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Morphological characters, phenolic and flavonoid contents of *Vitex trifolia* accessions from Lamongan District, Indonesia

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Abstract. *Nisa A, Kurniawati A, Faridah DN. 2023. Morphological characters, phenolic and flavonoid contents of* Vitex trifolia *accessions from Lamongan* District, Indonesia. *Biodiversitas 24: 1635-1641.* Indonesia is a country with high biodiversity. *Legundi* (*Vitex trifolia* L.) is one of the plant species that has long been used as a raw material for traditional herbal medicine. This study aims to determine the morphological, agronomic, leaf production, and total phenolic and total flavonoid content of the *V. trifolia* accession from Lamongan District, East Java, Indonesia. Morphological and agronomic characterizations were observed using qualitative and quantitative parameters. The colorimetric method determined the total phenolic and flavonoid content in *V. trifolia* extract. The results showed differences in morphological and agronomic characters between accessions. The Bluluk accession had the highest leaf production that did not differ from the Pucuk, Sekaran, and Kedumpring accessions, while the Modo accession had the lowest. There was no difference in total phenol and flavonoid content between accessions. Leaves had the highest total phenolic content (3210.57 GAE mg/100 g dry powder), followed by twigs (1331.98 GAE mg/100 g dry powder) and fruit (973.77 GAE mg/100 g dry powder). Leave also contained the highest flavonoid content (1792.90 QE mg/100 g dry powder). The highest phenolic and flavonoid content in the leaves of *V. trifolia* may support its use in traditional medicine.

Keywords: Cluster analysis, fruit, leaves, production, twigs

INTRODUCTION

Indonesia is a country with high biodiversity, including various medicinal plants. Medicinal plants as raw materials for herbal medicines are based on local knowledge passed down from generation to generation (Hidayat and Hardiansyah 2012). *Legundi* (*Vitex trifolia* L.) is one of the plant species that has been used as herbal medicine. *V. trifolia* is a shrub plant that grows in the wet tropics. It originated in East Africa (POWO 2022) and is distributed in South Africa, Asia, Australia, and Asia Pacific (Heim 2015). *V. trifolia* has several local names, namely *langgundi* (Minangkabau), *gandasari* (Palembang), *lagundi* (Malay), *lagondi* (Sunda), l*egundi* (Central Java), *langghundi* (Madura), *galumi* (Sumba), s*angan* (Bima), *laura* (Makassar), *lawarani* (Bugis), *man jing zi* (China) (Herbie 2015). *Vitex* is the largest genus in the Verbenaceae family which consists of 250 species distributed throughout the world. *Vitex* (bush leaves) commonly used in herbal medicine are *Vitex agnus-castus* L. (sacred tree) scattered along the coast in the Mediterranean region, Southern Europe, and Southeast Asia. *V. trifolia* is distributed in Asian countries and Vietnam (Rani and Sharma 2013).

The pharmacological effects of *V. trifolia* leaves include being a sedative, fever medicine, inflammation, increasing body weight, healing wounds, anti-repellent against mosquitoes (Arpiwi et al. 2020), antibacterial (Sukarsih et al. 2021), anti-HIV, anti-cancer activity (Gong et al. 2021), antioxidant (Musa et al. 2020) and antipyretic (Chan et al. 2016). Empirically *V. trifolia* leaves are used to reduce pain, rheumatism, and asthma, treat wounds, and as a urine laxative, treat fever, and insecticide. *V. trifolia* leaves contain vitexicarpin (casticin), which has an antiproliferative and apoptotic activity that is efficacious as a tracheoplasmotic (Chan et al. 2018). Casticin is a phytochemical compound found in the leaves, roots, flowers, and fruit of *V. trifolia*. It has anti-inflammatory, anticancer, antibacterial, and antioxidant (Shukri and Hasan 2021). The flower of *V. trifolia* is used to treat fever. In addition, *V. trifolia* is widely used as an antibacterial, sedative, and rheumatic drug in Asian countries (Tiwari et al. 2013). *V. trifolia* fruit is used as an anti-inflammatory (Fang et al. 2018), inhibits the division and growth of tumor cells, and relieves headaches, rheumatism, migraines, sore eyes, and colds (Natheer et al. 2012). China and Korea used dried *V. trifolia* fruit to remedy asthma and allergic diseases (Chan et al. 2016). *V. trifolia* can be used as an antiasthma, allergy, and anticancer (Hanudin 2015).

Vitex trifolia leaves in Indonesia have been used in the pharmaceutical industry, especially traditional medicines in the modern herbal medicine industry. Standardized raw materials could be started from the cultivation aspect, especially good agricultural practices (GAP). The potency of *V. trifolia* could be carried out by characterizing *V. trifolia* accessions that grow in Indonesia. Quantitative characters include plant height, number of stems, number of leaves, leaf length, and leaf width. The parameters of qualitative characteristics were leaf color, leaf shape, stem color, rhizome color, and texture of rhizome skin (Setiadi et al. 2017). Morphological and agronomic characteristics are used to evaluate and select plants (Occhiuto et al. 2014). The growth and production become the basis for cultivation technology to produce good quality and standardized products.

Vitex trifolia plants from each location are local accessions that have not been morphologically described. The current problem is that there is no information regarding the characterization or description of local *V. trifolia* accessions, so it is necessary to study the production and the content of natural compounds which characterize the quality of *V. trifolia* products. Therefore, character identification from the juvenile phase to mature and productive plants are needed to provide information on the production potential of each local *V. trifolia* accession, especially from Lamongan District, East Java, Indonesia. This study aims to identify morphological and agronomic characters and to determine the accession with the highest leaf production and the total phenol and flavonoid content in various plant parts.

MATERIALS AND METHODS

Study sites

The research was conducted in February-November 2021 at the Cikabayan Experimental Garden and Post-Harvest Laboratory of Department of Agronomy and Horticulture, Faculty of Agriculture, Institut Pertanian Bogor, Indonesia.

Materials

Stem cuttings of *V. trifolia* were taken from 5 places in Lamongan District, Indonesia, i.e.: Pucuk, Sekaran, Kedumpring, Modo, and Bluluk. The equipment used is material tools for plant cultivation. The chemical used in this study were ethanol (MSDS), aquadest, Iron (III) chloride hexahydrate (MSDS), sodium hydrogen carbonate (Merck 1.06329.1000), Folin-Ciocalteau (Merck 1.09001.0500), quercetin (Sigma) 50 mg and gallic acid standard.

Research design

This research consists of two experiments. The first experiment used a one-factor Randomized Block Design (RBD), namely *V. trifolia* accession. There are five accessions, namely the Pucuk accession, Sekaran accession, Kedumpring accession, Modo accession, and Bluluk accession, collected from Lamongan District, East Java, Indonesia. These accessions were propagated and then planted in Bogor District, West Java, Indonesia. Each treatment had three replications, so there were 15 experimental units. The second experiment used a 2-factor nested plot design (NPD). Five *V. trifolia* plant accessions (Pucuk, Sekaran, Kedumpring, Modo, and Bluluk) and three types of plant parts (leaves, twigs, fruit), where plant parts are nested in accessions. Each treatment has three replications, so there were 45 experimental units.

Morphological and agronomical characteristics

Several morphological characteristics were observed. They include leaf shape, leaf tip shape, leaf base shape, color and texture of the upper surface of the leaf, color, and texture of the fruit surface of the leaf, flower appearance time, flower petal color, stem color, and stem surface texture (Tjitrosoepomo 2008). The agronomic characteristics include plant height, number of leaves, branches, age at the start of flowering, and leaf production. Observations were made every two weeks, and leaf harvest was carried out 17 weeks after planting or 119 days.

Extraction

Leaves were taken from number one to three of the youngest ends. Leaf, twig, and fruit samples were dried at 60°C for 24 hours until the moisture content was approximately 10%. Furthermore, the sample was blended, sifted, and stored in a closed container with silica. Samples were weighed ±1-2 g of the powder and then dried in the oven at 105ºC for 5 hours or to constant weight to determine the moisture content. Two g of simplicia powder was macerated in 70% ethanol solvent. After maceration, the sample was homogenized using a vortex for 15 minutes, followed by 15 min sonication. The sample was re-macerated for 24 hours in a dark room, then centrifuged for 15 minutes at 5000 rpm. The filtrate was evaporated until the extract volume was 10 mL (Koley et al. 2018).

Analysis of total phenolic and flavonoid content

Total phenolic content was determined using the Folin-Ciocalteu reagent with the standard curve of gallic acid from various concentrations, namely 2 ppm, 5 ppm, 35 ppm, 50 ppm, 75 ppm, and 100 ppm. The final unit of analysis is expressed as mg gallic acid equivalent (GAE)/100 g of dry powder (Vongsak et al. 2013). Analysis of the total flavonoid content was determined by the aluminum chloride ($AlCl_3$) colorimetric method. $AlCl_3$ reagent with hydroxyl groups from flavonoid compounds forms complex yellow compounds (Pothitirat et al. 2009). The results are then expressed in mg Quercetin Equivalent (QE)/100 g of dry powder (Vongsak et al. 2013).

Data analysis

Data were analyzed using the F test. Differences between treatments were tested by Tukey's Honest significant difference (HSD) test at a 5% significance level. It is analyzed using the R Studio. The cluster analysis was carried out using SPSS version 23.

RESULTS AND DISCUSSION

The geographic locations of origin for each *V. trifolia* accession are presented in Table 1. All accessions were obtained from the lowlands; *V. trifolia* grow from 100 to 1,000 m above sea level (asl) (SCV 2022). Based on the Meteorological, Climatological, and Geophysical Agency (BMKG) climate data from the Bogor climatology station, weather conditions during the planting show that in February-August 2021, rainfall ranges from 115.6-510.3

mm per month^{-1} with average temperature and humidity per month ranging from 25-16ºC and 74-86%. Based on these data, the climatic conditions at planting are suitable for *V. trifolia* growth.

Morphological characters and cluster analysis

Leaf morphological characters are presented in Figure 1. The characters of the leaves in five *V. trifolia* accessions were not different. The leaves were elliptical trifoliate compound leaves with pointed ends and bases. *V. trifolia* flowers are borne in panicles or clusters up to 18 cm long. The flower of all *V. trifolia* accessions is purple, consisting of a tube with five lobes; the middle lobe is larger than the others, has four white stamens with a purple head, and two stamens are longer than the crown (Figure 2). Different accession had different lengths of time to flower. Bluluk accession was the fastest accession to flower, i.e., 66.44 days, followed by Modo accession, which needs 75 days to produce the flower. Accession of Pucuk, Sekaran, and Kedumpring begin to flower around 80-83 days after planting, so they are categorized as the late flowering category (Figure 3). Differences in the length of time to the flower emergence can be seen from the grouping of accessions on the dendrogram of the cluster analysis (Figure 4). The cluster analysis results of the five accessions showed three groups at a coefficient of variance of 0.80%. The first cluster consists of three accessions, i.e., Pucuk, Sekaran, and Kedumpring. The second cluster consists of the Bluluk accession, and the third cluster is the Modo accession.

Agronomy characters

The agronomic characteristics of the five accessions significantly differed in plant height, number of leaves, branches, and dry weight. The Bluluk accession had the highest plant height from the whole observation. However, it was not significantly different from the Pucuk, Sekaran, and Kedumpring accessions, while the Modo accession had the lowest plant height (Figure 5). The number of leaf branches indicates that the Bluluk accession has a significantly higher number of branches than the Modo accession but is not significantly different from the Sekaran accession (Figure 6). The number of leaves in the five accessions was significantly different, and the Bluluk accession had the highest number of leaves, significantly different from the Kedumpring and Pucuk accessions (Figure 7). Plant height, number of branches, and number of leaves affect the total yield of the *V. trifolia* accessions (Table 2).

Table 1. The geographic location of origin of the *Vitex trifolia* accessions

Origin of accession	Latitude (ºN)	Longitude (ºE)	Altitude (m asl.)
Pucuk, Lamongan	7'97'7	112.13'10	19.71
Sekaran, Lamongan	7'42'9	112.26'12	17.00
Kedumpring, Lamongan	7'23'6	122.04'44	26.74
Modo, Lamongan	7'27'7	112.13'10	55.88
Bluluk, Lamongan	6'51'6	112'33'12	63.56

Source: BPS Data for Lamongan District, East Java 2018

Figure 1. *Vitex trifolia* leaf characters. A. Leaf shape; B. Young leaves; C. Mature leaves

Figure 2. *Vitex trifolia* flower and fruit. A. Emerging flowers at 65 days after planting (DAP); B. Flowers at 9 days after 1st emergence; C. Full bloom flowers (blooming for 1-2 days); D. Fruits at 25-30 days after the flower fall

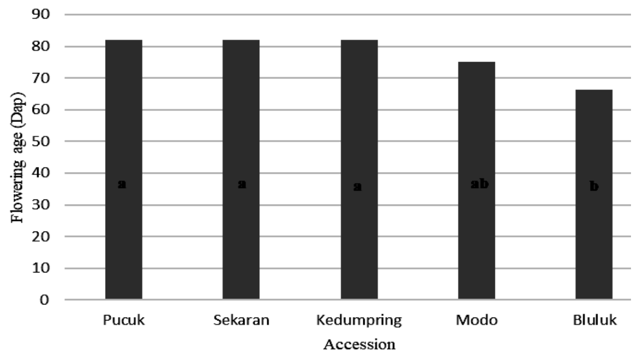

Figure 3. The length of time to flower in five *Vitex trifolia* accessions

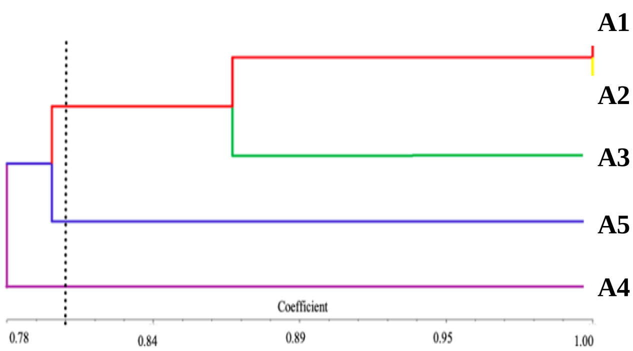

Figure 4. Dendogram of five *Vitex trifolia* accessions: A1: Pucuk; A2: Sekaran; A3: Kedumpring; A4: Modo; A5: Bluluk

The results showed that the fresh leaves' weight was twice that of twigs. Meanwhile, the dry weight of the leaves is lower than the weight of the twigs due to the water evaporation that occurs during the drying process and affects the dry weight (Syafrida et al. 2018).

Total phenolic and total flavonoid content

The total phenolic and flavonoid content of leaves, twigs, and fruit are presented in Figure 8. Plant parts affect the total phenol and flavonoid content. Leaves had the highest total phenolic and total flavonoid content, followed by twigs and fruit, Twigs and fruit with similar total flavonoid content. The total phenolic and flavonoid content of the five *V. trifolia* accessions is shown in Figure 9. Total phenolic and total flavonoid content in five *V. trifolia* accessions were not significantly different.

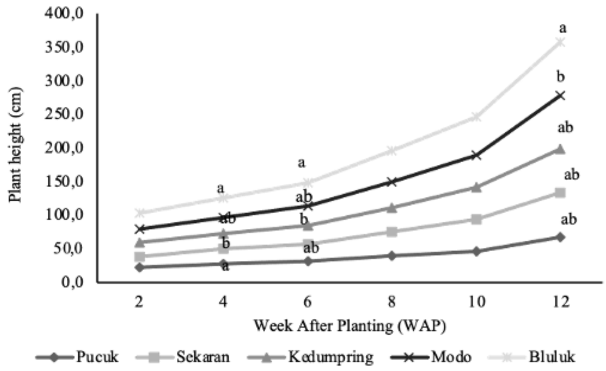

Figure 5. Plant height of five *Vitex trifolia* accessions

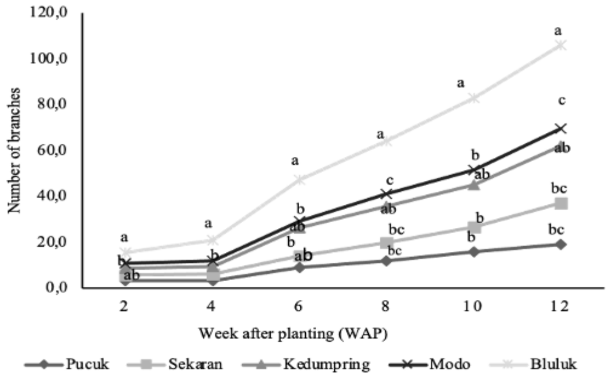

Figure 6. Number of branches of five *Vitex trifolia* accessions

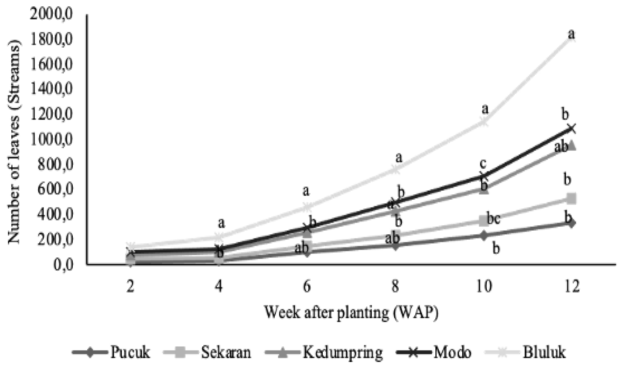

Figure 7. Number of leaves of five *Vitex trifolia* accessions

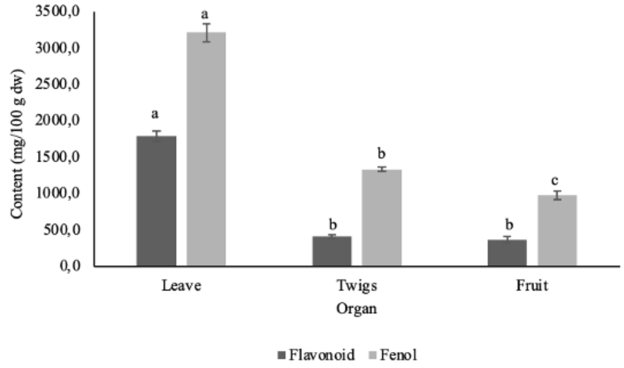

Figure 8. Total phenolic and flavonoid content of the leaves, twigs, and fruit of *Vitex trifolia*

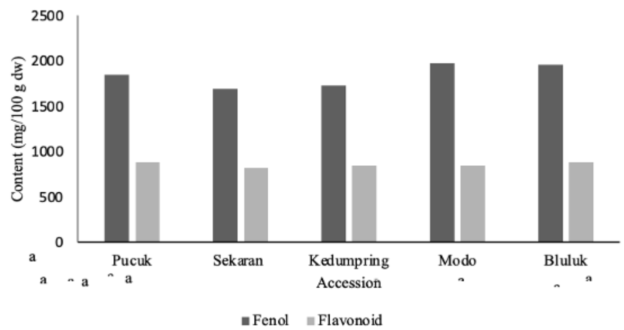

Figure 9. Total phenolic and flavonoid content of five *Vitex trifolia* accessions

Table 2. The wet and dry weight of five *Vitex trifolia* accessions

Accession	Fresh weight			Dry weight		
	Leaves (g)	Twigs (g)	Total (g)	Leaves (g)	Twigs (g)	Total (g)
Pucuk	4662,67^{a}	2134,33^{a}	6797,00^{a}	813,00^{ab}	1018,33^{a}	1831,33^{a}
Sekaran	5313,66^{a}	2349,33^{a}	7663,00^{a}	937,37^{ab}	1235,67^{a}	2173,03^{a}
Kedumpring	6506,67^{a}	3570,00^{a}	10076,67^{a}	1192,10^{a}	1514,30^{a}	2706,43^{a}
Modo	895,67^{b}	437,00^{b}	1332,67^{b}	202,67^{b}	257,03^{b}	459,70^{b}
Bluluk	6783,00^{a}	3467,33^{a}	10250,33^{a}	1267,87^{a}	1505,67^{a}	2773,53^{a}
F Test	**	**	**	**	**	**

Note: numbers followed by the same letter in the same column are not significantly different at the 5% HSD test

Discussion

Morphological characteristics of the leaves and flowers of V. trifolia *accessions*

Leaves. Leaves are the most crucial plant part for plant metabolism through photosynthesis. The leaves have four functions, i.e., absorption, assimilation, transpiration, and respiration (Putriani et al. 2019). Leaf characters are essential in identifying plant species and are related to the phytochemical content (Salvana et al. 2019). *V. trifolia* leaves are compound leaves that consist of three to five separate leaflets. The shape of the *V. trifolia* leaf is an elliptical, pointed tip and base, green on the upper and lower surfaces, with a rough and hairy lower surface. The *V. trifolia* leaves of the Pucuk, Sekaran, Kedumpring, Modo, and Bluluk accession have narrow and pointed leaf shapes. Leaf morphology is fundamental in identifying and classifying plant species (Masungsong et al. 2022).

Flowers. *V. trifolia* plants have many flowers (planta multiflora) located at the tip of branches. *V. trifolia* flowers grow terminally in groups, including the complete flower, because they have petals, corolla, pistils, and stamens. The *V. trifolia* flowers of the Pucuk, Sekaran, Kedumpring, Modo, and Bluluk accessions have the same stamens. Each flower has four stamens near the middle of the corolla tube and between, with longer stamens with a hairy pistil. The fruit is a watery or dry drupe with a rigid wall and a diameter of about 6 mm. The flower morphology of the five accessions is similar. The flower morphology of *V. trifolia* is identical to *Vitex negundo* L. and *Vitex rotundifolia* L.F, which has a terminal inflorescence with five purple lobes covered with hairs (POWO 2022).

Vitex trifolia flowering time ranges from 66 to 82 days or 10-12 weeks after planting. Accessions that start flowering on days 60-70 are classified as fast flowering; 70-80 is relatively fast, and 80-90 days as slow flowering. The leaves of *V. trifolia* have been used as herbal medicine. Therefore, *V. trifolia* is expected not to flower quickly. Fast-flowering accession needs cultivation techniques to delay the generative phase, for example, by pruning the crown. The time required for flower buds to fully bloom in the five accessions was ±13 days. Flower development begins with the emergence of tiny buds between the leaves of the apical terminal growing point and the axillary terminal growing point. Flowers bloom in the afternoon, around 14.00 to 17.00, with a purple crown, then the flower closes. The next day the flowers bloom again, then the crown falls off during the day. The emergence of fruit followed the fall of the crown.

Cluster analysis

The cluster analysis showed that five *V. trifolia* accessions were grouped into three groups. The grouping of *V. trifolia* accession based on the flowering time with a coefficient of 78-100% variation. Based on these results, the Modo accession is distantly related to the Pucuk, Sekaran, and Kedumpring accessions with a similarity coefficient of 80%. Grouping relates to genetic, breeding systems, and phenotypic similarities (Popoola et al. 2016). According to Raiswati et al. (2018), the grouping of *tempuyung* accessions (*Sonchus arvensis* L.) based on ecological similarity of accession origin, stomatal density, chlorophyll content, plant height, number of internodes, number of leaves, dry leaf weight, fresh leaf weight, and total flavonoids. The coefficient line close to zero shows the similarity of a character, and the coefficient line farther away from zero had low character similarity. For example, the sweet tuber shape character with a similarity coefficient of 0.96 has a dissimilarity value of only 4% (Hetharie et al. 2018). With a coefficient of 0.80, *V. trifolia* has a 20% dissimilarity in flowering characters. Therefore, the similarity level of the five accessions of *V. trifolia* was categorized as high.

Agronomy characters (plant height, number of branches, number of leaves, and yield)

Bluluk accession had the highest plant height but did not differ significantly from Pucuk, Kedumpring, and Sekaran accessions until six weeks after planting. Meanwhile, the Modo accession has a lower plant height than the Bluluk accession but is similar to the Pucuk accession. Plant height determines leaf production because the taller the plant, the higher the leaves emerge. The taller the plant, the farther apart the nodes are and the wider the leaf size (Nurlela et al. 2022). The taller the plant, the more nodes from which leaves emerge. *V. trifolia* was harvested by pruning at a certain height above the ground. Therefore, the taller the plant, the more leaves can be harvested from the stems and branches. However, the management of plant height must be considered so that it remains affordable and easy to harvest.

The number of twigs correlates to leaf production. Research by Febjislami et al. (2018) on *Orthosiphon stamineus* Benth. plants showed that the number of *O. stamineus* leaves correlates with the number of nodes and twigs because the leaf of the *O. stamineus* plant grows in pairs on each node. *V. trifolia* leaves are arranged in pairs facing each other, with the next leaves' position alternating, resembling the *O. stamineus* leaves. Plants with the most significant height gain and twigs have the highest number of internodes, which results in increasing leaf production. The weight of fresh leaves was 50% higher than that of fresh stem weight. However, the dry weight of stems is 75% higher than leaves. It is due to the water content of twigs that is usually lower than in the leaves. The number of twigs affects the increase in the dry weight of a plant's stem. The number of twigs determines the number of nodes related to the production of leaves harvested on the *O. stamineus* (Febjislami et al. 2018).

Total phenol and total flavonoid content

The analysis of five *V. trifolia* plant accessions on the leaf, twig, and fruit organs with ethanol solvent showed that leaves had the highest phenolic content, i.e., 3210.57 mg GAE/100 g of dry leaf powder. The total phenolic content of n-hexane extract of the leaves, twigs, and fruit of *V. trifolia* was 3612, 1302, and 696 mg GAE/100 g dry extract, respectively. The total phenolic content in leaves, twigs, and fruit methylene chloride extract was 16,008, 5998, and 3521 mg GAE/100 g dry extract, respectively. Furthermore, the total phenolic content of the ethyl acetate extract of leaves (29,726), twigs (22,789), and fruit (2420) mg GAE/100 g dry extract. Results of analysis with methanol extract of leaves (17203), twigs (11287), and fruit (1697) mg GAE/100 g dry extract (Khamweera and Janupinthusophon 2021). Ethanol is a solvent with a low boiling point that tends to be safe and has a high degree of polarity. Hexane is a stable non-polar solvent that does not quickly evaporate (Munawaroh and Handayani 2010). A study by Sembiring et al. (2018) showed that the highest phenolic compounds in *Caesalpinia bonduc* L. Roxb were found in ethanol extract of leaves (146.64 mg GAE/g crude extract), and the lowest was in fruit extract (70.34 mg GAE/g). The total phenolic content in *V. trifolia* leaves accession from Lamongan was higher.

Phenolics are included in polar compounds, and phenolics will readily dissolve in polar compounds, namely acetone. Ethanol is a semi-polar solvent that can dissolve polar and non-polar compounds; ethyl acetate is a semi-polar solvent that cannot attract polar compounds. Pratt and Hudson (1990) stated that phenolic compounds consist of large molecules with various structures, the main characteristic of which is an aromatic ring with a hydroxyl group. Phenolic is acidic due to the nature of the easily detached H+ group. Flavonoid compounds are non-polar compounds, but flavonoids have a sugar group that causes them to dissolve quickly in polar or semi-polar. Kar et al. (2006) stated that flavonoid compounds are non-polar compounds commonly found in plant stems. Flavonoid compounds dissolve in polar solvents such as methanol, ethyl acetate, etc.

Phenolic compounds are a source of antioxidants that effectively suppress free radicals. The mechanism of phenolic compounds as antioxidants is through the components of the phenol group that pair with free radicals by donating their hydrogen atoms through electron transfer. This process converts phenols into phenoxyl radicals. Phenoxyl radicals can stabilize themselves through a resonance process so that a chain reaction does not occur in forming radicals (Janeiro and Brett 2004). Phenolics in plants are important as protective compounds when environmental stresses occur, such as high light, low temperatures, pathogenic infections, and nutritional deficiencies, and they cause the formation of free radicals in plants (Lattanzio 2013). In addition, many groups of phenolic compounds have activities as antibacterial, anticancer, anti-inflammatory, cardioprotective, skin protector from UV rays (Tungmunnithum et al. 2018), and antidiabetic (Albuquerque et al. 2021).

The analysis of total flavonoids showed that leaves, twigs, and fruit contained flavonoids, and the highest flavonoid content was obtained from the leaves (1792.90 mg QE/100 g dry powder). A previous Dwinatari and Murti (2015) study showed that the highest vitexicarpin content is found in leaves 1-3. Vitexicarpin is a flavonoid compound contained in *V. trifolia* leaves with anti-asthmatic properties. Flavonoids are natural compounds known to have anticancer, antioxidant, anti-pathogenic bacteria, anti-inflammatory, anti-cardiovascular, and free radical scavengers (Arifin and Ibrahim 2018). Leaf extracts of *V. negundo* and *V. trifolia* L. have a high total phenolic and flavonoid content that could be an essential source of antioxidants (Saklani et al. 2017). A study by Sembiring et al. (2018) on *gorek* plants (*Caesalpinia bonduc* L. Roxb.) showed that the highest total flavonoid content was obtained from the leaves (31.05±0.35 mg QE/g), while the lowest was in the roots (12.55±0.08 mg QE/g). Prayoga et al. (2019) stated that the total flavonoids in pepe leaves (*Gymnema reticulatum* Br.) resulting from ethanol extraction were 2010 mg QE/100 g dry extract. The total flavonoid content of insulin leaves (*Smallanthus sonchifolius* (Poepp. & Endl.) H.Rob.) is 2842 mg QE/100 g dry extract. The flavonoid content in pepe and insulin leaves was lower than in *V. trifolia* leaves.

The total flavonoid content in *Halimium halimifolium* (L.) Willk. leaves was higher, while the total flavonoid content was lower in flowers (Rebaya et al. 2014). The total flavonoid content in the leaves of *H. halimifoliu* is also higher than interest (Rebaya et al. 2014). The content of flavonoids in bitter leaves (*Vernonia amygdalina* Del.) increased at longer harvest intervals (Betty et al. 2021). Each part of the plant, such as leaves, stems, and roots, has different physiological functions, so the levels of metabolites are also different (Alfarabi et al. 2022). The total phenolic content in *V. negundo* leaves was 8971 mg GAE/100 g dry extract, and in *V. trifolia* leaves was 7720 mg GAE/100 g dry extract. The total flavonoids in *V. negundo* leaves are 6311 mg QE/100 g dry extract, and the flavonoid content in *V. trifolia* leaves is 5740 mg QE/100 g dry extract (Saklani et al. 2017). The total phenols and flavonoid content of *V. trifolia* leaves were three times higher than in this study. Plant parts of *V. trifolia* (leaves, twigs, and fruits) had significantly different total flavonoids. However, plant accession did not significantly affect the total phenolic and flavonoid content.

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