 232 ppm, and combination of banana stump and bean sprout ZPT extract (6:4) of 210 ppm. Variation A (10:0) has an IC₅₀ value of 475 ppm, which is classified as weak, while variation F (0:10 gr) has an IC₅₀ value of 442 ppm, which is classified as weak. This shows that the composition of the ingredients plays an important role in antioxidant synergism, not only the total phenolic content. The phenolic compounds generated in higher amounts within the sample of natural ZPT combinations act as effective antioxidants through two main mechanisms: they donate hydrogen atoms or electrons from

their hydroxyl groups to unstable free radicals, converting those radicals into more stable and non-reactive molecules, and simultaneously the phenolic structure itself is stabilized by resonance within the aromatic ring, preventing further chain reactions. This dual action directly lowers the reduction potential of reactive oxygen species (ROS) such as DPPH, thereby inhibiting oxidative chain reactions and reflecting the increased antioxidant activity observed in samples with higher total phenolic content[30]. This phenomenon can show a strong correlation between TPC and antioxidant activity, where phenolic compounds will act as electron donors in neutralizing free radicals by reducing their reduction rate in sugarcane plants[31]. Another study stated that the application of synthetic GA3 and auxin with each concentration of 0.5 mg/l, can increase total phenolics up to 1.7 times more than the control [32].

The results of the total phenolic value and antioxidant activity or IC₅₀ were analyzed using the SPSS tool in the form of a normality test, to determine if the results were normally distributed. Continued by the Duncan Multiple Range Test (DMRT) with a significance level of 5%, the DMRT test is used to see the significance between each variation A to F on the total phenolic value and antioxidant activity can be described in Table 2 below:

Table 2. Statistical test results between total phenolics and antioxidant activity of the combination ZPT

Combination Variance	Phenolics Total (mgGAE/g) Average	IC ₅₀ (ppm) Average
Variance A	5.49±0.046 ^a	476 ± 11.50 ^a
Variance B	6.89 ±0.098 ^b	242 ± 1.52 ^b
Variance C	8.8 ± 0.126 ^c	207 ± 16.70 ^c
Variance D	6.09 ± 0.338 ^d	423.38 ± 12.52 ^d
Variance E	8.02 ± 0.098 ^e	231.6 ± 0.55 ^e
Variance F	6.08 ± 1.201 ^f	453 ± 2.88 ^f

In the DMRT test results column above, it states that the total phenolic test results and antioxidant activity test results have significant differences from variations A to F, demonstrated a distinct synergistic effect compared to the other combinations, suggesting that the specific proportion of auxin, gibberellin, and other phytohormones present in this mixture promoted a more efficient activation of the phenylpropanoid pathway. This pathway is responsible for the biosynthesis of phenolic compounds, and its activation is often regulated by signalling cascades involving these growth regulators. In contrast, combinations such as Variance A and F, with lower TPC values and weaker antioxidant capacities as indicated by their higher IC₅₀ values, reflect a less favorable balance of phytohormones, possibly leading to suboptimal enzyme activation and limited phenolic accumulation. Interestingly, Variance D and E showed relatively high TPC, but their IC₅₀ values were not as low as expected, indicating that although phenolic biosynthesis was enhanced, the qualitative profile of phenolic compounds or their bioavailability might differ due to competitive or antagonistic hormonal interactions. From an application perspective, the synergistic effect of the C combination can be strategically utilized to develop biostimulant formulations aimed at improving the metabolic quality and stress resistance of horticultural crops. By increasing phenolic accumulation and antioxidant capacity,

these formulations can improve growth performance, productivity, yield stability, and tolerance to oxidative stress in economically valuable crops such as chilli peppers. Where it is stated that the combination of natural food ingredients with different phenolic content can produce synergistic, additive, or even antagonistic effects depending on the proportion, pH, and matrix interactions of each[33].

Conclusion

Of all the variations of the combination of ZPT A to F, the combination variation that showed the dominant total phenolic test value and antioxidant activity was combination variation C with the composition of banana stem and bean sprout extract (6:4), which recorded the highest total phenolics at 8.8 mg GAE/g and an antioxidant activity of 207 ppm. These findings imply that the synergistic interaction of natural phytohormones in this combination has the potential to enhance the physiological quality and stress tolerance of horticultural crops such as chili and tomato, thereby supporting improved growth performance, yield stability, and resilience against oxidative stress in cultivation.

Author's Contribution

Jovan Pratama: contributed to drafting the research concept, developing the research methodology, collecting data, analyzing data, and writing the manuscript.. Mirwa Adiprahara Anggarani: contributed to drafting the research concept and evaluating the authors in writing the manuscript.

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