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Full Length Article

Extraction and Characterization of Antimicrobial Compounds from Different Soybean Varieties (*Glycine max*) under Varying Plant Shade Intensity

Deddy Wahyudin Purba[1,2*], Suswati[3] and Zulheri Noer[3]
[1]*Doctoral Program in Agricultural Sciences, Universitas Medan Area, Medan 20122, Indonesia*
[2]*Faculty of Agriculture, Universitas Asahan, Kisaran 21212, Indonesia*
[3]*Faculty of Agriculture, Universitas Medan Area, Medan 20122, Indonesia*
[*]For correspondence: deddywahyudin086@gmail.com
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Abstract

Cultivating soybeans in an environment with appropriate lighting is crucial for the healthy development of the soybean industry as it significantly influences the content of secondary metabolites. This study aims to analysis the impact of different shading treatments on soybean leaf metabolites. This study was conducted from August to November 2023. The study involved extracting metabolic/isoflavone substances from soybean leaves through maceration and assessing variations in secondary metabolic substances under different shading intensities, namely no shading (S_0), shading on non-producing plants (S_1), and shading on producing plants (S_2). The soybean varieties used were local varieties in Indonesia (*Glycine max* L.), consisting of Anjasmoro (V_1), Mutiara 1 (V_2), Denasa 1 (V_3), Denasa 2 (V_4), Dena 1 (V_5) and Dena 2 (V_6). The bioactivity test method against *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) bacteria involved paper disc diffusion, observing the diameter of the inhibition zones produced. Shading treatments impact leaf area, root length, and dry root weight morphology, with Anjasmoro (V_1) emerging as the superior variety, exhibiting strong growth irrespective of lighting conditions. The Gas Chromatography-Mass Spectrometry (GC-MS) analysis indicates the existence of the most abundant phytochemical compounds, including n-hexadecanoic acid and undecanoic acid. The Fourier Transform Infrared (FTIR) analysis identified that the leaf extract contains strong out-of-plane bending vibrations of C-H for substituted benzene rings, indicating the existence of phenols and flavonoids in the soybean leaf extract. In the antimicrobial test, the inhibition zone diameter generated by the soybean leaf extract exhibited the greatest inhibitory power in the chloroform fraction against *E. coli*, measuring 13.50 mm from the S_1V_5 sample, and against *S. aureus*, measuring 10.43 mm from the S_1V_2 sample. Therefore, shading on non-producing plants (S_1) is the most effective treatment for the production of antibacterial compounds. In addition, statistical analysis of the antimicrobial index against *E. coli* indicates a significant difference among shading treatments ($P < 0.05$).

Keywords: Antibacterial properties; Secondary metabolites; Shading effects; Soybean leaf compounds
Abbreviation: S_0V_1, No shading with Anjasmoro variety; S_0V_2, No shading with Mutiara 1 variety; S_0V_3, No shading with Denasa 1 variety; S_0V_4, No shading with Denasa 2 variety; S_0V_5, No shading with Dena 1 variety; S_0V_6, No shading with Dena 2 variety; S_1V_1, Shading on non-producing plants with Anjasmoro variety; S_1V_2, Shading on non-producing plants with Mutiara 1 variety; S_1V_3, Shading on non-producing plants with Denasa 1 variety; S_1V_4, Shading on non-producing plants with Denasa 2 variety; S_1V_5, Shading on non-producing plants with Dena 1 variety; S_1V_6, Shading on non-producing plants with Dena 2 variety; S_2V_1, Shading on producing plants with Anjasmoro variety; S_2V_2, Shading on producing plants with Mutiara 1 variety; S_2V_3, Shading on producing plants with Denasa 1 variety; S_2V_4, Shading on producing plants with Denasa 2 variety; S_2V_5, Shading on producing plants with Dena 1 variety; S_2V_6, Shading on producing plants with Dena 2 variety

Introduction

Light is a crucial environmental factor that holds substantial sway over the growth and development of plants. It stimulates plants to undergo diverse physiological, morphogenetic, and metabolic adaptations to cope with shifting light conditions (Liu *et al.* 2018a; Liu *et al.* 2018b; Huang *et al.* 2021). Consequently, environmental factors closely tied to plant growth and secondary metabolism often include lighting (Radušienė *et al.* 2012). Plants of the same species require varying light conditions throughout their growth stages, prompting farmers to utilize diverse technical methods to

adjust intensity with the aim of meeting specific growth needs (Huang *et al.* 2016). Environmental factors, e.g., light, play a key role in influencing plant growth, development, and secondary metabolic processes (Janská *et al.* 2010; Zoratti *et al.* 2014), regulating growth rates, organ development, and plant functions and behaviors (Akari *et al.* 2014).

Changes in light conditions due to shading significantly affect photosynthesis, growth patterns, morphology, anatomical structures, various aspects of cellular physiology and biochemistry, as well as flowering timing and overall plant productivity (Dai *et al.* 2009; Deng *et al.* 2012). Under conditions of light deficiency, plant growth is disrupted due to a lack of ATP and the energy supply needed for photosynthesis (Niinemets 2015; Valladares and Niinemets 2018). Plant responses to light deficiency or shading are related to physiological, biochemical, anatomical, and leaf morphology processes (Valladares and Niinemets 2018). Shading is commonly used to manage light intensity and can decrease the active radiation accessible for plant photosynthesis. This decrease in light availability can consequently influence both the photosynthesis process and photomorphogenesis in plants (Dennis *et al.* 2020; Liu *et al.* 2020; Xu *et al.* 2020).

Plants generally contain active compounds as secondary metabolites. Secondary metabolite compounds are chemicals that typically possess bioactivity and function as protective agents for plants against pests, diseases, either for the plant itself or its environment (Maya *et al.* 2015). Soybean is one of the food commodities rich in plant-based protein that contains bioactive compounds (Isanga and Zhang 2008). Currently, the growth of industries using soybeans as a raw material continues to expand, leading to an increased demand for soybeans, coupled with the public's awareness of nutritional adequacy (Zainuddin *et al.* 2022).

There are several challenges in soybean cultivation under tree canopies, including competition for nutrients, water, and light. Low light intensity is a major obstacle in the development of soybeans as an understory crop beneath tree canopies, especially due to shading from the main plants. Spatiotemporal shading has been found to influence the accumulation of anthocyanins, proanthocyanidins, and sucrose in black soybean seeds (Dennis *et al.* 2020). In this study, various shading treatments, including no shading and shading under coconut trees that have not produced, were applied on various soybean varieties known for their resistance and tolerance. The objective is to gain a deep understanding of the influence of various levels of shading intensity on soybean leaf metabolites to determine an appropriate shading level beneficial for soybean growth and metabolite accumulation. To achieve this goal, we identified a range of metabolite components in soybean leaves, classified these metabolic substances, and determined the trends of change in crucial metabolic substances under different levels of shading intensity. Therefore, this study aims to analyze the impact of different shading treatments on the secondary metabolite profile of soybean plants.

Materials and Methods

Experimental details and treatments

Experimental material: The materials employed for this research included soybean seeds (*Glycine max* L.) of the following varieties: Anjasmoro (V_1), Mutiara 1 (V_2), Denasa 1 (V_3), Denasa 2 (V_4), Dena 1 (V_5), and Dena 2 (V_6). Additionally, a fertilizer mixture consisting of 25% manure (*Setia Tani*, Tangerang, Indonesia) and 75% water hyacinth compost (obtained from the local community in Medan Labuhan, Indonesia) was used. The materials employed for cultivating the soybeans in small polybags included soil, rice husks, and a small quantity of charcoal obtained from *PT Bukit Asam Tbk*, Indonesia. Ethanol 70% was also utilized as a solvent in the extraction process. In addition, for antibacterial testing, the materials included Mueller Hinton Agar (MHA), methanol solvent, chloroform, sterile 9% NaCl, paper disks (diameter: 6 mm), distilled water, and erythromycin. The bacterial strains prepared were *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*).

The instruments used in this study included the following: Gas Chromatography-Mass Spectrometry (GC-MS; Shimadzu QP 2010), Fourier Transform Infrared (FTIR; Agilent Cary 630), a rotary evaporator (IKA, RV 3 V), an oven, autoclave, hot plate with magnetic stirrer, laminar airflow (LAF) hood, flame source (for sterilization), petri dishes, vortex mixer, analytical balance (Mettler Toledo), calipers, and mortar.

Treatments: This study was conducted from August to November 2023 in the coconut plantation area of Medan City, while GC-MS analysis was performed at the Integrated Research Laboratory of Universitas Sumatera Utara, and FTIR analysis and antibacterial analysis were conducted at the Plant Protection Laboratory at Universitas Medan Area. The soybean planting method is based on the research conducted by Widiastuti and Latifah (2016). The treatments consisted of shading levels (S) as the primary treatment, with three treatment levels, namely no shading (S_0), shading on non-producing plants (S_1), and shading on producing plants (S_2). Soybean seeds of the V_1, V_2, V_3 V_4, V_5 and V_6 varieties were used for the experiment. Eighteen treatment combinations (3×6) with two replications was used, so the total was 36 research plots × 9 plants per plot = 324 plants, of which 2 were destructive samples. The size of each plot was 1.5 × 2 m. The plants were cultivated using artificial planting media in small polybags, supplemented with fertilizers. Watering was carried out once a day, with a volume ranging from 440 to 550 mL per plant (Makarim *et al.* 2017). Soybean pods form optimally within a temperature range of 26.6–32.0°C (Widiastuti and Latifah 2016). Harvesting was done after 90 days when the soybean plants are ready. Leaf samples from the soybean plants were then prepared for isoflavone testing.

Morphology measurements

The method for assessing soybean morphology combines research methodologies outlined by He *et al.* (2023) and Vaccaro *et al.* (2022). Morphology measurements were taken when the soybeans were ready for harvest, specifically at 90 days. Parameters examined included leaf area, root length, and the dry weight of the roots. Leaf area was determined through the analysis of digitized images using ImageJ software (NIH, Bethesda, MD, USA), while lengths were manually measured with a ruler. Dry weights were obtained using an analytical balance following a drying process at approximately 90°C until a consistent weight was achieved.

Metabolite extraction for GC-MS analysis

A total of 100 g of young leaf samples were crushed, macerated in 250 mL of 70% ethanol for 24 h, filtered and the obtained filtrate was collected. The choice of young leaves was made because secondary metabolites found in young leaves are more advantageous, thus increasing the probability of achieving elevated levels of is flavonoids (Liu *et al.* 2018). The residue was combined with 100 mL of 70% ethanol and left to macerate for 24 h (Kudou *et al.* 1991). After this period, it was filtered, and the filtrate was collected. The remaining residue underwent a second filtration following its mixing with another 100 mL of 70% ethanol. The filtrate obtained from this maceration process was then concentrated using a rotary evaporator, yielding a concentrated extract. The concentrated extract was heated for 30 mins at 50°C to produce an ethanol extract. The results were obtained as yellow filtrate for 0-day soybean leaf fermentation. The obtained filtrate was then evaporated at 50°C in a rotary evaporator until a concentrated extract was acquired or nearly all ethanol was evaporated. This extract was then placed in an oven at 40°C to evaporate any remaining solvent before being weighed to determine the extraction yield.

GC-MS analysis

GC-MS testing was employed using a RTx5MS column to identify compound contents in the extract (Wang *et al.* 2018). The column, with a length of 30 m, had injector and detector temperatures set at 250°C, and operated within a temperature range of 50–300°C. The temperature increased gradually from 50 to 120°C at a rate of 4°C/min, held for 1 min, followed by a faster increase from 120 to 300°C at a rate of 6°C/min, held for 5 min, with a total retention time (Rt) of 60 min. Helium was used as the carrier gas. Compound identification utilized Wiley/NIST Library software (Fernandes and Maharani 2019).

Initially, the sample was dissolved in ethanol solvent, followed by injecting 1 µL of this sample into the GC instrument inlet. The inlet conveys the sample to the column through the helium mobile phase. The stationary phase of the RTx-5MS column, composed of 5% diphenyl and 95% dimethyl polysiloxane, facilitates the separation process.

The concentration data from this analysis were analyzed using the Kruskal-Wallis test to address this hypothesis:

H1: There is a significant difference in the concentration of metabolite compound extracts among the three treatment groups.

FTIR analysis

FTIR analysis was carried out on soybean leaf extract with an objective of identifying functional groups in the compounds.

Antibacterial activity test

The antibacterial activity was evaluated by examining the growth inhibition of bacteria, specifically *S. aureus* and *E. coli*, using the agar disk diffusion method (Kirby-Bauer) (Sun *et al.* 2019). For the medium, 6.8 g of MHA was used, which was first autoclaved and then heated on a hot plate while stirring with a magnetic stirrer (Choi *et al.* 2019). Pure bacterial cultures were revitalized on the solid medium by streaking *E. coli* and *S. aureus*-containing needles in a zigzag pattern near a flame. Two bacterial cultures were prepared for each bacterium. This process was conducted under sterile conditions in a Laminar Airflow Hood (LAF) and incubated at 37°C for 24 h (Teow *et al.* 2016).

Additionally, a statistical analysis was conducted on the antimicrobial index data to address these hypotheses: H2: There is a significant difference in the antibacterial compound extract abilities of the three treatments against *E. coli*. H3: There is a significant difference in the antibacterial compound extract abilities of the three treatments against *S. aureus*.

The hypothesis testing utilized the Kruskal-Wallis test, which is a non-parametric method for comparing three or more independent groups. Prior to conducting the Kruskal-Wallis test, two prerequisite analyses were performed: normality and homogeneity tests. These tests were conducted to assess whether the data meets the assumptions required for parametric statistical tests. The significance value used for all tests was set at 0.05.

Results

Morphology

The results for soybean plant morphology on day 90 are presented in Table 1, 2 and 3 for leaf area (cm^2), root length (cm) and dry weight of roots (g), respectively. Analysis reveals that across all shading treatments, variety V_1 (Anjasmoro) consistently demonstrates superior performance in terms of leaf area, root length, and dry

weight of roots. Notably, V_1 consistently exhibits the highest leaf area and root dry weight compared to other varieties. Additionally, while V_4 (Denasa 2) shows the longest root length under shading conditions (S_1 and S_2), variety V_5 (Dena 1) displays the longest root length in the absence of shading (S_0). These findings suggest that Anjasmoro may be the most resilient variety, showing robust growth regardless of shading conditions.

Table 1: Soybean leaf area on day 90 (cm^2)

Varieties	Shading		
	S_0	S_1	S_2
V1: Anjasmoro	2230.4	2471.5	2521.2
V2: Mutiara 1	2179.6	2278.6	2368.5
V3: Denasa 1	2034.6	2380.2	2520.1
V4: Denasa 2	2178.9	2231.4	2432.6
V5: Dena 1	1989.5	2068.5	2231.6
V6: Dena 2	1876.3	2138.6	2248.6

Table 2: Soybean root length on day 90 (cm)

Varieties	Shading		
	S_0	S_1	S_2
V1: Anjasmoro	53.90	42.50	23.50
V2: Mutiara 1	48.70	33.75	26.80
V3: Denasa 1	41.92	30.45	22.50
V4: Denasa 2	61.77	46.88	31.33
V5: Dena 1	68.80	48.70	34.72
V6: Dena 2	56.35	32.30	26.33

Table 3: Dry weight of soybean roots on day 90 (g)

Varieties	Shading		
	S_0	S_1	S_2
V1: Anjasmoro	11.86	10.72	8.76
V2: Mutiara 1	9.76	9.12	7.65
V3: Denasa 1	8.33	7.98	7.32
V4: Denasa 2	10.23	9.23	6.22
V5: Dena 1	6.72	7.42	6.06
V6: Dena 2	7.83	7.21	5.32

GC-MS analysis results

The chemical compounds resulting from the GC-MS analysis of extracts from various varieties of soybean leaves and different shading variations can be observed in Table 4. It is evident that in the no-shading variations (S_0), variety V_1 exhibits several peaks corresponding to alkaloid compounds like thymol, with a concentration of 7%. Conversely, other varieties (V_2–V_6) predominantly produce saturated acids such as n-hexadecanoic acid, ranging from 20 to 45%. Under shading on non-producing plants (S_1) conditions, all varieties display peaks corresponding to antioxidant compounds from the flavonoid group, particularly hydroxy-1. Variety V_2 stands out with the highest concentration of flavonoids, reaching up to 35.2%. However, shading on producing plants (S_2) leads to the presence of non-flavonoid compounds (*e.g.*, 11-bromoundecanoic acid and azelaoyl chloride) across all varieties (V_1–V_6). Their presence slightly interferes with the presence of hydroxy-1 compounds, evidenced by their highest concentration being around 26.1%.

Statistical analysis using data on metabolite concentrations in soybean leaves was conducted to address H1, including tests for normality and homogeneity. The normality test revealed that the data are non-normally distributed but homogenous. This led to the Kruskal-Wallis test as shown in Table 5, where the Asym. Sig. value is 0.085, exceeding the significant threshold of 0.05. Therefore, H1 is rejected, indicating no difference between treatments in metabolite concentrations.

FTIR results

The FTIR spectra of soybean extracts with three shading variations (no shade, shade on non-producing plants, and shade on producing plants) and several soybean varieties can be observed in Fig. 1, 2 and 3.

Based on Fig. 1, 2 and 3, the FTIR spectrum profiles exhibit distinctive patterns, and they share similar spectrum patterns. Differences are noticeable in the absorbance values and wavelength intensity readings of the FTIR spectra. This indicates that the compounds present in the treatments are not significantly different. Interpretation of the FTIR spectrum reveals several functional groups within the soybean extract. At peaks of 3317–3628 cm^{-1}, the presence of N-H groups indicates the presence of amine compounds. Within the wavenumber range of 2532–3130 cm^{-1}, the

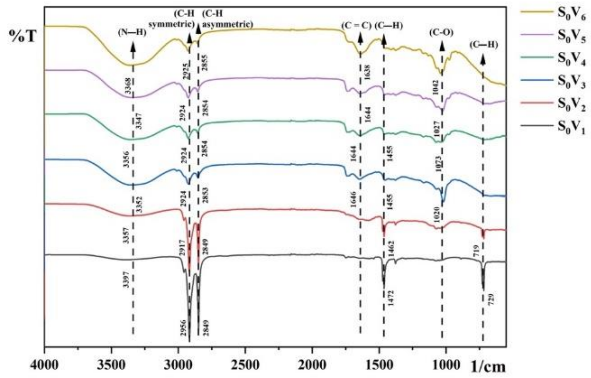

Fig. 1: FTIR spectra of soybean extracts extracted from the no shade treatment

presence of -OH groups suggests bonds with acidic compounds. The stretching of single bonds in the C-H group occurs at 2855–2963 cm^{-1}, indicating the presence of alkane compounds. Additionally, C-H groups appear at 3010–3087 cm^{-1}, indicating the presence of alkene compounds. Moreover, the FTIR spectrum shows stretching of double bonds, specifically the C = O group of ketone compounds at the wavenumber 1734 cm^{-1}, and the presence of C = C aromatic bonds at the wavenumber range of 1591–1604 cm^{-1}. These interpretations are supported by the results of phytochemical screening, where the N-H or amine group signifies the presence of alkaloid metabolites. The alcohol

functional group indicates the presence of steroid and tannin compound metabolites. Additionally, the C=C aromatic group indicates the presence of flavonoid and tannin compound metabolites. In conclusion, the shading treatments do not yield distinct FTIR spectra for each variety.

Antibacterial activity test results

Antibacterial activity was assessed by culturing *S. aureus* and *E. coli* bacteria on MHA media in petri dishes. Filter paper discs, measuring 6 mm in diameter, were treated with leaf extracts from various soybean varieties (*Glycine max* L.) with 3 repetitions. The negative control used distilled water. The positive control in the antibacterial test used the antibiotic erythromycin. The antibacterial activity of extracted compounds from various soybean varieties under varying shade treatments against *S. aureus* and *E. coli* can be observed by the formation of inhibition zones after incubating the petri dishes for 24 h at 37°C. The inhibition zones formed in the antibacterial test for soybean leaves of various varieties without shade can be seen in Fig. 4 and 5 (*E. coli* and *S. aureus*).

Table 6 shows information about the diameter of the inhibition zones of soybean leaf extracts against *E. coli* and *S. aureus* bacteria. It is observed that the inhibition zone diameter produced by the leaf extracts from various soybean varieties using the paper disc method against *E. coli* is larger.

The statistical analysis on normality and homogeneity tests of the antimicrobial index for *E. coli* and *S. aureus* was conducted prior to the Kruskal-Wallis test. The normality test for both datasets shows normal distribution, except for S. aureus under treatment S_2. The homogeneity test for all datasets indicates homogeneous variance ($P > 0.05$). Based on these results, the decision to address the three hypotheses involves employing the Kruskal-Wallis test. The test outcomes for each dataset are presented in Table 7 and 8.

Based on the Asymp. Sig. data from the Kruskal-Wallis tests in Table 7 and 8, only the Kruskal-Wallis test data for the antimicrobial index against *E. coli* has a value below the significance level of 0.05. The acceptance criteria for the hypotheses are met, specifically, if the significance value obtained from the Kruskal-Wallis test is < 0.05. Therefore, only H2 is accepted, while H3 is rejected. This indicates a significant difference in the antibacterial compounds extracted using the three different treatments, with the optimal treatment being S_2, involving shading in soybean varieties on non-producing plants.

Discussion

The research results indicate that expanding soybean cultivation areas under shading on a large scale is crucial for the development of healthy soybeans. Varied shading intensities resulted in alterations in the morphology and bioaccumulation of soybean plants. Across all soybean

Table 7: Results of the Kruskal-Wallis test for the antimicrobial index data of the three treatments against *E. coli*

Test Statistics [a,b]	
	Antimicrobial Index (*E. coli*)
Kruskal-Wallis H	8.588
DF	2
Asymp. Sig.	0.014

[a] Kruskal Wallis test; [b] Grouping variable: treatment

Table 8: Results of the Kruskal-Wallis test for the antimicrobial index data of the three treatments against *S. aureus*

Test statistics[a,b]	
	Antimicrobial Index (*S. aureus*)
Kruskal-Wallis H	5.746
DF	2
Asymp. Sig.	0.057

[a] Kruskal Wallis test; [b] Grouping variable: treatment

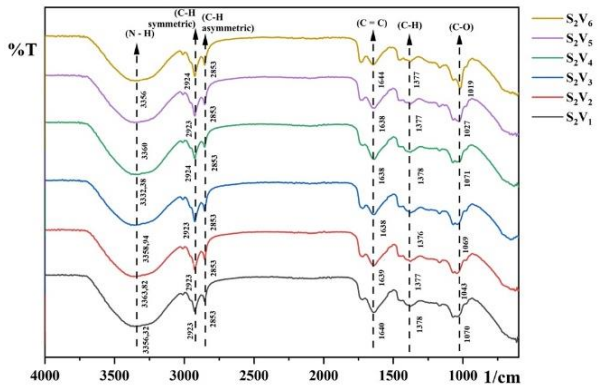

Fig. 3: FTIR spectra of soybean extracts extracted from the shade on producing plants treatment

varieties (V_1–V_6) subjected to shading treatments S_0 and S_1, there was a notable reduction in leaf area compared to those treated with S_2. Notably, V_1 exhibited the largest leaf area, measuring 2521.2 cm². Diminished light intensity can decelerate both photosynthesis and the breakdown of trehalose 6-phosphate (Tre6P), which serves as a growth stimulant in plants. As a result, plants might produce larger leaves to optimize light absorption (Göbel and Fichtner 2023). For root length, we observed varying outcomes across different shading intensities. In treatment S_0, variety V_5 exhibited the longest root, measuring 68.80 cm in length. Meanwhile, Variety V_4 displayed the longest root length under shading conditions (S_1 and S_2), with lengths of 41.88 cm and 31.33 cm, respectively. Moreover, V_1 emerges as the top-performing variety in terms of dry root weight (g) across all conditions of S_0, S_1 and S_2. The dry root weight of V_1 reaches 11.86 g. These results from all tests indicate that V_1 (Anjasmoro variety) exhibits vigorous growth irrespective of shading conditions. The growth of plants, encompassing both leaf and root development, can be constrained by competition for carbon resources (Vaccaro *et al.* 2022). Achieving a balance between the utilization and storage of fixed carbon in leaves becomes crucial. When an excess of

Table 5: Results of the Kruskal-Wallis test for the concentration of metabolite compound extracts among the three treatments

Test Statistics [a,b]	
	Metabolite Compound Percentage
Kruskal-Wallis H	4.938
DF	2
Asymp. Sig.	0.085

[a] Kruskal Wallis test; [b] Grouping variable: treatment

Table 6: Inhibition zone diameter and antimicrobial index of antibacterial activity test for various varieties of soybean leaves

Sample Code	Inhibition Zone Diameter (mm)		Antimicrobial Index	
	S. aureus	*E. coli*	*S. aureus*	*E. coli*
S_0V_1	8.36	10.00	0.40	0.66
S_0V_2	8.40	9.36	0.40	0.56
S_0V_3	9.00	10.70	0.50	0.78
S_0V_4	9.53	10.20	0.59	0.70
S_0V_5	9.03	11.97	0.51	0.99
S_0V_6	8.90	9.87	0.48	0.64
S_1V_1	10.03	11.57	0.67	0.93
S_1V_2	10.43	11.70	0.74	0.95
S_1V_3	8.70	11.77	0.45	0.96
S_1V_4	9.60	11.23	0.60	0.87
S_1V_5	10.17	13.50	0.70	1.25
S_1V_6	9.90	11.20	0.65	0.87
S_2V_1	8.30	10.07	0.38	0.68
S_2V_2	8.57	11.00	0.43	0.83
S_2V_3	9.97	10.01	0.66	0.68
S_2V_4	8.60	9.80	0.43	0.63
S_2V_5	9.97	9.97	0.66	0.66
S_2V_6	8.73	10.10	0.45	0.68

Note: The diameter of the paper discs used is 6 mm

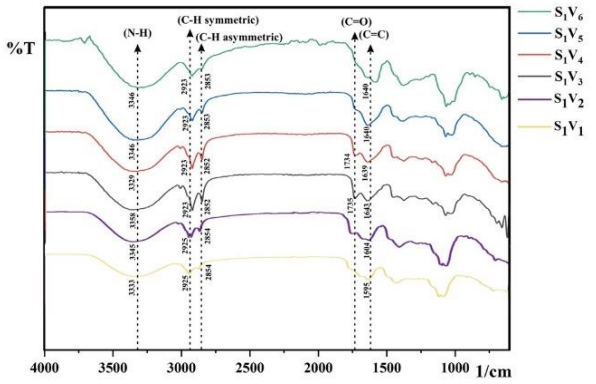

Fig. 2: FTIR spectra of soybean extracts extracted from the shade on non-producing plants treatment

newly fixed carbon is allocated to the synthesis of sucrose, it does not lead to a significant growth increase (He *et al.* 2023).

Research findings have demonstrated that shading significantly influences leaf gas exchange, leaf pigments, and the secondary metabolites in plants (Yusof *et al.* 2021). Based on the results of GC-MS analysis, extracts from soybean leaves under no shading (S_0) conditions in various varieties (V_1–V_6) contain alkaloids. In addition, the results show that thymol compound appears at the second peak, with a retention time of 7.559 mins and a concentration of

Table 4: GC-MS analysis results of soybean leaf extracts

Sample Code	Retention Time (minutes)	Compounds	Concentration (%)
S_0V_1	1.39	Hydrazine	1.810
	7.55	Thymol	7.016
	18.04	Nonanedioic acid, monemethyl	6.199
	18.69	n-Hexadecanoic acid	20.331
	20.49	Undecanoic acid, hydroxy-, 1	26.107
	20.77	Octadedcanoic acid	3.23
	20.85	Tetradecanoic acid	2.20
	20.98	11-Hexadecen-1-ol, (z)	6.046
	21.11	Cyclopentadecanol	1.779
	21.25	Cis-9-Tetradecan-1-ol	2.356
	24.36	Hexadecanoic acid, 2, 3 – dihyd	2.750
S_0V_2	17.95	Undecanoic acid, hydroxy-, 1	2.687
	18.42	n-Hexadecanoic acid	31.768
	20.18	Undecanoic acid, hydroxy-, 1	27.809
	20.35	Octadecanoic acid	19.233
	20.86	11-Hexadecen-1-ol, (z)-	2.307
S_0V_3	17.92	Azelaoyl chloride	5.719
	18.26	n-Hexadecanoic acid	43.605
S_0V_4	1.42	Hydrazine	84.319
	18.34	n-Hexadecanoic acid	8.083
	20.17	Undecanoic acid, hydroxy-, 1	2.200
S_0V_5	1.42	Hydrazine	79.873
	18.32	n-Hexadecanoic acid	12.548
	20.18	Undecanoic acid, hydroxy-, 1	2.583
S_0V_6	18.15	n-Hexadecanoic acid	4.820
	19.96	Undecanoic acid, hydroxy-, 1	1.430
S_1V_1	1.42	Acetic acid, hydroxy-	43.632
	17.93	Azelaoyl chloride	6.852
	18.26	n-Hexadecanoic acid	34.272
	20.08	Undecanoic acid, hydroxy-, 1	11.13
S_1V_2	1.42	1, 2-Ethanediol	4.190
	18.46	n-Hexadecanoic acid	36.858
	20.25	Undecanoic acid, hydroxy-, 1	35.206
	20.42	Octadecanoic acid	15.904
S_1V_3	1.42	Hydrazine	94.866
	18.16	n-Hexadecanoic acid	5.134
S_1V_4	20.25	Undecanoic acid, hydroxy-, 1	33.242
	18.47	n-Hexadecanoic acid	38.823
	20.43	Octadecanoic acid	14.961
S_1V_5	1.40	Hydrazine	79.304
	17.91	Azelaoyl chloride	5.895
	18.14	Tridecanoic acid	1.341
	18.30	n-Hexadecanoic acid	5.076
	19.59	3-Cyclohexene-1-acetaldehyde	1.894
	20.16	Udecenoic acid, hydroxy-,1	4.302
S_1V_6	1.40	Silane	72.881
	17.92	Azelaoyl chloride	8.500
	18.29	n-Hexadecanoic acid	6.910
	20.17	Udecenoic acid, hydroxy-,1	7.718
S_2V_1	1.43	Acetic acid, hydroxy	2.272
	6.23	3-Cyclohexen-1-ol, 4-methyl	3.207
	6.77	1, 6-octadien-3-ol, 3, 7-dimet	3.016
	9.22	Thymol	20.321
	17.91	Undecanoic acid, hydroxy-, 1	3.298
	18.25	n-Hexadecanoic acid	12.371
S_2V_2	1.402	Carbonic dihydrazide	11.738
	17.92	Azelaoyl chloride	7.215
	18.30	n-Hexadecanoic acid	36.346
	20.07	Undecanoic acid, hydroxy-, 1	21.912
	20.24	11-Bromoundecanoic acid	17.952
S_2V_3	1.411	Carbonic dihydrazide	26.265
	17.92	Undecanoic acid, hydroxy-, 1	8.315
	18.27	n-Hexadecanoic acid	31.564
S_2V_4	1.40	Carbonic dihydrazide	20.241
	17.93	Undecanoic acid, hydroxy-, 1	9.06
	18.28	n-Hexadecanoic acid	36.084
	20.07	Undecanoic acid, hydroxy-, 1	19.71
	20.22	11-Bromoundecanoic acid	11.514
S_2V_5	1.42	Acetic acid, hydroxy	83.052
	17.93	Azelaoyl chloride	16.948
S_2V_6	1.42	1, 2-Ethanediol	89.167
	17.93	Azelaoyl chloride	7.875
	19.69	Undecanal	2.151

7.016%. The phytochemical group present in soybean leaf extract from shading on non-producing plants (S_1) in all varieties (V_1–V_6) is flavonoids, with the highest concentration of 35.2% observed in variety V_2. Flavonoids are a group of hydroxy phenols with high antioxidant activity, exhibiting various bioactivities, including antibacterial, anticancer, anti-inflammatory, immune system enhancement (Tungmunnithum *et al.* 2018), and anti-diabetic properties (Sarian *et al.* 2017).

Moreover, GC-MS testing results indicate the presence of n-hexadecanoic acid, which is a saturated fatty acid (Eastwood 2003), at the eighth peak, with a retention time of 18.693 mins and a concentration of 20.331%. Furthermore, the analysis reveals the presence of undecanoic acid, hydroxy-, 1 at the seventeenth peak, with a retention time of 20.731 mins and a concentration of 26.107%. However, shading on producing plants (S_2) leads to the presence of non-flavonoid or organic halide compounds, such as 11-bromoundecanoic acid and azelaoyl chloride, in all varieties (V_1–V_6). Their presence causes a slight disruption in the presence of hydroxy-1 compounds, as indicated by their highest concentration reaching around 26.1% in variety V_2. Moreover, treatments involving shading on non-producing plants (S_1) and shading on producing plants (S_2) result in the production of flavonoid and phenol compounds with antioxidant properties. In conclusion, the most efficient treatment for generating antioxidant compounds is shading on non-producing plants (S_1).The FTIR analysis results identify that the soybean leaf extract contains strong out-of-plane bending vibrations of C-H for substituted benzene rings, indicating the presence of phenols and flavonoids in soybean leaf extract (Corcoran *et al.* 2022). The identification of benzenoid compounds through FTIR spectroscopy supports the findings from phytochemical examination, detecting the presence of phenols and flavonoids.

The antibacterial test results showed that the positive control, represented by the antibiotic erythromycin, exhibited antibacterial activity against both *S. aureus* and *E. coli*, while the negative control, consisting of distilled water, showed no antibacterial activity. The extract demonstrated antibacterial activity, as indicated by the formation of clear zones around the paper discs after incubation for 24 h at 37°C (Daneshzadeh *et al.* 2019).

The largest inhibitory zone diameter produced by leaf extracts from various soybean varieties without shading treatment (S_0) against *S. aureus*, was 9.53 mm, and against *E. coli*, it was 11.97 mm. The smallest effective concentration inhibiting bacterial growth was observed in the chloroform fraction against *S. aureus* (8.36 mm) and against *E. coli* (9.36 mm). The inhibitory zone diameters obtained from each fraction against *S. aureus* indicate that the leaf extract from various soybean varieties without shading treatment (S_0) has weak antibacterial activity, while it exhibits strong antibacterial activity against *E. coli*. The largest inhibitory zone diameter produced by leaf extracts from various soybean varieties under shading treatment against *S. aureus*, was 10.43 mm, and against *E. coli*, it was 13.5 mm. The smallest effective concentration inhibiting bacterial growth was observed in the chloroform fraction against *S. aureus* (8.7 mm) and against *E. coli* (11.20 mm). The inhibitory zone diameters against *S. aureus* indicate that the leaf extract from various soybean varieties under shading treatment has weak antibacterial activity, while it exhibits strong antibacterial activity against *E. coli*.

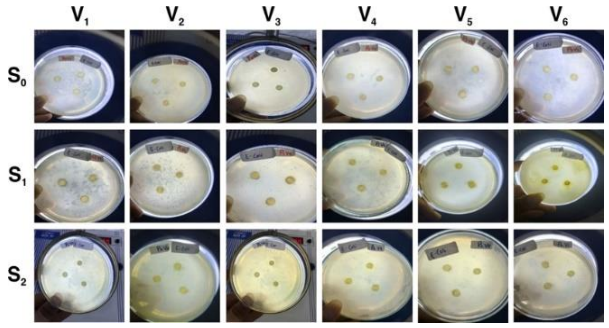

Fig. 4: Inhibition zones in the antibacterial test against *E. coli* with various soybean extracts and shading treatments

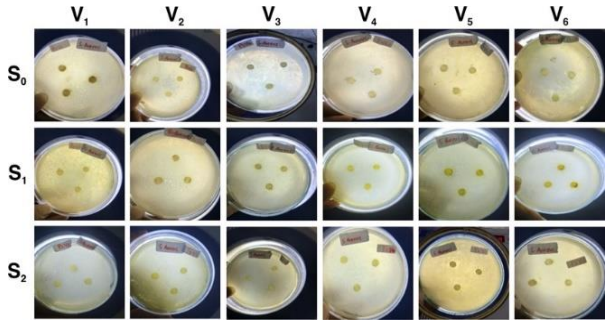

Fig. 5: Inhibition zones in the antibacterial test against *S. aureus* with various soybean extracts and shading treatments

The largest inhibitory zone diameter produced by leaf extracts from various soybean varieties under shading treatment against *S. aureus*, was 9.97 mm, and against *E. coli*, it was 10.07 mm. The smallest effective concentration inhibiting bacterial growth was against *S. aureus* (8.3 mm) and against *E. coli* (9.8 mm). The inhibitory zone diameters against *S. aureus* and *E. coli* indicate that the leaf extract from various soybean varieties under shading treatment has weak antibacterial activity. According to Souza *et al.* (2020), antibacterial activity < 5 mm is considered weak, 5–10 mm is moderate, 11–20 mm is strong, and > 20 mm is very strong. As a result, shading on non-producing plants (S_1) emerges as the most efficient treatment, with the Mutiara 1 variety (V_2) proving to be superior against *S. aureus*. Meanwhile, the Dena 1 variety (V_5) exhibits strong antibacterial activity against *E. coli*. The ability of soybean leaf extract to inhibit bacteria is attributed to the presence of secondary metabolites in soybean leaves such as alkaloids, tannins, steroids, terpenoids, and flavonoids, which have the capability to inhibit the growth of bacteria. The primary

mechanism through which alkaloid compounds impede bacterial growth is by interfering with the formation of peptidoglycan components in the bacterial cell walls. This disruption ultimately leads to the lysis or breaking down, of the bacterial cells (Daneshzadeh *et al.* 2019).

Conclusion

This study combines traditional Indonesian soybean cultivation with modern scientific analysis to investigate the impact of shading techniques, like using non-fruit-bearing coconut trees, on local soybean varieties. The research reveals that different shading intensities affect the soybean leaves' metabolic substances, identifying important phytochemicals and demonstrating their potential antimicrobial properties against bacteria like *S. aureus* and *E. coli*. The results demonstrated the highest inhibitory efficacy against *S. aureus*, which is 10.43 mm (S_1V_2), and against *E. coli*, which is 13.50 mm (S_1V_5). Therefore, shading on non-producing plants (S_1) stands out as the most effective treatment, highlighting Mutiara 1 (V_2) and Dena 1 (V_5) as the best varieties. Significantly, it finds that certain shading treatments enhance these antimicrobial properties, bridging traditional agricultural practices with modern science, and offering new insights for sustainable farming and natural antibacterial agents.

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Author Contributions

DWP and S conceptualized the study and developed the methodology. DWP conducted the investigation and visualized the data. ZN provided resources and supervised the study. DWP and S wrote the original manuscript. ZN revised the manuscript. All authors agreed to the final version of the manuscript.

Conflicts of Interest

All authors declare no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable to this paper.

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