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Phenolic and flavonoid content with agro morphological characters of 12 accessions of *Justica gendarussa* grew in Indonesia

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Abstract. *Marliani N, Artika IM, Rafi M, Syukur M, Galingging RY, Nurcholis W. 2022. Phenolic and flavonoid content with agro morphological characters of 12 accessions of* Justica gendarussa *grew in Indonesia. Biodiversitas 23: 5101-5107. Justicia gendarussa* Burm. f is a medicinal plant used in traditional and modern medicine. This study was conducted to determine the agro morphological characters, phenolic and flavonoid content of 12 accessions of *J. gendarussa* plants obtained from various locations in Indonesia and then grown in the same conditions and places. The agro morphological characteristics include plant height, number of leaves, number of branches, wet weight, and dry weight of leaves and stems. Total phenolic content (TPC) and total flavonoid content (TFC) were analyzed by spectrophotometric method. The results showed significant differences in the characters of 12 accessions of *J. gendarussa* ($p<0.05$). Accessions of BK4 (Bekasi 4) and KP (Kejayan Pasuruan) had the best agro morphological characteristics. Accessions of BK4 (Bekasi 4) and BG (Bogor) had the best growth rates. The highest TPC value was obtained from TG (Trenggalek) accession (0.752 mg GAE/g DW) and the highest TFC (1.899 mg QE/g DW from LK (Langkat) accession. The best accessions based on agro morphological characters were BK4 (Bekasi 4) and KP (Kejayan Pasuruan), and based on the content of polyphenols were TG (Trenggalek) and LK (Langkat). The accessions of BK4, BG, TG, and LK could be further developed into superior varieties of *J. gendarussa*.

Keywords: Accession, agro morphological, flavonoid, *Justicia gendarussa*, phenolic

INTRODUCTION

Medicinal plants with standardized quality raw materials are urgently needed in Indonesia, along with the development of the medicinal industry. Developing herbal medicines requires several steps to produce safe and effective drugs, from selecting the correct plant species, determining the right growth conditions, and quantifying active substances to evaluating clinical trials in vitro and in vivo (Liu et al. 2018). *Justicia gendarussa* Burm.f (Acanthaceae) is a plant native to China and has spread to various countries such as Thailand, India, and Indonesia (Kavitha et al. 2014).

The *J. gendarussa* has a round woody stem morphology. The leaves are light green and dark green with a pinnate shape, flat leaf edges with a leaf width of 1-3.5 cm, and a single lanceolate measuring 3-12 cm. In general, *J. gendarussa* has small white flowers arranged in closed grains, spreading and ending at the end of the stalk (Syamsul et al. 2015).

The *J. gendarussa* is one of the plants that have the potential to be developed as a drug due to its metabolite content and pharmacological activity. Previous studies have reported the content of phenolic content, alkaloids, isoflavonoids, terpenoids, amino benzyl (Marliani et al. 2022), isoquinoline, triterpenoids, and alcohol (Sonal and Maitreyl 2011). The main active compounds of *J. gendarussa* that are also used as chemical markers are gendarusin A, gendarusin B, and the amide group (justidrusmides A-D) (Mnatsakanyan et al. 2018). The *J. gendarussa* has been traditionally used to treat wounds, stomach pain, coughing, and asthma (Roy and Joseph 2022) and as a male contraceptive agent (Indrawati et al. 2022). The pharmacological activity of *J. gendarussa* includes antioxidant, anti-inflammatory, hepatoprotective, cytotoxic, and antimicrobial (Phatangare et al. 2017; Mahmud et al. 2020).

The quality of medicinal plants is affected by the amount and type of active compounds produced. Zaynab et al. (2018) stated that environmental factors, i.e., biotic (pathogens and human treatment) and abiotic (temperature, light, nutrition, and humidity), genetic and geographical factors affect the plant growth stage. Environmental factors also affect metabolite concentrations in the plant. Sampaio et al. (2011) reported that environmental temperature and micronutrients had a major effect on the production of phenolic compounds in *Lafoensia* leaves. In the study of Ieamkheng et al. (2022), the leaves of *Maranta* *arundinacea* showed higher total flavonoid, phenolic, and tannin content than the stems in the dry season. In addition, there are differences in the chemical compounds of *M. arundinacea* from Thailand and Cambodia that relates to genetic diversity. Genetic diversity provides a genetic basis for the selection in plant breeding (Asha et al. 2016). Another factor that influences the concentration of compounds in plants is the age of the plant (Ieamkheng et al. 2022). In Indonesia, the superior quality of *J. gendarussa* varieties has not been developed; therefore, exploration for obtaining plant varieties with the best efficacy is urgently needed.

The leaves of *J. gendarussa* contain phenolics and flavonoids (Kuber 2021; Marliani et al. 2022). Phenolics and flavonoids have important biological roles, such as plant growth regulation, defense mechanisms against pathogens and predators, plant pigmentation, protection from UV rays, and nitrogen fixation mechanisms. In addition, phenolics and flavonoids could also act as an antioxidant. Antioxidants are important components in the body's system that can inhibit free radical reactions leading to the prevention of degenerative diseases (Maddu 2019). Marliani et al. (2022) showed that *J. gendarussa* leaves have the potential as a source of antioxidants. This study aimed to obtain superior accession based on growth characteristics, biomass, total phenolic content, and total flavonoids. The present study provides scientific information that may be useful for breeding *J. gendarussa* commercial plant varieties.

MATERIALS AND METHODS

Plant material

Twelve plant accessions of *J. gendarussa* were collected from various regions in Indonesia (Table 1). Plant specimens were identified by a taxonomist in the Biopharmaca Conservation and Cultivation Station, Tropical Biopharmaca Research Center, Bogor Agricultural University. The field experiment was carried out in the Biopharmaca Conservation and Cultivation Station, Tropical Biopharmaca Research Center, Institut Pertanian Bogor, Bogor, Indonesia. This study was arranged in a one-factor randomized block design with three replications. All treatments were given a dose of 300 kg/ha of urea fertilizer, SP-36 200 kg/ha, KCl 200 kg/ha, and fertilizer cow manure at 20 tons/ha. Cow manure was applied before planting, while urea, SP-36, and KCl were applied after planting. The harvest process is carried out four months after planting, in August 2021.

Growth observation

Growth observation was carried out according to the procedure of Nurcholis et al. (2021) with modifications. The growth parameters include plant height, number of leaves, number of branches, wet plant weight, and plant dry weight 16 weeks after planting. Plant height measurements were carried out from the base of the stem to the top of the two tallest plants. All leaves were counted as the number of leaves, while the number of branches was counted on all branches at the height of ≥5 cm.

The wet weight of stems and leaves was measured by weighing the cleaned plants. Meanwhile, the dry weight of the plant was carried out by drying the cleaned stems and leaves in the oven at 70°C until a constant weight was obtained.

Sample extraction

Leaves of *J. gendarussa* were ground and sieved using an 80-mesh sieve. Next, the dry powder was extracted using water and hexane as solvent. Briefly, 4 g of dry powder of each accession was macerated with 40 mL of solvent at room temperature. After 24 hours, the extract mixture was filtered using filter paper. Next, the extract was concentrated using a rotary vacuum evaporator (HAHNVAPOR, Korea). Finally, the stock solution of extract (0.2 g/mL) was used to determine the total phenolic and flavonoid content.

Table 1. Collection site of twelve accessions of *Justicia gendarussa* based on google earth

Specimens	Sample code	Province	Location	Geographic coordinates	
				Latitude (S)	Longitude (E)
BMK0492042021	BG	West Java	Ciomas, Bogor	6°36'09"	106°45'52"
BMK0493042021	BK1	West Java	BP2TOOP, Bekasi	6°18'24"	107°02'35"
BMK0494042021	BK2	West Java	Bekasi City	6°17'05"	106°58'12"
BMK0495042021	BK3	West Java	Tk. Mustika Jaya, Barokah Jaya Abadi, Bekasi	6°17'35"	107°00'04"
BMK0496042021	BK4	West Java	Tabulampot Indonesia, Bekasi	6°17'51"	107°01'48"
BMK0497042021	MG1	East Java	UPT. Lab Herbal Materia Medic, Pesanggrahan, Batu	7°52'03"	112°31'09"
BMK0498042021	MG2	East Java	Batu, Malang	7°52'30"	112°32'17"
BMK0501042021	TM	Central Java	B2P2TOOT Tawang Mangu, Karanganyar	7°39'48"	111°08'02"
BMK0500042021	TG	East Java	Alta Local Herb, Trenggalek	8°06'21"	111°42'02"
BMK0502042021	LK	North Sumatra	Langkat	3°45'36"	98°13'54"
BMK0499042021	KP	East Java	Kejayan Pasuruan	7°44'08"	112°50'07"
BMK0503042021	JB	East Java	Jember	8°20'50"	113°32'49"

Note: BK1: Bekasi 1; BK2: Bekasi 2; BK3: Bekasi 3; BK4: Bekasi 4; MG1: Malang 1; MG2: Malang 2; TM: Tawang Mangu; TG: Trenggalek; LK: Langkat; KP: Kejayan Pasuruan; JB: Jember

Analysis of total phenolic content (TPC)

The total phenolic content was analyzed using the Folin-Ciocalteu method (Yanuarti et al. 2017). First, 20 µL of the extract was added to 120 µL of Folin-Ciocalteu 10% into a 96-well microplate and incubated for 5 minutes. After that, 80 µL 10% Na_2CO_3 was added and incubated again for 30 minutes in a dark room. The absorbance was measured at a wavelength of 750 nm using a microplate reader (Spectrostar Nano, BMG LABTECH). Gallic acid was used as a standard curve at various 20-300 ppm concentrations. Total phenolic content was expressed as milligrams of gallic acid equivalent per gram dry weight (mg GAE/g DW).

Analysis of total flavonoid content (TFC)

Total flavonoid content was determined based on the Colorimeter method by Yanuarti et al. (2017). A total of 120 µL of distilled water was added to a 96-well microplate, then added with 10 µL of extract, 10 µL of 10% $AlCl_3$, 10 µL of glacial acetic acid, and 50 µL of pro-analytic ethanol. The mixture was incubated for 30 minutes in a dark room. The absorbance was measured at a wavelength of 415 nm using a microplate reader (Spectrostar Nano, BMG LABTECH). Quercetin at a concentration variation of 25-500 ppm was used to prepare a standard curve. Total flavonoid content was expressed as milligrams of quercetin equivalent per gram dry weight (mg QE/g DW).

Data analysis

Statistical analysis was performed by analysis of variance (ANOVA) followed by Duncan's test to identify significant differences between accessions of *J. gendarussa* ($p < 0.05$). In addition, the Pearson Correlation test between growth characteristics and total phenolic and flavonoid content was carried out.

RESULTS AND DISCUSSION

Twelve accessions of *J. gendarussa* were planted until 16 weeks old. The growth parameters include plant height, number of leaves, number of branches, wet weight, and dry weight of plant are presented in Table 2. There are significant differences in growth parameters among the 12 accessions of *J. gendarussa* ($p<0.05$). Plant height ranged from 51.27 to 79.03 cm. The plant height of BK4 accessions was the tallest and significantly differed from that of LK accessions. The number of leaves per plant ranged from 129.36 to 251.66; KP accessions were the highest (251.66 leaves per plant). The number of branches ranged from 5.67 to 11.00 per plant, with the highest number being TM and significantly differing from LK. Based on the parameters of plant height, the number of leaves, and the number of branches, it can be concluded that BK4 and KP accessions have the highest growth development, so these accessions might have the potential to be superior varieties for plant breeding.

Twelve accessions of *J. gendarussa* have different morphological characters, i.e., leaf color, stem color, and plant height. Figure 1 shows the differences in leaf and stem color of *J. gendarussa* accessions. Accession of BK1 had green leaves with brownish red leaf veins (Figure 1A and 1B), while MG2 accessions had whitish green leaf color with brownish green leaf veins. In addition, other accessions of TG and LK had green leaves and green veins. There were two different stem types of 12 accessions of *J. gendarussa*. Figure 1C represents the shape and color of green stems from accessions TG and LK, while accessions BG, MG1, MG2, BK1, BK2, BK3, BK4, KP, JB, and TM accessions had a dark brown stem. Meanwhile, there were rusty leaves during plant growth due to pathogens attacking leaves, so the leaves became dry and fell off (Figure 2).

Table 2. Plant growth parameters of 12 of *Justicia gendarussa* accessions

Accessions code	Plant height (cm)	Number of leaves/plant	Number of branches/plants
BG	71.35 ± 9.47abc	206.55 ± 56.22abcd	9.55 ± 1.50abc
BK1	53.97 ± 5.69d	172.11 ± 34.18abcd	10.50 ± 0.60ab
BK2	62.86 ± 6.93cd	147.93 ± 47.15bcd	5.91 ± 0.65c
BK3	77.21 ± 7.91ab	222.77 ± 60.69ab	8.90 ± 1.69abc
BK4	79.03 ± 2.03a	238.61 ± 42.90a	8.83 ± 1.64abc
MG1	64.18 ± 8.40bcd	168.78 ± 41.50abcd	8.27 ± 1.42abc
MG2	52.94 ± 2.53d	129.36 ± 34.81d	6.45 ± 0.78bc
TM	58.09 ± 4.15cd	174.61 ± 40.36abcd	11.00 ± 1.33a
TG	58.94 ± 15.02cd	221.50 ± 64.27abc	10.11 ± 3.18ab
LK	51.27 ± 8.23d	143.66 ± 54.07bcd	10.72 ± 5.23a
KP	78.42 ± 6.73a	251.66 ± 11.18a	10.55 ± 1.17ab
JB	60.97 ± 6.91cd	131.92 ± 45.16cd	5.67 ± 2.59c

Note: The average value in each column marked with a different letter is significantly different at $p<0.05$ with Duncan's test. BK1: Bekasi 1; BK2: Bekasi 2; BK3: Bekasi 3; BK4: Bekasi 4; MG1: Malang 1; MG2: Malang 2; TM: Tawang Mangu; TG: Trenggalek; LK: Langkat; KP: Kejayan Pasuruan; JB: Jember

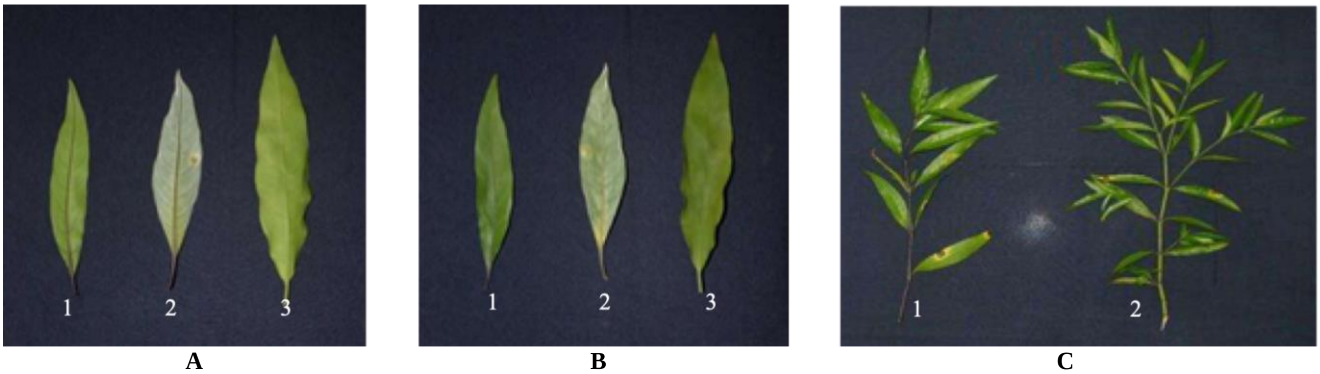

Figure 1. The morphological characteristics of *Justicia gendarussa* leaf and stem were observed at 16 weeks of age. A. leaf margin, B. leaf surface, C. stem with branches

Figure 2. *Justicia gendarussa* rusty leaves

Table 3. Biomass of leaf and stem of 12 accessions of *Justicia gendarussa*

Accession code	Wet weight (g)		Dry weight (g)	
	Leaves	Stem	Leaves	Stem
BG	73.50 ± 26.71ab	67.44 ± 12.13a	29.28 ± 2.11a	13.11 ± 4.00ab
BK1	46.16± 19.44bcd	24.94 ± 19.33c	10.33 ± 1.36cde	6.22 ± 4.02bc
BK2	30.94 ± 12.11cd	17.27 ± 7.39c	8.94 ± 4.09cde	8.17 ± 6.24abc
BK3	62.61 ± 3.17bc	50.50 ± 10.97ab	16.55 ± 1.70bc	12.22 ± 3.51ab
BK4	100.33 ± 33.93a	56.50 ± 16.97a	27.22 ± 6.03a	10.77 ± 5.12abc
MG1	52.94± 26.71bcd	30.78 ± 21.24bc	15.55 ± 3.75bcd	9.72 ± 4.29abc
MG2	31.49 ± 3.40cd	18.55 ± 1.70c	7.50 ± 1.60e	3.99 ± 0.28c
TM	43.99 ± 8.12bcd	21.00 ± 5.59c	12.94 ± 2.27bcde	6.50 ± 1.58bc
TG	48.72 ± 19.93bcd	26.05 ± 15.40c	14.38 ± 8.16bcde	6.99 ± 3.68bc
LK	26.22 ± 8.81d	12.61 ± 3.96c	8.61 ± 3.26de	3.61 ± 1.13c
KP	72.11 ± 6.46ab	59.94 ± 11.82a	19.78 ± 3.25b	14.22 ± 3.12a
JB	47.44± 19.19bcd	27.83 ± 14.58bcd	11.61 ± 4.35cde	7.11 ± 3.16bc

Note: The average value in each column marked with a different letter was significantly different at $p<0.05$ with Duncan's test. BK1: Bekasi 1; BK2: Bekasi 2; BK3: Bekasi 3; BK4: Bekasi 4; MG1: Malang 1; MG2: Malang 2; TM: Tawang Mangu; TG: Trenggalek; LK: Langkat; KP: Kejayan Pasuruan; JB: Jember

Plant biomass is used to determine plant productivity. There are significant differences in wet and dry weights of leaves and stems of *J. gendarussa* ($p<0.05$) (Table 3). The highest wet weight of leaves was obtained from BK4 accession (100.33±33.93 g), and the highest dry weight was 29.28±2.11 g from BG accession.

Meanwhile, the highest wet weight of stem was obtained from BG accession (67.44±12.13 g), and the highest stem dry weight was 14.22±3.12 g from KP accession. LK accessions had the lowest weight of wet stem (12.61±3.96 g) and dry weights (3.61±1.13 g). The lowest weight of wet leaf was 26.22±8.81 g from LK accessions, and the lowest weight of dry leaf was 7.50±1.60 g from MG2 accessions. The results indicate that accession has a significant effect on plant biomass. BK4 and BG accessions had the highest biomass compared to other accessions, and LK accession had the lowest biomass. The low biomass value of LK accession is due to a pathogen attack that causes rusty leaves and falls off before harvest time,

Polyphenols are compounds with antioxidant activity (Tungmunnithum et al. 2018), so they can be used to evaluate their pharmacological potential. TPC and TFC content of 12 accessions of *J. gendarussa* leaf varied significantly (Table 4). The highest TPC was obtained in TG accession (0.752 mg GAE/g DW), while the lowest was obtained in MG2 accession (0.314 mg GAE/g DW). The TFC values of 12 accessions of *J. gendarussa* were not significantly different (p<0.05). However, LK accession showed the highest value of TFC (1,889 mg QE/g DW), and the lowest was obtained in MG2 accession (1,204 QE/g DW). Therefore, it is recommended that the TG and LK accessions with the highest TPC and TFC could be used as superior accessions.

The results showed a positive correlation ($p < 0.01$, r value 0.80) between plant height (TT) and the number of leaves (JD), whereas the correlation between plant height (TT) and the number of branches (JC) was r 0.047. Correlation between plant height (TT) with wet weight (BB) and dry weight (BK) showed a positive correlation with values of 0.86 ($p < 0.001$) and 0.79 ($p < 0.01$), respectively. Meanwhile, a positive correlation was also shown between number of leaves (JD) and number of branches (JC) ($p < 0.1$, 0.53), wet weight (BB) ($p < 0.01$, r 0.81) and dry weight (BK) ($p < 0.01$, r 0.75). Correlation between number of JC, BB and BK, 0.21 and 0.26, respectively, while BB and BK showed a correlation of 0.92 ($p < 0.001$). The correlation between total phenolic content (TPC) and total flavonoid (TFC) with growth parameters is presented in Figure 3. The correlation coefficient between TPC with TT, JD, JC, BB, and BK were -0.27, - 0.036, 0.31, -0.1.9, and -0.081. While the correlation coefficient between TFC with TT, JD, JC, BB, and BK were 0.28, 0.27, 0.32, 0.16, 0.022, respectively. The positive correlation between flavonoids and plant growth indicates that plant growth influences flavonoid content., Flavonoids are also affected by plant growth. Accession of *C. aeruginosa* with its agro morphological characteristics positively correlated with phenolic content (Khumaida et al. 2019). A study by Ferreira et al. (2018) showed that plant age and plant height of Yacon leaves showed a negative correlation with TPC extract.

Table 4. Total phenolics and flavonoid content of 12 accessions of *Justicia gendarussa*

Accession code	Total phenolic (mg GAE/g DW)	Total flavonoid (mg QE/g DW)
BG	0.611±0.077ab	1.337±0.250a
BK1	0.655±0.078ab	1.685±0.135a
BK2	0.586±0.019ab	1.528±0.327a
BK3	0.576±0.126abc	1.443±0.173a
BK4	0.506±0.111abc	1.405±0.420a
MG1	0.547±0.341abc	1.491±0.646a
MG2	0.314±0.072c	1.204±0.501a
TM	0.599±0.078ab	1.395±0.166a
TG	0.752±0.128a	1.598±0.478a
LK	0.700±0.141ab	1.899±0.822a
KP	0.438±0.019bc	1.235±0.455a
JB	0.660±0.201ab	1.472±0.152a

Note: TPC: total phenolic content, TFC: total flavonoid content, DPPH: 2,2-diphenyl-1-picrylhydrazyl, FRAP: ferric reducing antioxidant power. BG: Bogor; BK1: Bekasi 1; BK2: Bekasi 2; BK3: Bekasi 3; BK4: Bekasi 4; MG1: Malang 1; MG2: Malang 2; TM: Tawang Mangu; TG: Trenggalek; LK: Langkat; KP: Kejayan Pasuruan; JB: Jember. The average value in each column marked with a different letter was significantly different at p<0.05 with Duncan's test

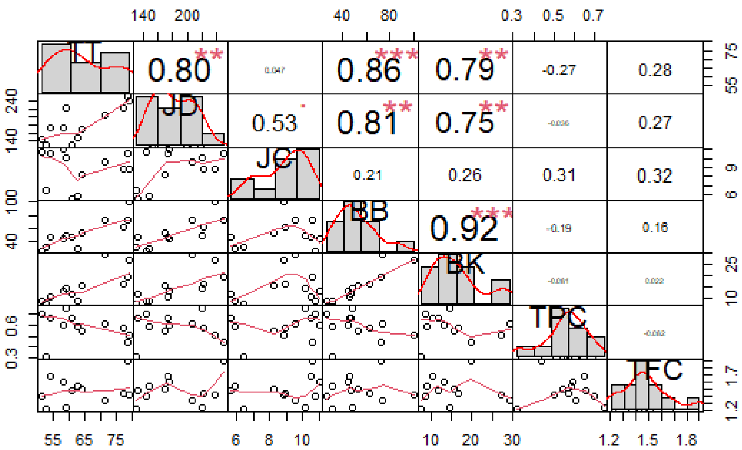

Figure 3. The correlation coefficient of: TT: plant height, JD: number of leaves, JC: number of branches, BB: wet weight, BK: dry weight, TPC: total phenolic content, and TFC: total flavonoid content. ˙, *, **, ***: significant level at p-value of 0.1, 0.05, 0.01, 0.001, respectively

Discussion

Plant observations were carried out at 4 months or 16 weeks of planting. It is due to the maximum number of leaves on the stem occurring at 4 to 6 months old. The younger stock could store more carbohydrates and other nutrients (Maheswari and Nivetha 2015). Previous research by Nurcholis et al. (2021) showed that *J. gendarussa* treated with manure and NPK had increased plant height, the number of leaves, and the number of branches (Nurcholis et al. 2021). However, the growth parameters in this study were higher. Plants have different responses between species and within species because plant growth is highly dependent on the adequacy of nutrients in the soil, which are usually obtained through plant roots (Morgan and Connolly 2013).

In general, *J. gendarussa* has green, white, or gray leaves (Chandra and Lo 2021) and dark green with dark purple to shiny brown stems (Putri et al. 2020). Pathogens often attack the leaves of *J. gendarussa*. Nearly 20 species of a pathogen called Puccinia attack the Justicia genus (Kumar et al. 2017). It is usually caused by the fungus *Puccinia hwaitessi* Berk. Et Br. results in leaves becoming dry leaves and falling off and continuing on dry branches (OpeTe 2022).

Secondary metabolites could be useful and important for plant defense systems against pathogen attacks and environmental stresses (Yang et al. 2018). Due to the bioactivities of secondary metabolites, they are increasingly used as therapeutic drugs. Phenolic compounds consist of several classes, such as flavonoids. Flavonoids have strong antioxidant properties. Flavonoids are naturally found in plants and are considered to benefit human health; and exhibit a wide range of activities, namely anti-inflammatory, antiviral, antibacterial, and various other degenerative diseases. Several studies have reported variations in the concentration of metabolites caused by environmental factors.

Place of cultivation, climate, altitude of planting location, temperature, and exposure to sunlight affects the production of secondary metabolites, including phenolics and flavonoids (Banerjee and Bonde 2011) qualitatively and quantitatively, and variations in bioactivity. For example, Nurcholis et al. (2021) reported that combining NPK fertilizer and manure significantly impacts the sugar content and growth productivity in *J. gendarussa*. In addition, it affects the levels of phenolics and flavonoid compounds. In this study, 12 accessions of *J. gendarussa* had various agro morphological characteristics, including plant height, number of leaves, number of branches, wet weight, and dry weight of the plant. In addition, each accession contained phenolics and flavonoids. Accessions BK4, BG, TH, and LK had the best plant growth and total phenolic and flavonoid content so that they could be further developed into superior varieties of *J. gendarussa*.

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