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Phenolic and Antioxidant Activity of Moringa-Bean Sprouts Extract as PGRs for Soybeans

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Abstract: The demand for soybeans continues to increase, but local production remains insufficient, requiring strategies to enhance productivity and quality, such as the application of Plant Growth Regulators (PGRs). Natural PGRs derived from moringa leaves and mung bean sprouts, which are rich in auxins and cytokinins, have the potential to accelerate growth and stimulate the production of secondary metabolites. This study investigated various proportions of moringa leaf and mung bean sprout extracts to evaluate their phenolic content, antioxidant activity, and potential as natural PGRs in soybean cultivation. The extracts were analyzed using the Folin–Ciocalteu method for total phenolics and the DPPH assay for antioxidant activity. Among the treatments, Variation E, with a higher proportion of mung bean sprout extract, proved to be the most effective, enhancing both antioxidant activity and phenolic compound production. These findings highlight the novelty of combining moringa leaf and mung bean sprout extracts as a natural PGR strategy, demonstrating promising potential to support soybean growth and improve crop quality.

Keywords: Antioxidant Activity; Bean Sprouts; Moringa Leaves; PGRs; Total Phenolics.

Introduction

Soybean demand in Indonesia continues to increase every year, driven by population growth and the rising consumption of soybean-based products, including tofu, tempeh, soy sauce, soy milk, and animal feed [1]. National consumption rose from 1.56 million tons in 2015 to 2.97 million tons in 2019, while domestic production decreased from 964,183 tons to 424,190 tons during the same period, leading to greater dependence on imports [2]. This imbalance highlights the need for strategies that can improve both the productivity and quality of soybean crops. One such approach is the application of Plant Growth Regulators (PGRs), which can optimize plant growth and development, ultimately increasing yield [3].

Moringa leaves are a natural material with potential use as a PGR. Moringa leaf extract contains 662.17 ppm auxin, 66.50 ppm zeatin, and 417.88 ppm gibberellin [4]. The application of moringa as a PGR has been proven to increase phenolic content by up to 78% and improve the overall yield of moringa seedlings compared to the control [5]. The use of moringa extract on bean plants has also been shown to significantly enhance antioxidant activity and antioxidant enzymes [6]. In addition to moringa leaves, mung bean sprout extract is also a promising natural source of PGRs, containing 96.26 ppm cytokinin, 1.68 ppm auxin, and 39.94 ppm gibberellin [7]. Its application has been reported to increase phenolic content and antioxidant activity in cayenne pepper plants [8]. This extract has also been proven to enhance phenolic levels and antioxidant activity in sugarcane plants [9]. When the two extracts are combined, the auxin from moringa leaves and the cytokinin from sprouts complement each other in increasing endogenous PGR levels in plant cells. A combination of 100 g/L mung bean sprout extract and 100 g/L moringa leaf extract has been reported to have a positive effect on mangosteen growth [10].

Combinations of PGRs from plant extracts containing auxin and cytokinin have the potential to influence phenolic compound synthesis and enhance antioxidant activity [11]. Auxin plays a role in stimulating the formation of phenolic compounds, while cytokinin can activate enzymes involved in the phenylpropanoid biosynthesis pathway [12]. The interaction of these two hormones can increase total phenolic content and antioxidant activity, which protect plant cells from free-radical damage, maintain cellular function stability, slow down ageing, improve harvest quality, and strengthen the plant's natural defence system against diseases [13].

Based on the above, further research is needed to confirm that the combination of moringa leaf extract and mung bean sprout extract contains total phenolic content and antioxidant activity with the potential to be developed as a PGR for soybean plants. Therefore, the researcher is interested in examining the total phenolic content and antioxidant activity of this extract combination. It is hoped that the results of this study will contribute to the utilization of moringa leaves and sprouts, as well as support their potential development as a PGR for soybean crops.

Research Methods

Materials

Moringa leaves, bean sprouts, 96% ethanol, distilled water, gallic acid, 7.5% Na_2CO_3, Folin–Ciocalteu reagent, and DPPH were used in this study. All reagents were of analytical grade.

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Equipment

The main instruments included a blender, 100-mesh sieve, oven, vacuum filtration set, rotary evaporator, vortex mixer, micropipette, volumetric flasks, beakers, centrifuge tubes, analytical balance, and a UV–Vis spectrophotometer.

Extract Preparation

Fresh moringa leaves and bean sprouts (10 kg each) were cleaned and dried until the moisture content was <10% (±3 days), then oven-dried at 110°C for 20 minutes to achieve a constant weight. The dried materials were ground, sieved (100 mesh), and stored in airtight containers. Extraction was performed by maceration with 96% ethanol (1:5 w/v) for 72 hours, followed by vacuum filtration and evaporation at 40–60 °C to yield a concentrated extract. The extracts were stored in dark glass bottles.

Preparation of Extract Combinations

Six combinations of moringa leaf and bean sprout extracts were prepared in different ratios (Table 1). Each mixture was diluted with distilled water in a 1000 mL volumetric flask to reach the desired concentrations.

Table 1. Variations in the concentration of combined moringa leaf and bean sprout extracts

Variation	Moringa Leaves (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 Phenolic Content

A gallic acid calibration curve (10, 20, 30, 40, and 50 ppm) was used as the standard. 1 mL of extract (1000 ppm) was mixed with 2 mL of 10% Folin–Ciocalteu reagent and 2 mL of 7.5% Na_2CO_3, incubated for 15 min, and measured at 767.5 nm. The total phenolic content (mg GAE/g) was calculated using:

$$\text{Total Phenolic} = \frac{C \,.\, V \,.\, fp}{g}$$

Where C = concentration (mg/mL), V = sample volume (mL), fp = dilution factor, and g = sample mass (g).

Determination of Antioxidant Activity

The sample was dissolved in ethanol at graded concentrations of 50, 100, 150, 200, and 250 ppm. Then, 4 mL of the sample solution was mixed with 1 mL of a 40 ppm DPPH solution, vortexed for 1 minute, and left to stand in the dark for 30 minutes. The sample was measured at λ 517 nm using a UV–Vis spectrophotometer in triplicate. The absorbance values obtained were used to calculate the percentage inhibition (%I) using the following formula:

$$\%I = \frac{(\text{Absorbance of blank} - \text{Absorbance of sample})}{\text{Absorbance of blank}} \text{ x } 100\%$$

Subsequently, a linear curve between %I and sample concentration was constructed to obtain the linear regression equation (y = ax + b), which was then used to determine the IC_{50} value.

Data Analysis

All experiments were performed in triplicate, and the results are expressed as mean ± standard deviation (SD). Data were analyzed using analysis of variance (ANOVA) to assess the effects of different extract ratios. When significant differences ($p < 0.05$) were detected, Duncan's Multiple Range Test (DMRT) was applied for post-hoc comparisons.

Safety and Ethical Considerations

All procedures involving ethanol and DPPH were conducted in a well-ventilated laboratory, utilising appropriate personal protective equipment (PPE), including gloves, lab coats, and safety goggles. Waste materials were disposed of in accordance with institutional safety protocols.

Results and Discussion

This study was conducted in four stages: extract preparation, formulation of concentration variations, total phenolic analysis, and antioxidant activity analysis. The extraction of moringa leaves and sprouts produced a thick, dark extract. The dark color was caused by the increased concentration of active compounds such as phenolics and chlorophyll due to solvent reduction, as well as mild oxidation reactions during the heating process [14] [15]. The thick extracts of moringa leaves and mung bean sprouts were then combined in varying ratios, ranging from 100% moringa to 100% sprouts. The extracts showed a color change to brownish green in moringa-rich combinations and yellowish in sprout-rich combinations, reflecting differences in pigments and secondary metabolite profiles. These variations indicate possible differences in bioactive compound content, consistent with previous reports on plant extract mixtures used as natural biostimulants [16].

The determination of total phenolic content aims to measure the amount of phenolic compounds in the combination of moringa leaf and bean sprout extracts and to assess their potential as antioxidants. Phenolic compounds have antioxidant properties because their hydroxyl groups can donate hydrogen atoms to neutralize free radicals through electron transfer [17]. The analysis was carried out quantitatively using the Folin–Ciocalteu method with gallic acid as the standard. Determination of total phenolic content began with identifying the optimum wavelength for the gallic acid standard solution [18]. Subsequently, absorbance was measured at various concentrations using a wavelength of 767.5 nm. The measurement results were used to construct a calibration curve showing the effect of concentration on absorbance, from which a linear equation was obtained. The gallic acid calibration curve is shown in Figure 1.

Figure 1 presents the gallic acid standard curve, which shows a strong linear relationship ($y = 0.0119x + 0.0912$, $R^2 = 0.9894$) between concentration and absorbance. This high R^2 value indicates that 98.94% of the variation in absorbance is explained by the gallic acid concentration. The

curve is used to calculate total phenolic content in moringa leaf and bean sprout extracts by substituting sample absorbance into the equation, adjusting for sample weight and solvent volume, and expressing the result as Gallic Acid Equivalent (GAE).

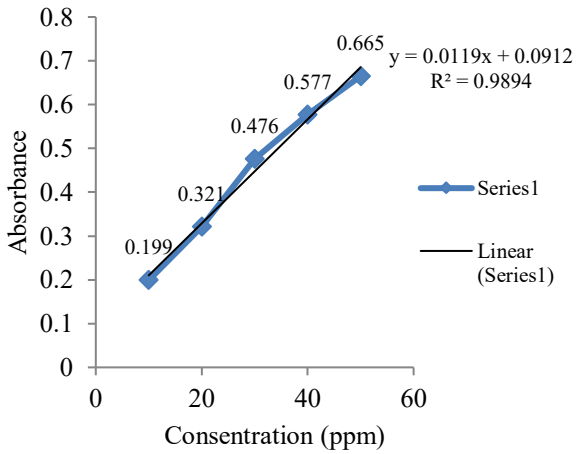

Figure 1. Gallic acid standard curve

Table 2. Total phenolic content of combined moringa leaf and bean sprout extracts

Sample	Total Phenolic (mg GAE/g)
A	6.98 ± 0.177[a]
B	6.47 ± 0.046[a]
C	8.02 ± 0.475[b]
D	8.35 ± 0.651[b]
E	22.95 ± 0.715[d]
F	12.94 ± 0.570[c]

Note: Values followed by the same letter in the column indicate no significant difference according to the 5% DMRT test.

Based on Table 2, the total phenolic content of the combined moringa leaf and mung bean sprout extracts varied significantly across the six formulations (A–F). Variations A and B, dominated by moringa extract, produced relatively low phenolic contents (6.98 and 6.47 mg GAE/g, respectively) with no significant difference between them ($p > 0.05$). Variations C and D, containing balanced ratios of moringa and mung bean sprout extracts, showed a moderate increase (8.02 and 8.35 mg GAE/g), suggesting a partial synergistic effect in phenolic accumulation. Variation F, which was sprout-rich, reached a higher phenolic concentration (12.94 mg GAE/g), indicating that mung bean sprouts contributed phenolic compounds that were more abundant or more efficiently extracted than those in moringa alone.

Interestingly, variation E exhibited the highest total phenolic content (22.95 mg GAE/g), surpassing all other combinations. This sharp increase indicates a synergistic interaction between growth hormones derived from moringa leaves, such as auxins, cytokinins, and phenolic precursors, and cytokinins from sprouts, which together may enhance both the biosynthesis and stabilization of phenolic compounds. Mung bean sprouts are known to be rich sources of phenolic compounds and antioxidants, consistent with the data in Table 2 showing a positive correlation between increasing sprout proportions and phenolic content as well as antioxidant activity [19]. Furthermore, evidence from multiple studies confirms the role of moringa leaf extract as a natural biostimulant, where foliar applications in quinoa and soybean significantly improved growth, yield, and antioxidant enzyme activity, thereby strengthening the practical prospects of this extract combination as an environmentally friendly natural plant growth regulator [20].

In plant physiology, phenolic compounds function as antioxidants as well as signalling molecules, protecting cells from oxidative stress while regulating growth and defence mechanisms [21]. This aligns with previous studies showing that PGRs derived from natural extracts with high phenolic and antioxidant content can act as effective biostimulants and growth modulators [22]. Research on soybean and other legumes has demonstrated that treatments rich in cytokinins and phenolics can stimulate leaf expansion, delay senescence, and enhance crop yield under stress conditions by neutralizing reactive oxygen species (ROS) and activating defence gene expression [23].

Biologically, the increased phenolic content results from complementary metabolic contributions, where moringa leaves provide phenolic precursors and hormone-like compounds, while mung bean sprouts supply abundant cytokinins that enhance the activity of enzymes in the phenylpropanoid pathway responsible for secondary metabolite production [24]. This synergy explains the peak observed in Variation E, where hormonal signalling and substrate availability are optimally balanced. The superior phenolic content in Variation E highlights its potential as a natural PGRs formulation for soybean cultivation.

Antioxidants in plants function to protect cells from free radicals generated by environmental stress. These compounds maintain the stability of membranes, enzymes, and molecules, thereby helping plants become more resistant to extreme environmental. Antioxidant activity analysis was carried out using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method and measured with a UV-Vis spectrophotometer. DPPH is a stable free radical characterized by a purple color due to the presence of a nitro group (NO_2). When a compound or extract containing antioxidants is added to the DPPH solution, the antioxidants donate a hydrogen atom or electron, reducing DPPH to its reduced form, DPPH-H, which is pale yellow in color [25]. Each sample was tested at five concentrations (50–250 ppm) with 4 mL, mixed with 1 mL of 40 ppm DPPH, and incubated in the dark for 30 minutes. Absorbance at 517 nm was measured in triplicate using a UV-Vis spectrophotometer, and the percentage inhibition was calculated to assess the antioxidant activity.

Table 3. Antioxidant Activity of Moringa Leaf and Sprout Extract Combinations

Sample	IC_{50} (ppm)
A	248.78 ± 3.664[a]
B	274.05 ± 4.820[b]
C	216.99 ± 5.784[c]
D	203.86 ± 2.330[d]
E	165.20 ± 1.180[e]
F	196.28 ± 2.110[f]

Note: Numbers with the same letter in the column indicate no significant difference based on the 5% DMRT test.

Based on Table 3, the 5% DMRT test results show significant differences among the extract combinations in their ability to scavenge free radicals, as indicated by the IC_{50} values. The lower the IC_{50} value, the stronger the antioxidant

activity. According to the classification, IC_{50} < 50 ppm is categorized as very strong, 50–100 ppm as strong, 100–250 ppm as moderate, and >250 ppm as weak [26].

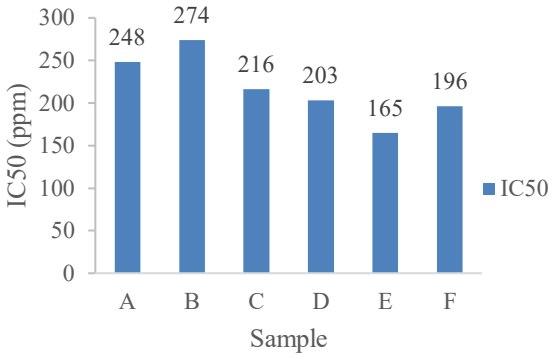

Figure 2. The antioxidant test results

Based on Figure 2, the most potent antioxidant activity, with the lowest IC_{50} value of 165 ppm, was found in variation E (a ratio of moringa leaves to bean sprouts of 2:8). This indicates that a higher proportion of bean sprouts in the mixture results in stronger antioxidant potential. Conversely, variation B had the highest IC_{50} value of 274.05 ppm, indicating the weakest antioxidant activity among the samples. On the other hand, variation A, which only contained moringa leaves, also showed a high IC_{50} value (248 ppm), possibly because the concentration of active compounds was too high to inhibit their optimal interaction with DPPH, a substance used in antioxidant testing. This result may be attributed to the high concentration of phenolic compounds and cytokinins in bean sprouts, which are believed to stimulate the production of antioxidant enzymes.

Extracts dominated by sprouts (cytokinin) have been shown to be superior in increasing phenolic compound levels and antioxidant activity compared to extracts containing more moringa (auxin), making this formulation highly suitable as a multifunctional natural PGRs. The addition of auxin and cytokinin hormones to plants can increase the concentration of endogenous PGRs in cells, which then stimulates increased production of secondary metabolites, including phenolic compounds and antioxidants [27]. PGRs have a significant effect on lentil plant growth as well as their phenolic content and antioxidant activity [28]. The combination of 1 ppm kinetin (cytokinin) and 1 ppm 2,4-D (auxin) has been shown to be effective in stimulating growth and increasing secondary metabolite content in Aceh patchouli callus of the Sidikalang variety [29].

The biological mechanism underlying this antioxidant activity involves phenolic compounds and cytokinins found in Moringa leaves and bean sprouts. Cytokinins are known to induce the biosynthesis of phenolic compounds by increasing the activity of the enzyme PAL. Meanwhile, auxin in Moringa leaves plays a greater role in cell elongation and vegetative growth, but its contribution to antioxidant formation is relatively lower compared to cytokinins [30]. This positive correlation aligns with the high phenolic content observed in the E variety, reinforcing the role of phenolic compounds in determining antioxidant activity. Antioxidants are essential for protecting plants from oxidative stress, increasing tolerance to environmental stress, and supporting growth and development [31].

This study, conducted under laboratory conditions, investigated the effects of PGRs derived from moringa leaves and bean sprouts on soybean plants, with a focus on total phenolic compounds and antioxidant activity. A major limitation is the lack of field trials to validate these results in real agricultural environments. Additionally, only a limited range of extract ratios was tested, which may not capture the full potential of these PGRs combinations. Future research should include field validation, broader testing of extract concentrations, and assessments of long-term impacts on soybean growth, yield, and stress resistance to better understand and optimize the use of these natural PGRs in sustainable crop production.

Conclusion

The combination of moringa leaf extract and mung bean sprouts showed strong potential as a natural plant growth regulator (PGR) for soybean cultivation. Variety E (2 g/L moringa and 8 g/L mung bean sprouts) produced the highest total phenolic content and the strongest antioxidant activity, indicating a positive correlation between phenolic content and free radical scavenging ability. These findings introduce a sustainable alternative to synthetic PGRs and provide a basis for improving the growth and resilience of soybean yields. Further field trials, scale-up, and testing on other crops are recommended to validate and expand this application.

Author's Contribution

Putri Nur Itsnaini: Collect data and compile the article; Mirwa Adiprahara Anggarani: Responsible person and article compiler

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