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Research Article

TLC Fingerprint Analysis and Evaluation of α-Glucosidase Inhibitory and Free Radical Scavenging Activity of *Justicia gendarussa* from Different Growth Locations

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Abstract

Justicia gendarussa is known to have antioxidant and antidiabetic properties, which are related to the composition and concentration of its metabolites and influenced by differences in growth location. This study aims to evaluate changes in metabolite profiles and biological activities, such as the inhibition of α-glucosidase and free radical scavenging in *J. gendarussa* genotypes from Bogor, Cianjur, and Sukabumi. Changes in metabolite profiles were assessed using thin-layer chromatography (TLC) fingerprint analysis combined with principal component analysis (PCA). Bioautography TLC using DPPH was performed to confirm the presence of antioxidant compounds. In the TLC fingerprint analysis of *J. gendarussa*, we obtained 14 bands with good resolution. PCA successfully grouped *J. gendarussa* extracts based on growth location. The percentage of α-glucosidase inhibitory and free radical scavenging activity was significantly different, with the highest percentage of inhibition of α-glucosidase shown by the IIIS genotype (99.23%) and the highest free radical scavenging (91.35%) demonstrated by the IIB genotype. The results of TLC bioautography confirmed the presence of antioxidant compounds in *J. gendarussa* represented by bands with Rf 0.04, 0.32, and 0.80. This study concludes that growth location differences influence the variations in metabolite profiles, α-glucosidase inhibitory activity, and free radical scavenging activity of *J. gendarussa* genotypes.

Keywords: α-Glucosidase, antioxidant, fingerprint, *Justicia gendarussa*

1. INTRODUCTION

Justicia gendarussa Burm.f. is a plant belonging to the Acanthaceae family that can grow to a height of 0.8-1.5 m with long, flat leaves [1]. This plant is widely found in Indonesia, Sri Lanka, India, Malaysia, China, Thailand, Vietnam, and Pakistan [2]. Traditionally, *J. gendarussa* has been used to treat colds, headaches, pharyngitis, bronchitis, dyspepsia, and antifertility [3]. Phytochemical content detected in *J. gendarussa* includes carbohydrates, glycosides, alkaloids, flavonoids, phenols, tannins, and saponins [4]. The main compounds reported to be detected in the methanol extract of *J. gendarussa* leaves include gendarusin A, gendarusin B, justidrusamide A, and justidrusamide B [5]. The other compounds, including kaempferol, apigenin, naringenin, and vitexin, have also been present in *J. gendarussa* [1]. *J. gendarussa* has several pharmacological effects, including antioxidants [6,7], antibacterial [8,9], anti HIV [10,11], antifertility [12], and antidiabetic [13,14].

Differences in growing locations, harvesting processes, and post-harvest handling can affect the composition and levels of metabolites in plants [15], which impact the level of biological activity. Therefore, this study evaluated the metabolite profile through fingerprint analysis using thin-layer chromatography (TLC) and its effect on the biological activity caused. Fingerprint analysis offers advantages because it provides comprehensive information about the metabolites detected and characterizes the

character of the sample [16]. TLC was chosen due to its advantages, such as ease of sample preparation, consistency, stability, quality control of herbal products, short time, low cost, and small sample size[17].

Previous studies using fingerprint analysis with TLC on medicinal plants include *Sida rhombifolia* [17], *Phyllanthus niruri* [16], *Curcuma xanthorrhiza, Zingiber cassumunar, C. longa* [18], *Orthosiphon stamineus* [15,19], *C. mangga* [20], and *Psidium guajava* [21] for identification and authentication of medicinal plants. The identification of *J. gendarussa* using TLC has been previously reported [22], but method validation has yet to be carried out. Accordingly, this study modified the validated TLC fingerprint analysis method based on validation parameters of stability, specificity, precision, and robustness. Furthermore, this method evaluates the metabolite profiles of three types of *J. gendarussa* genotypes based on the growing locations: Bogor, Cianjur, and Sukabumi. The results of the TLC fingerprint analysis were then processed using chemometrics, specifically principal component analysis (PCA), to determine the grouping based on geographical origin.

The biological activity of *J. gendarussa* was also evaluated by determining the percentage of α-glucosidase inhibition and scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals, as well as TLC bioautography of its antioxidants. In previous studies, *J. gendarussa* was reported to have antidiabetic activity *in vivo* [14]. Therefore, this study aims to evaluate the changes in metabolite profile and biological activities of three types of *J. gendarussa* genotypes from Bogor, Cianjur, and Sukabumi through TLC fingerprint analysis combined with PCA and evaluation of α-glucosidase inhibitory and free radical scavenging activity.

2. RESEARCH METHODS

Materials and Instruments

The tools used are glass instruments commonly used in chemistry laboratories, analytical balances (Aczet CY 224C, Mumbai, India), 96-well microplates (Costar REF 3590, Kennebunk, United States), microplate readers (Biotek Epoch, Santa Clara, United States), oven (Memmert GmbH+Co. KG, Schwabach, Germany), rotary evaporator (Buchi, Flawil, Switzerland), filter paper nylon pore size 0.45 µm, diameter 25 mm (Whatman, Marlborough, United States), micropipette (Thermo Scientific, Waltham, United States), Branson 1510 ultrasonicator (Branson Ultraschall, Dietzenbach, Germany), CAMAG Linomat 5 semiautomatic applicator TLC (CAMAG, Muttenz, Switzerland), CAMAG Reprostar 3 (CAMAG, Muttenz, Switzerland), WinCATS software (CAMAG, Muttenz, Switzerland), twin-through and flat bottom type chromatography chambers (CAMAG, Muttenz, Switzerland).

The materials used in this research were samples of *J. gendarussa* leaves with 3 types of genotypes, such as: green and white leaves (I), green (II), and black (III) stem, respectively from 3 different regions. The samples were collected from the Biopharmaca Conservation and Cultivation Unit of Tropical Biopharmaca Research Center IPB University, Bogor (B) located at 6.55 South Latitude and 106.72 East Longitude, IPB University Pasir Sarongge Cianjur Experimental Garden (C) located at 6.77 South Latitude and 107.05 East Longitude and farmer-managed fields in Nagrak Selatan Sukabumi village (S) located at 6.7 South Latitude and 107.05 East Longitude. *Ruellia simplex* C. Wright was obtained from the Biopharmaca Conservation and Cultivation Unit of Tropical Biopharmaca Research Center, IPB University, Bogor. All samples were 3 months old and were identified by Mr. Taopik Ridwan, a botanist from Tropical Biopharmaca Research Center IPB University, Bogor. Silica gel 60 F_{254} TLC plates and TLC saturation pad were obtained from Merck (Darmstadt, Germany). Ethanol, methanol, acetonitrile, acetone, dichloromethane, chloroform, *n*-hexane, toluene, dimethyl sulfoxide, potassium dihydrogen phosphate, dipotassium hydrogen phosphate from Merck (Darmstadt, Germany), and pro analysis grade, α-glucosidase, p-nitrophenyl -α-D-glucopyranoside (pNPG), 2,2 – diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Aldrich (St. Louis, United States).

Optimization of Mobile Phase Composition

Various solvents (methanol, ethanol, acetonitrile, ethyl acetate, chloroform, acetone, toluene, dichloromethane, *n*-hexane) of 10 mL each were prepared and saturated in each chromatography chamber. *J. gendarussa* leaf extract was applied to a TLC plate to separate the compounds using the single solvent mentioned previously. Afterward, 2 to 3 solvents are selected and mixed to obtain the best mobile phase composition for compound separation using TLC. The optimum mobile phase composition was selected, producing the most significant number of separated compounds with a separation resolution of ≥ 1.5. Derivatization was done to determine the presence of separated compound bands by staining with 10% sulfuric acid in methanol. Then, the plate was dried in the oven at 105 °C for 10 minutes and documented before and after derivatization at a wavelength of 366 nm.

Application and Development of *J. gendarussa* Extract with TLC

J. gendarussa leaf extract was applied to the TLC plate using CAMAG Linomat 5 with WinCATS software. The application was carried out with a band length of 6 mm, a volume of 8 µL, a spot flow rate of

80 nL/s, and a distance between the band of 4 mm. Previously; saturation paper was inserted together with the mobile phase into the chromatography chamber for the saturation process for 30 minutes. Next, the TLC plate was developed in a chromatography chamber, and the optimum mobile phase was obtained. After the development process, the TLC plate is dipped into the dye reagent and then documented using CAMAG Repostar 3 at a wavelength of 366 nm.

Validation of the Fingerprint Analysis Method Using TLC

Method validation was performed by following the procedures used by Reich and Schibli [23]. The parameters determined are stability, specificity, precision, and robustness. For the stability test, observations were made by visual inspection for 1 hour after the color reagent was applied. Next, the separation of the analyte in the sample solution and on the TLC plate was observed by applying the sample to 4 lines. Line 1 involved applying the sample to the plate, which was left for 3 hours. Lines 2 and 4 involved applying a fresh sample, and line 3 involved leaving the sample in the solutions for 3 hours before application to the plate. In addition, stability tests were carried out during 2-dimensional solvent development. Specificity was determined by comparing the TLC fingerprints of *J. gendarussa* with *R. Simplex* leaves with the same leaf morphology. The method's precision was tested by repeating procedure 2.2 with the extracted sample 3 times. Each extract replication was applied to the plate 3 times. This was repeated for the other 3 plates carried out on the same day. Intermediate precision was determined by repeating procedure 2.2 for three different days. The robustness was evaluated by observing the solvent development distance at 7 and 8 cm and between different chamber types (twin through and flat bottom). All separation results were documented before and after derivatization with color reagents at 366 nm.

Fingerprint Analysis Using TLC

Fingerprint Analysis Using TLC refers to the procedure used by Rafi *et al*. [17] with slight modifications. Leaf samples from three types of *J. gendarussa* genotypes from Bogor, Cianjur, and Sukabumi were each made into powder with a size of 80 mesh. The leaf powder was then extracted using methanol by sonication with a ratio of powder to extraction solvent of 1:10 for 30 min. Next, the filtrate was applied to the TLC plate.

Determination of the Percentage of α-Glucosidase Inhibition

Inhibition of α-glucosidase from *J. gendarussa* leaf extract was performed following the procedure described by Yuliana *et al* [24]. The extract concentration used was 10,000 µg/mL. Inhibition of α-glucosidase was determined by measuring the absorbance of the extract solution using a microplate reader at a wavelength of 410 nm with acarbose as a positive control and three replicates. The calculation formula uses the equation below.

$$\% \text{ inhibition} = \frac{(\text{Blank absorbance} - \text{Sample absorbance})}{\text{Blank absorbance}} \times 100\% \quad (1)$$

Determination of the Percentage of DPPH Free Radical Scavenging Inhibition

The percentage of antioxidant free radical scavenging was performed using the DPPH method following the procedure used by Zang *et al.*[7] The extract solution was made with a concentration of 1000 µg/mL with ethanol solvent. The absorbance of the solution was then read at a wavelength of 517 nm using a microplate reader. The analysis was performed in 3 replications with ascorbic acid as a positive control. The calculation formula uses the equation below.

$$\% \text{ inhibition} = \frac{(\text{Blank absorbance} - \text{Sample absorbance})}{\text{Blank absorbance}} \times 100\% \quad (2)$$

TLC Bioautography for Detection of Antioxidant Compounds

The bioautography TLC method for detecting antioxidant compounds refers to the procedure used by Batubara *et al*. [25] with slight modifications. The compounds were separated from the extracts of the three types of *J. gendarussa* genotypes using TLC. After drying, the TLC plates were dipped in 500 µg/mL DPPH solution and incubated at room temperature for 1 hour. The yellowish band that forms indicates the presence of antioxidant compounds.

Data Analysis

The results of TLC fingerprint analysis were converted into a densitogram using ImageJ software version 1.53t / Java 1.8.0 64-bit (Bethesda, Maryland, United States). The grouping of *J. gendarussa* is based on the growing location of PCA using the Unscrambler X software version 10.1 (Camo, Oslo, Norway). The α-glucosidase inhibition value and the percentage of antioxidant free radical scavenging were processed using two-way Analysis of Variance (ANOVA) and tukey's test using the statistical package for the social sciences (SPSS) version of IBM SPSS Statistics 27 (New York, United States) to determine significant differences from the percentage value of α-glucosidase inhibition and DPPH free radical scavenging in three types of *J. gendarussa* genotypes based on growing location. A significant difference was defined at the 95% confidence level ($p < 0.05$).

3. RESULTS AND DISCUSSION

Optimization of Mobile Phase Composition

The TLC technique utilizes a stationary phase of silica gel 60 F254 and an optimized mobile phase to achieve as many separate bands as possible, with an inter-band resolution of ≥ 1.5. The mobile phase was optimized through trial and error using solvents with varying polarity levels (non-polar, semi-polar, and polar). Based on the 9 single mobile phases tested, chloroform gave separation results with 7 bands, the highest among other single solvents, shown in **Figure 1**. Furthermore, combining several single solvents with different ratios with a mixture of chloroform and ethanol (9:1) produced 13 bands (**Figure 2**). Further optimization of the mixture resulted in 14 bands with a ratio of 9.5:0.5. Therefore, chloroform-ethanol with a ratio of 9.5:0.5 was chosen as the optimum mobile phase for separation using TLC for *J. gendarussa* leaves. This combination produces a balanced band position because the more polar a solvent is, the more polar compounds will move further from the point of upward expansion, and the non-polar compounds are stuck at the bottom[17]. Apart from the mobile phase, the number of bands formed is also influenced by the type of detection. In this study, 10% sulfuric acid in methanol was used as a derivatization reagent to visualize component bands under UV 366 nm because sulfuric acid reacts with almost all chemical components and detects various secondary metabolites [17,23]

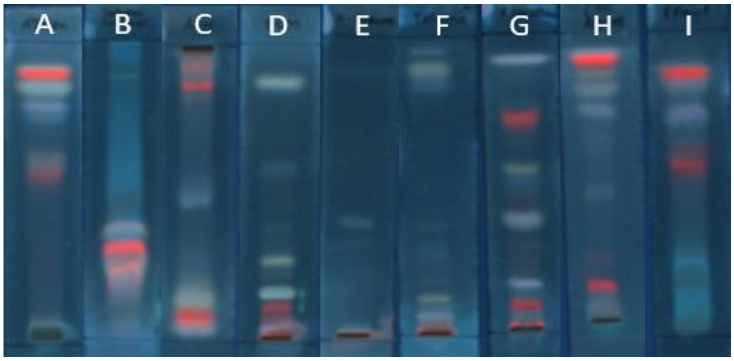


Figure 1. TLC chromatogram from *J. gendarussa* leaves extract using single eluent (A) acetone, (B) methanol, (C) acetonitrile, (D) DCM, (E*)* n-hexane, (F) toluene, (G) chloroform, (H) ethyl acetate, (I) ethanol.

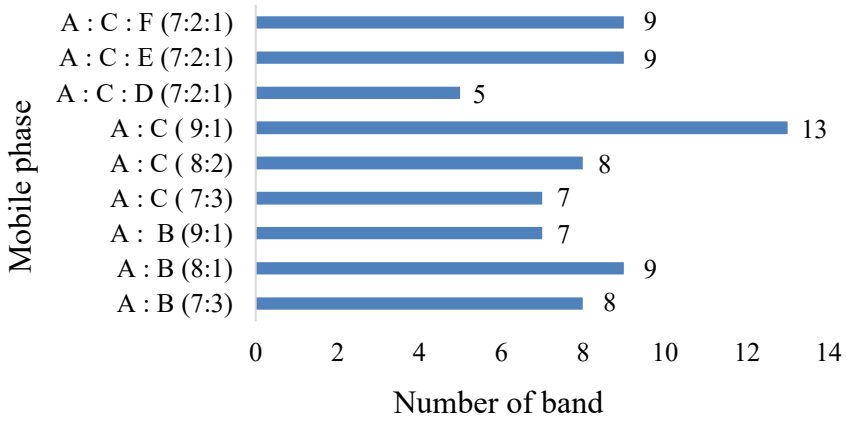


Figure 2. Results of mixed optimization of 2 and 3 mobile phases, such as chloroform (A), acetonitrile (B), ethanol (C), ethyl acetate (D), toluene (E), DCM (F) for TLC analysis of *J. gendarussa* leave extract based on the number of bands.

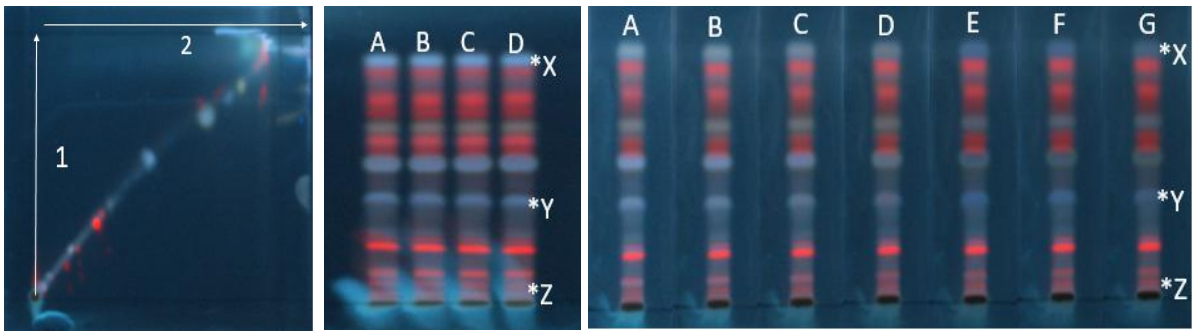
Validation of The Developed TLC Finggerprint Analysis Method on *J. gendarussa* Leaves.

The validation parameters determined include stability, specificity, precision, and robustness, which are evaluated based on the criteria used by Reich and Schibli [23]. Validation of the TLC fingerprint method on *J. gendarussa* leaves was observed using three bands representing the top (X), middle (Y), and bottom (Z) areas.

Stability

The stability of the analyte during chromatography is shown in **Figure 3(a)**, which illustrates that the analyte remains stable throughout the chromatography process. All detected spots or components align along the diagonal line, indicating the movement of the stain during the two-dimensional solvent development on the TLC plate. The stability test of the analyte on the plate and in solution in **Figure 3(b)** shows that the analyte remains stable, as

evidenced by the consistent fingerprint pattern with the same number of bands, intensity, and color, and the Rf value meets the acceptance requirements (≤ 0.05) on all four lines[23]. The Rf values of the observed bands are 0.82 (X band, upper area), 0.33 (Y band, middle area), and 0.07 (Z band, lower area). Therefore, it can be concluded that the *J. gendarussa* leaf extract is considered stable for at least 3 hours in solution and on plates. **Figure 3(c)** displays the results of the visual stability test of the analyte after derivatization. The result shows that the analyte remains visually stable for up to 60 minutes, as there is no change in color and number of bands for 60 minutes after treatment with the color reagent. This indicates that detection and documentation of separation results can still be performed up to 60 minutes after the color reagent is administered.



(a) (b) (c)

Figure 3. (a) TLC chromatogram of analyte stability test during chromatography (1) First solvent development process, (2) Second development process. (b) TLC chromatogram of the analyte stability test on the plate and in solution in (A) lane 1, (B) lane 2, (C) lane 3, and (D) lane 4. (c) TLC chromatogram of analyte stability test visualization results on (A) 0 minutes, (B) 2 minutes, (C) 5 minutes, (D) 10 minutes, (E) 20 minutes, (F) 30 minutes, and (G) 60 minutes. For images (b) and (c), observations were made on three bands in the *J. gendarussa* leaf extract, which represent the top (X), middle (Y), and bottom (Z) areas.

Specificity

The specificity test compared *J. gendarussa* with *R. simplex*. The TLC plate resulting from specificity analysis (Figure 4) shows typical bands of *J. gendarussa*, namely a bright red band (A) with Rf 0.23 and two faint red bands (C) with Rf 0.13 and (D) with Rf 0.06, which is not found in *R. simplex* leaves. On the other hand, *R. simplex* leaves have a distinctive green band (B) with an Rf of 0.19, which is not found in golden *J. gendarussa*. These results can be used to differentiate between the two plants to prevent adulteration of *J. gendarussa* from *R. simplex* leaves.

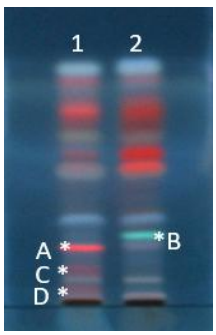


Figure 4. TLC chromatogram of specificity test on the extract of *J. gendarussa* leaves (lane 1) and *R. simplex* leaves (lane 2). Detection at a wavelength of 366 nm after adding sulfuric acid as a derivatization reagent.

Precision and intermediate precision

The precision of the same-day and different-day methods (intermediate precision) is shown, respectively, in **Figures 5** and **6**. The results show that the precision test of TLC fingerprint analysis of *J. gendarussa* leaf extract meets the acceptance requirements, namely, the position, number, and intensity of the color of the stable bands and the difference in Rf (ΔRf) from three representative bands, respectively between X, Y, and Z bands, namely, ≤ 0.02 and ≤ 0.05 for the precision of the same and different day methods. Therefore, the precision test results for both same-day and different-day methods are acceptable. The acceptance requirements for determining the precision of the different day methods are greater than those of the same-day method because the laboratory environment is challenging to maintain during the experiment [17].

Robustness

The robustness parameters were evaluated based on chamber type and development distance for chromatography in *J. gendarussa* extracts. The criteria for this parameter are the number, position, and color of the constant bands with a difference in the value of Rf ≤ 0.05. The evaluation based on chamber type in **Figure 7 (a)** shows that the results of elusion with twin-trough and flat bottom chamber have a difference in Rf value ≥ 0.05, and the ribbon pattern is different, with the upper band (X) not resulting from the elusion process with the flat bottom chamber. This can be due to different saturation levels and changes in relative humidity in different chamber [17]. The different

saturation levels of the moving phase are because the shape of the flat bottom chamber is larger than the twin-through chamber, which causes different surface areas. Therefore, it is likely that the same saturation time will produce different saturation levels in both chambers. Hence, the robustness parameter does not meet the criteria based on different chamber types; therefore, this method is specific to TLC fingerprint analysis with a twin-through chamber. The robustness parameters based on the development distance of 7 and 8 cm (**Figure 7 b**) show the constant band's number, position, and color with a difference in the value of Rf ≤ 0.05 so that the robustness based on development distance is acceptable.

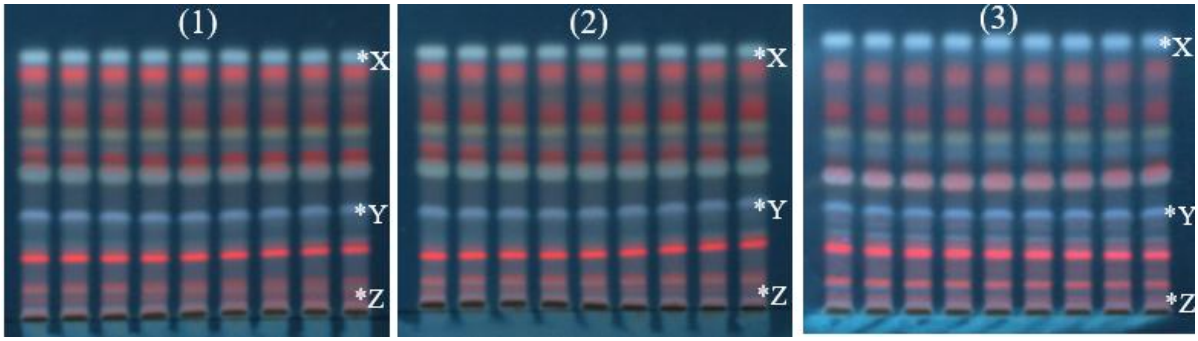


Figure 5. TLC chromatogram of precision determination on the same day (1) first plate, (2) second plate and (3) third plate

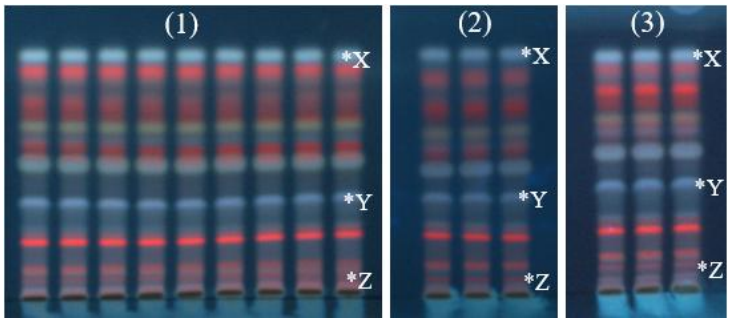
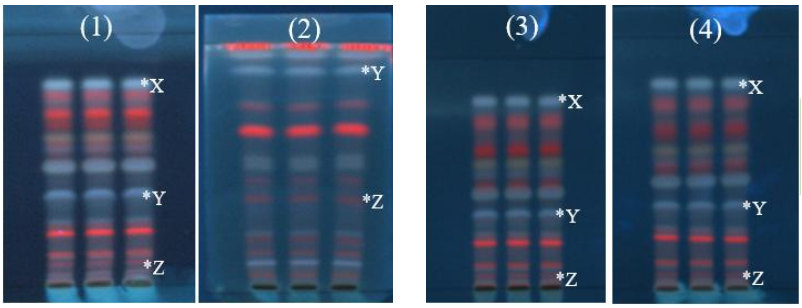


Figure 6. TLC chromatogram for determining precision on different days (1) first day, (2) second day, and (3) third day



(a) (b)

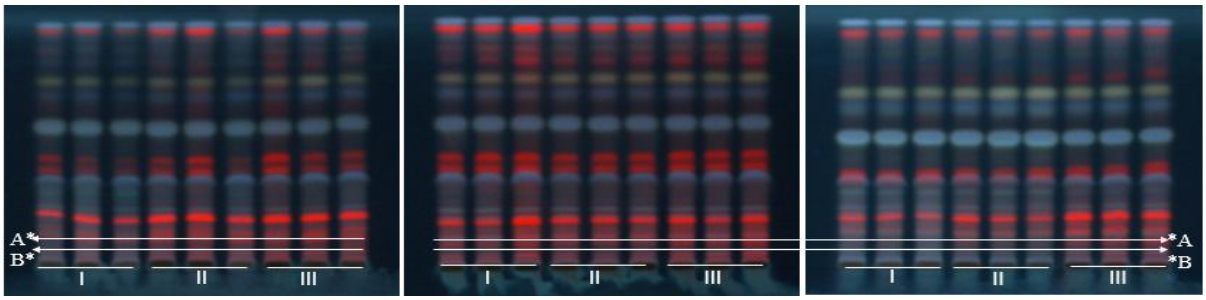
Figure 7. (a) TLC Chromatogram robustness test based on chamber type (1) twin-trough chamber, (2) flat bottom chamber and (b) Chromatogram of TLC robustness test based development distance (3) development distance of 7 cm, (4) development distance of 8 cm, in 3 bands on *J. gendarussa* leave extract representing the upper (X), middle (Y), and lower (Z) regions after derivatitation in 366 nm UV light detection.

Fingerprint Analysis of Three Types of *J. gendarussa* Leaf Extract Genotypes from Three Different Locations Chemometrically

Figure 8 shows the results of TLC fingerprint analysis on three types of *J. gendarussa* leaf genotypes from Bogor, Cianjur, and Sukabumi. The fingerprint patterns produced from the three locations are almost identical. Only the difference in color intensity in bands A and B from the Cianjur location in Figure 8 (b) is more clearly visible compared to bands A and B from the Sukabumi and Bogor locations in Figure 8 (c) and (a). On the TLC plates from the Bogor and Sukabumi locations, bands A and B still appeared but had different color intensities for each genotype. The color intensity indicates the concentration of the compound. On the TLC plate from the Sukabumi location, band B only appeared on the genotype (III) black stem. This suggests the existence of genetic variation, namely, genotype as an intrinsic factor and growing location as an extrinsic factor, which influences the chemical composition of plants, specifically secondary metabolite [26,27]. This was

previously proven, where the content of phenolic acids and tanshinones in *Salvia miltiorrhiza* was found to be influenced by differences in location and genotype [28]. Next, the TLC fingerprint analysis results were converted into a densitogram to produce area values using Image-J software. The area value is used in PCA analysis. Combining data processing with PCA helps evaluate complex and similar chromatogram data [19]. PCA reduces data dimensionality, improves interpretation, and identifies similarities/dissimilarities of samples as well as specific marker compounds [29]. TLC fingerprint analysis, combined with chemometrics using PCA, has been widely used in previous research to group herbal plants based on differences in location, including *Orthosiphon stamineus* Benth [19], and *Curcuma longa* L. [30], *Curcuma xanthorrhiza* [31].

In the PCA score plot (**Figure 9**), PC 1 and PC 2 were generated, accounting for 93% of the data's diversity, as explained by 83% of PC 1 and 10% PC 2. PC 1 explains the largest source of variation in the dataset, while PC 2 has the next most significant variation[32]. The results of the principal component diagram show two suitable dimensions if the sum of PC 1 and PC 2 is more significant than 70% [32]. The results of the PCA score plot in **Figure 9** successfully grouped the three types of *J. gendarussa* genotypes from Bogor, Cianjur, and Sukabumi in different quadrants. *J. gendarussa* from the Sukabumi area is on the border of quadrants I and IV, the Cianjur area is in quadrant II, while the Bogor area is in quadrant III. This shows a change in the metabolite profile resulting from each TLC fingerprint analysis of extracts of three types of *J. gendarussa* genotypes based on growing location. The growing locations were chosen based on differences in altitude [33] , namely Bogor (lowlands, 141 masl), Sukabumi (medium plains, 493 masl), and Cianjur (highlands, 1083 masl). In this study, the altitude of different growing locations can be a factor influencing differences in composition profiles and metabolite concentrations in a plant [33].The distance between samples shows the similarity between samples. The closer the samples are to the PCA score plot, the greater the similarity between the samples [34]. The overall PCA plot shows that each genotype originating from the same area exhibits a similar composition and metabolite profile, as indicated by the distance between adjacent genotypes and their placement in the same quadrant.



(a) (b) (c)

Figure 8. (a) Chromatogram of TLC fingerprint analysis on *J. gendarussa* leaf extract from Bogor, (b) Cianjur, and (c) Sukabumi areas from three genotypes of green and white leaves (I), green (II), and black stems (III).

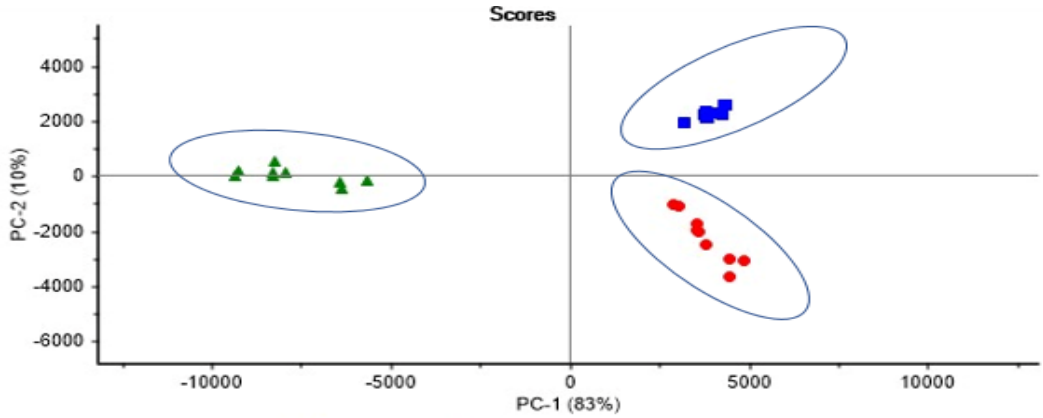


Figure 9. PCA score plot of *J. gendarussa* leaf extract with three types of genotypes from ■ the Bogor, ● Cianjur, and ▲ Sukabumi areas.

Inhibition of α-glucosidase and DPPH free radicals scavenging and TLC bioautography of *J. gendarussa* Plants

The biological activities determined in this study were α-glucosidase inhibition, the percentage of DPPH free radical scavenging, and bioautography TLC for identifying antioxidant compounds. The two-way ANOVA test of α-glucosidase and antioxidant inhibition values produced significantly different values ($p < 0.05$), with the highest α-glucosidase inhibition value shown by the black stem genotype from Sukabumi (IIIS) at 99.23 % (**Figure 10**) and the highest antioxidant inhibition at 91.35 % (**Figure 11**) showed the green stem genotype from Bogor (IIB). This study demonstrates that the same genotype yields different results for α-glucosidase inhibition and antioxidant values at various locations. For example, in determining the percentage of α-glucosidase enzyme inhibition, the black stem genotype from Sukabumi produced the highest value. However, it produced relatively low values in the Bogor area, at 11.75%, and in Cianjur, at 22.44%. The results of determining the percentage of DPPH free radical capture of genotype I from Bogor produced a value of 74.99%, significantly different from genotype I from Sukabumi, which was 54.35%. This indicates that different growing locations affect the metabolite composition of *J. gendarussa*, thereby influencing the resulting biological activity. Internal factors such as planting age and the part of the plant harvested, as well as external factors including soil type, altitude, rainfall, light intensity, planting distance, fertilization, harvest time, and post-harvest processing, can all influence the quality of plants from a specific location. Grow[34]. Different geographical origins will affect the growth conditions of a plant. In previous studies, different growth conditions in *Marrubium vulgare* L. have been shown to cause variations in phenolic acid content and antioxidant activity [35]. Differences in α-glucosidase inhibition and antioxidant values produced by the same genotype at different locations can also be caused by interactions between genotypes and the environment. Abiotic stress factors in plants influenced by the environment (salinity, heat, drought, flooding, heavy metals, and plant nutrients) can also increase the expression levels of related genes involved in secondary metabolite formation [36].

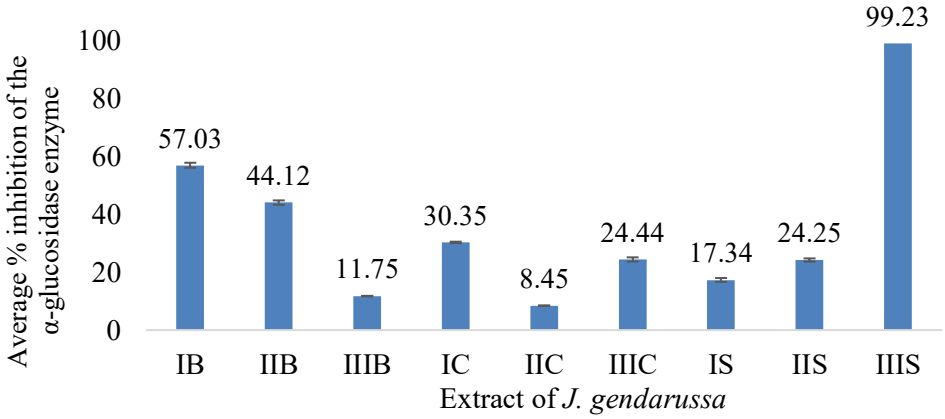


Figure 10. Average % inhibition of the α-glucosidase enzyme from three genotypes I (green and white leaves), II (green stems), III (black stems) shows significantly different results from each area of Bogor (B), Cianjur (C), and Sukabumi (S)

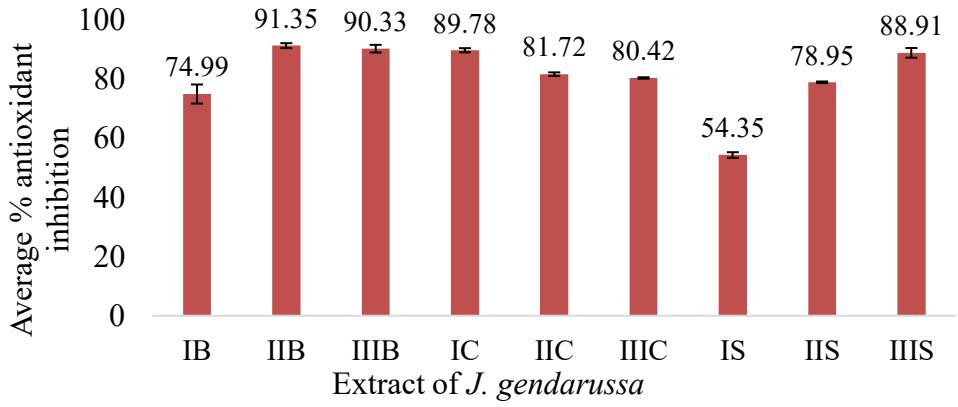
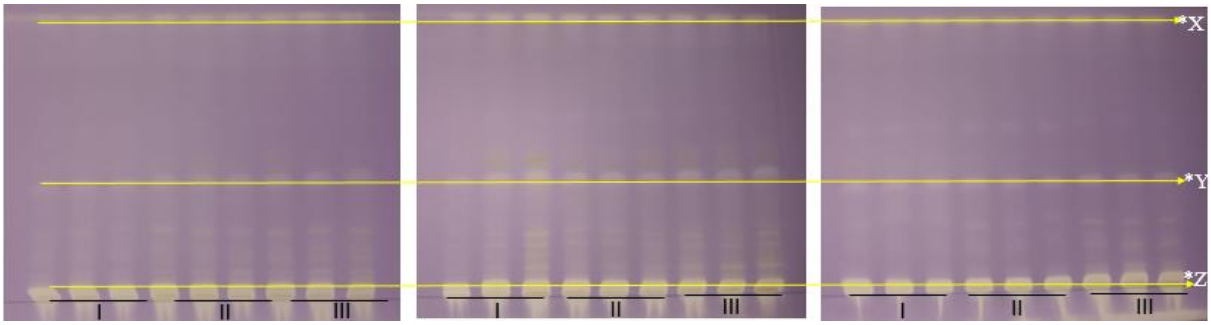


Figure 11. Average % antioxidant inhibition of three genotypes I (green and white leaves), II (green stems), and III (black stems) shows significantly different results from each area of Bogor (B), Cianjur (C), and Sukabumi (S)

Different genotypes produced different α-glucosidase inhibition and antioxidant values from the same or different locations (**Table 1**). This indicates that genotype affects the biological activity of a medicinal plant. In medicinal plants, the genotype determines the plant's genetic potential as a producer of active compounds [37]. Meanwhile, the phenotype is an expression of the genotype that is influenced by the environment[38]. Several previous studies have shown that genotype variation significantly affects the biological activity of a plant, including the evaluation of 15 genotypes of ethyl acetate extract of *Orthosiphon aristatus* leaves on antioxidant activity and photochemical content [39], evaluation of 25 chili genotypes (hybrids and parents) on total phenolic content (TPC) and total flavonoids content (TFC), antioxidants, and α-glucosidase inhibitor activity [40]. The effect of genotype and its interaction with the environment on plants at different growing locations was also explained in previous studies, namely on chili plants (*Capsicum annuum* L.) [38], *Salvia miltiorrhiza* [28], *Glycyrrhiza uralensis* [41], *Curcuma xanthoriza,* and *C. aeruginosa* [42].

The processing of Tukey's or Tukey's Honest Significant Difference (HSD) further test results from the determination of the percentage of α-glucosidase enzyme inhibition revealed significant differences at $p < 0.05$ in the three genotypes from each different location, except for genotypes IIIC and IIS. Likewise, the results of Tukey's further test on determining the percentage of DPPH free radical capture yielded significantly different results except for genotypes IIB, IIIB, IC, and IIIS, which had results that were not significantly different. In addition, genotypes IIC, IIIC, and IIS also yielded results that were not significantly different from each other. This can be caused by the similarity of environmental factors that influence their interaction with the genotype, resulting in similar metabolite compositions and profiles. In addition, it can also be caused by the similarity of genetic factors that influence plant growth [43]. This has an impact on the results of its biological activity, which shows results that are not significantly different.

Bioautography TLC was carried out to confirm the presence of antioxidant compounds. The formation of a yellow band in the pattern produced by TLC fingerprint analysis on *J. gendarussa* from various locations (**Figure 12**) indicates the detection of antioxidant compounds. The yellow color is formed in the X and Y bands with an average Rf of 0.80 and 0.32, respectively. The band that appears between the Y and Z bands is also yellow but has a lower color intensity. A thicker yellow color is shown in the Z band, with an average Rf value of 0,04. The thicker the yellow band, the stronger the antioxidant activity [25]. Differences in color intensity indicate different concentrations of compounds.



(a) (b) (c)

Figure 12. (a) TLC bioautography of antioxidants on leaf extracts from the Bogor, (b) Cianjur, and (c) Sukabumi areas from three genotypes of green and white leaves (I), green stems (II), and black (III)

Tabel 1. Results of determining the percentage of inhibition of the α-glucosidase enzyme and scavenging of DPPH free radicals (antioxidants)

Genotypes	Enzyme inhibition α-glucosidase	DPPH Free Radical cavenging (Antioxidant)
I B	57.03 ± 0.88^{g}	74.99 ± 3.22^{b}
II B	44.12 ± 0.76^{f}	91.35 ± 0.83^{d}
III B	11.75 ± 0.10^{b}	90.33 ± 1.29^{d}
I C	30.5 ± 0.25^{e}	89.78 ± 0.70^{d}
II C	8.45 ± 0.06^{a}	81.72 ± 0.58^{c}
III C	24.44 ± 0.73^{d}	80.42 ± 0.23^{c}
I S	17.34 ± 0.67^{c}	54.35 ± 0.95^{a}
II S	24.25 ± 0.56^{d}	78.95 ± 0.27^{c}
III S	99.23 ± 0.44^{h}	88.91 ± 1.64^{d}

The reported values are the mean ± SD of 3 replicate tests for each genotype code of green and white leaves (I), green stems (II), and black stems (III) from *J. gendarussa* extracts from Bogor (B), Cianjur (C) and Sukabumi (B). The mean ± SD in each extract in the same column followed by different superscript letters indicates significant differences at $p < 0.05$.

4. CONCLUSIONS

Based on TLC fingerprints combined with chemometric analysis, specifically PCA, it is evident that changes in the metabolite profiles of three types of *J. gendarussa* genotypes are influenced by the growing location. The three types of *J. gendarussa* genotypes from different growing locations also showed significantly different α-glucosidase inhibitory and antioxidant activities. Growing location and genotype variations influence changes in metabolite profiles and biological activities of *J. gendarussa*.

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