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THE FLAVONOID AND ALKALOID CONTENT OF CYCLOSORUS PARASITICUS (LINN.) FARWELL FERNS AT THE PLANTATION AREAS OF JEMBER REGENCY

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Abstract

Cyclosorus parasiticus (Linn.) Farwell is one potential medicinal ferns. The plant contains secondary metabolites, such as flavonoids and alkaloids. This study aims to determine the flavonoid and alkaloid content of *Cyclosorus parasiticus* (Linn.) Farwell in three plantation areas of Jember Regency. Plant samples were collected from Mount Gumitir coffee plantations, Pine Garahan Village and rubber and cocoa plantations in Tancak in Jember. Samples of stems and leaves were dried at room temperature and then blended to obtain a powder. One gram of powder samples was macerated in 90 ml of methanol for 3x24 hours then was concentrated with an evaporator to obtain a crude extract. The crude extract was tested qualitatively for flavonoids and alkaloids by the Willstätter and Dragendorff methods followed by quantitative tests with Spectrophotometric. The results showed that both flavonoids and alkaloids were found in stem and leaves of *Cyclosorus parasiticus* (Linn.) Farwell growing at three research locations. The flavonoids and alkaloids content found in leaves was higher than those in stem organs. The leaves of *Cyclosorus parasiticus* (Linn.) Farwell in Gumitir contain the highest flavonoids and alkaloids content than those in Tancak.

Key Words: Alkaloids, flavonoids, content, *Cyclosorus parasiticus*

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INTRODUCTION

Cyclosorus parasiticus (Linn.) Farwell is one of the thelypteridaceae fern tribe that has characteristics such as fibrous blackish brown root, green stem, hairy, brown base and scaly. In addition, both sterile and fertile leaves have the same shape (lanceolate) and size. Both leaf surfaces are hairy, pinnate vein leaves. The arrangement of the leaves is alternate, sori are superficial, round, arranged regularly cover the entire edge of the leaves (Duncan and Isaac, 1986; Cobb and Farnsworth, 2005; Nasution et al., 2018).

These ferns are generally grown in tropical and subtropical regions such as Indonesia, Malaysia, Asian, Queensland, Pacific to Tahiti, Hawaii, Africa, St. Helena and India as well as the northeastern and central parts of North America (Cobb and Farnsworth, 2005). According to Kinho (2009), *Cyclosorus parasiticus* is a terrestrial fern growing in open or shade areas.

Many people get benefits from this plant species for treatment such as antimalarial (Wei et al., 2016), itchy skin and muscle bruising, excessive body fluids, anticancer (Fang et al., 2011), anti-inflammatory (Tangavelou & Viswanathan, 2017), gout and rheumatism (Singh, 2003). Utilization of ferns *Cyclosorus parasiticus* as drugs is allegedly due to the content of its secondary metabolites (Astuti et al., 2013).

Previous research reported that *Cyclosorus parasiticus* contains several secondary metabolites, among them are flavonoids and alkaloids found in the leaves (Tangavelou and Viswanathan, 2017).

The content of secondary metabolites such as flavonoids and alkaloids in ferns varies depending on numerous factors such as the environment (ie temperature, humidity, pH, location, soil), extracted plant parts, age, harvesting extraction and methods (Chikmawati et al., 2013, and Nasution et al., 2018). According to Ahmad et al. (2015) the composition of these secondary metabolites varies in plant organs. The fern leaves may contain higher secondary metabolites compared to other organs. Differences in the composition and content of secondary metabolites are influenced by the growth environment (Chikmawati et al., 2013).

The fern species of *Cyclosorus parasiticus* in Jember, East Java is found in three locations: coffee plantations Mount Gunitir, pine forests Garahan village, and area of Tancak waterfall. The forest in Mount Gunitir is classified as Gunitir plateau that lies at an altitude of 700-850 meters above sea level (Astuti, 2018). Pine Forest in Garahan Village classified as mid plains located at an altitude between 550-551 meters above sea level (Makhmud, 2018). On the other hand, Tancak waterfall located in rubber plantations and cocoa

plantations Gunung Pasang Desa Suci subdistrict Panti is situated at an altitude of 300-900 meters above sea level (Baihaki, 2016). However, the flavonoid and alkaloid composition variability of the *Cyclosorus parasiticus* in those three locations have not been investigated yet. Therefore, it is necessary to investigate flavonoids and alkaloids compounds found in the *Cyclosorus parasiticus* (Linn.) Farwell fern in Mount Gunitir coffee plantations, Pine Garahan Village and Tancak Plantation in Jember. These three areas have height differences that may influence the content of flavonoids and alkaloids in *Cyclosorus parasiticus*.

MATERIALS AND METHODS

This research study was conducted in November 2018 until June 2019. Data sampling was carried out at three locations: coffee plantations of Mount Gunitir, pine plantation of Garahan village, and rubber and cocoa plantations of Tancak in Jember.

The tools and equipments used in this study were an analytical balance, petri dishes, shelves and test tube, flask, beakers, micropipette, beaker glass, glass bottles, rotary evaporator, Spectrophotometer UV-Visible, blender, sieve, GPS, Termohigrometer, Lux meter, Soil tester, and Anemometer.

The materials used in this study were filter paper, tissue, distilled water, methanol, ethanol 70%, FeCl₃, NaOHm, HCL 2N, Dragendorff reagent, Bromocresol green solvent, chloroform, AlCl₃, potassium acetate, and magnesium.

Research procedure

Sampling

One kilogram *Cyclosorus parasiticus* ferns samples were taken each from three locations, namely in the area of coffee plantation Mount Gunitir, pine forest area in Garahan Village Silo Subdistrict, and rubber and cocoa Tancak plantations Gunung Pasang Desa Suci, Panti subdistrict, Jember Regency.

Preparation and Sample Extraction

Cyclosorus parasiticus plant samples were separated between the stems and leaves. These samples then were washed with running water. The leaves and stems samples were cut into small pieces, and dried until a constant weight. These dried samples were mashed up by blending and sieving to obtain leaves and stems dust called simplicia. The simplicia subsequently were put into glass bottles and stored at a temperature of 180C. Simplicia was ready for secondary metabolites analysis.

As much as 1 gram of *C. Parasiticus* stem and leaves simplicia were soaked in

90 mL of methanol or a ratio of 1: 9 (Yudharini et al., 2016) for 3x24 hours. Afterward these simplicia were filtered and concentrated by rotary evaporator for \pm 15 minutes. The crude extractsof both leaves and stem obtained was ready to be analyzedfor secondary metabolites contents.

Qualitative Test of Flavonoids by Wilstatter Method and Alkaloids by Dragendorff Method

Qualitative test of flavonoid was done by adding 1 mL of crude extract, 1 mL of 70% ethanol, 0.1 grams of magnesium powder, and 10 drops of concentrated HCl. This mixed extract was shaken vigorously so that it changedcolor. Flavonoid positive test was indicated by the formation of red, yellow or orange (Mainawati et al., 2017).

Alkaloid test was conducted by taking a crude extract as much as 1 mL adding 2 mL of 2N HCl and then heating for 5 minutes and then filtering. The extract was added 2 drops of Dragendorffreagent. A positive result was indicated by the formation of alkaloids test precipitate of red brick (brown to orange) (Ning et al., 2016).

Quantitative Test of flavonoids and alkaloids:

Determination of the maximum wavelength (λ maks) of quercetin and berberine.

The compound used as a standard in determination of flavonoids level was quercetin, since quercetin is a flavonoid class of flavonolsthat have a keto group at atom C-4 and also the hydroxyl groups of atoms C-3 and C-5 which neighbors (Aminah et al., 2017). Determination of the maximum quercetin wavelength was done by running quercetin solvent at a wavelength range of 400nm, 405nm, 410nm, 415nm, 420nm, 425nm, 430nm, 435nm, 440Nm, 440Nm and 450 nm (Aminah et al., 2017).

The compound used as the standard determination of alkaloids level was berberine, since berberine is one of the alkaloids in the plant of turmeric extract (*Fibraurea tinctoria* Lour) (Utami et al., 2017). The determination of the maximum wavelength berberine was done by running berberine solvent at range of 200-400 nm. (Salama et al., 2017).

The results indicated that the maximum wavelength of quercetin was 435 nm, while the maximum wavelength of quercetin was 346 nm. The maximum wavelength was used to measure the absorbance of the extract *C.parasiticus* fernssample.

Making curve of standard quercetin and berberine

Standard quercetin was conducted in the following steps, weighing 10 mg of quercetin and dissolving in 10 mL of methanol in order to obtain the concentration of 1000 ppm stock solvent. One mL of this stock solvent was taken then was added 10 mL of methanol to get a concentration of 100 ppm. For standard solvent of quercetin 100 ppm, then made some concentration of 10 ppm, 20 ppm, 30 ppm, 40 ppm, 50 ppm and 60 ppm. Furthermore, from each of the standard that solvent concentrations of quercetin was added 0.5 mL and 1.5 mL of methanol, 0.1 mL of 10% AlCl₃ and 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. Samples of standard solvent were then incubated for 30 min at room temperature. Absorbance was determined using UV-Vis spectrophotometry at maximum wavelength (Aminah et al., 2017).

Production of standard berberine solvent is as follows, weighed 10 mg berberine and dissolved in 10 mL of methanol in order to obtain the concentration of 1000 ppm stock solvent. The 1 mL of stock solvent was taken and 10 mL of methanol was added to obtain a concentration of 100 ppm. Of berberine standard solvent 100 ppm, some concentration of 10 ppm, 20 ppm, 30 ppm,

40 ppm, 50 ppm and 60 ppm were made. Each standard berberine solvent concentration was put into a tube test and added 5 mL of phosphate buffer at pH 4.7 and 5 mL of BCG, then extracted with 5 mL chloroform (2 times) and chloroform phase was taken. The extract then was collected in the flask 10 mL of chloroform was then added up to the mark. The extraction results were then checked at the wavelength of maximum absorbance (Salama et al., 2017).

Assay of total flavonoid and alkaloid extract of *Cyclosorus parasiticus* (Linn.) Farwell ferns

Assay of total flavonoid content was done by weighing 10 mg of crude extract *C. parasiticus* and dissolving it in 10 mL of methanol, to obtain a concentration of 1000 ppm. 0.5 mL of the solvent was taken then successively was added 1.5 mL of methanol, 0.1 mL of 10% AlCl₃ and 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. The sample solvent was incubated for 30 min at room temperature. Absorbance was determined using UV-Vis spectrophotometry at maximum wavelength. Samples were made in three replications for each analysis and then absorbance measurement was averaged value (Aminah et al., 2017).

Assay of total alkaloid content was done by extracting viscously the fern

sample of 50 mg dissolved in 3 mL of HCl 2 N. The solvent was put into a tube test and was added 5 mL of phosphate buffer at pH 4.7 and 5 mL of BCG, then was extracted with 5 mL chloroform (2 times) and chloroform phase was taken. The extraction was collected in 10 mL flask, and added again chloroform to the mark (Salama et al, 2017). The solvent was then inspected its absorbance at a wavelength of 346 nm. Samples were made in three replications for each analysis and the result of absorbance measurement was calculated its average value.

Absorbance values were formulated into the regression equation of standard solvent of quercetin and berberine in order to obtain lower levels of total flavonoids and alkaloids as indicated by the percent total flavonoids and total alkaloids.

Abiotic Factors Measurement

Measurement of abiotic factors on the sampling locations include altitude, light intensity, humidity, and pH. Each measurement abiotic factor was repeated for three times and analyzed for the average. Determination of the altitude and position of the place was done by using the Global Positioning System (GPS). The light intensity was done by using a lux meter while the measurement of temperature and humidity was done by using hermohyrometer. Measurement of

pH and soil moisture measurements were by using soil tester.

Data analysis

Research data in the form of quantitative and qualitative data are compiled in table form. The qualitative data were the test results of secondary metabolites, flavonoids and alkaloids. Quantitative data of the percent content of secondary metabolites, flavonoids and alkaloids and average data measurement results of abiotic factors were calculated. Data from the test observations of secondary metabolites were analyzed descriptively and were associated with abiotic environmental factors where the *Cyclosorusparasiticus (Linn.)* Farwell fern grows.

RESULTS AND DISCUSSION

Qualitative test of flavonoid extract ferns *Cyclosorusparasiticus (Linn.)* Farwell

Qualitative test of secondary metabolites was done to ensure their flavonoid and alkaloid contained in the extract fern *Cyclosorusparasiticus(Linn)* Farwell. The test results of qualitative flavonoid and alkaloid extract this fern species can be seen in Table 1. The red or orange colors formed indicated that the leaves and stems of ferns *Cyclosorusparasiticus* positively contained

flavonoids. As supported by Mainawati et al. (2017) said that the plant extract containing flavonoids positive when it shown the formation of red, yellow or orange colors after adding magnesium chloride reagent indicated. The red color was indicated the reduction of the flavonoid result of concentrated hydrochloric acid and magnesium salts formed flavilium (Ahmad, 1986).

Qualitative test alkaloids in leaves and stems of ferns *Cyclosorus parasiticus* indicated red precipitate. It was proved

that the leaves and stems of ferns *C. parasiticus* positively contained alkaloids. The plant extract positively contains alkaloids after being given reagent Dragendorff that was indicated by the formation of deposits sorrel (brown to orange) (Ning et al., 2016). The formation of brick red precipitate as nitrogen formed a coordinate covalent bond with K⁺ metal ions. These deposits are potassium-alkaloid (Marliana et al., 2005).

Table 1. Results of the qualitative test flavonoid and alkaloid extract of ferns *Cyclosorus parasiticus* (Linn.) Farwell

part Plant	locations	flavonoids		Alkaloids	
		Color	info	Color	Info
Leaf	Tancak	yellowish orange	+	Brick red precipitate	+
	Garahan	Red	+	Brick red precipitate	+
	Gumitir	dark red	+	Brick red precipitate	+
rod	Tancak	Orange	+	Brick red precipitate	+
	Garahan	Red	+	Brick red precipitate	+
	Gumitir	Orange	+	Brick red precipitate	+

Note :+ : Positive contain flavonoids / alkaloids

- : Negative contain flavonoids / alkaloids

Determination of a standard curve of quercetin and berberine

The analysis of maximum wavelength absorption indicated that the maximum wavelength standards of quercetin was at a wavelength of 435 nm. It is consistent with research study done by Ahmad et al. (2015) has shown the maximum wavelength is 435 nm quercetin. While the results of measurements of the absorption maximum wavelength of berberine indicated that the wavelength maximum standards beberin was 346 nm as stated by Utami et al.

(2017) that the maximum wavelength is 346nm quercetin.

Absorbance measurement of quercetin standard solvent obtained was used to get a calibration curve of standard solvent of quercetin such as concentration curve graph versus absorbance. A calibration curve with a regression equation for absorbance quercetin is $y = 0,0047x + 0.0094$ (figure 1).

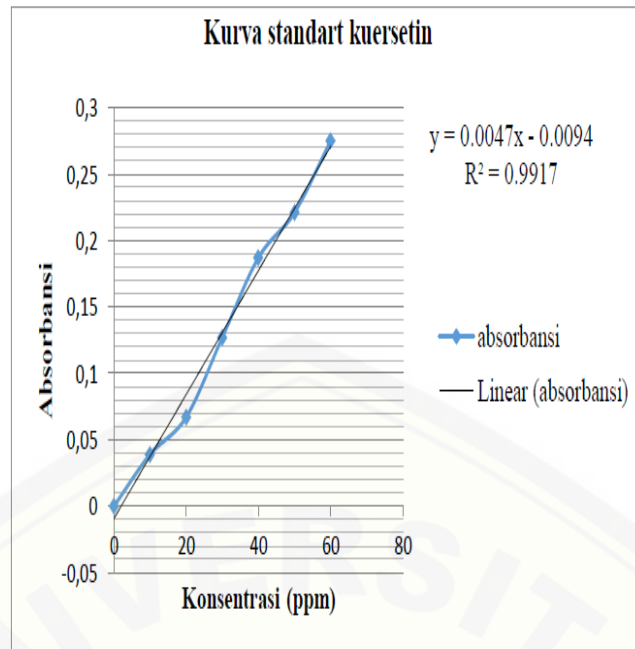


Figure 1. Standard curve of quercetin at a wavelength of 435 nm maximum

The results of the calibration curve of standard solvent of the compound quercetin was obtained linear relationship between the absorbance with concentration. It was indicated by the value of the correlation coefficient (r) of 0.9917 quercetin is close to 1.

of the calibration curve of standard solvent of the berberine compound was also obtained a linear relationship between the absorbance with concentration that was indicated by the value of the correlation coefficient (r) of 0.9937 honing berberine1. (Figure 2).

As for berberine regression equation $y = 0,0043x + 0.0099$ (Figure 2). The results

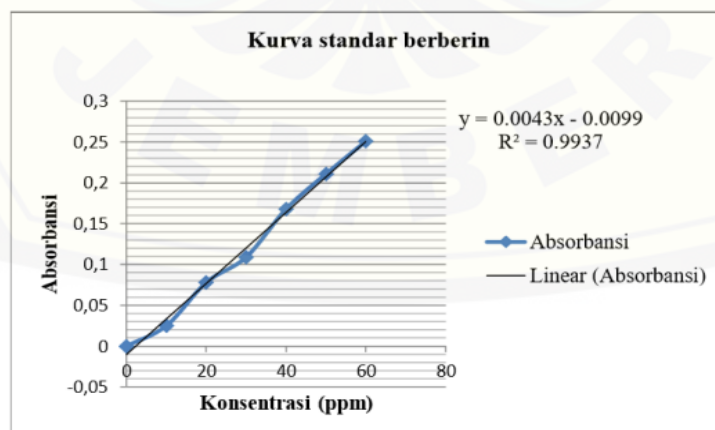


Figure 2. Standard curves of berberine at a wavelength of 346 nm maximum

Quantitative Test of alkaloid and flavonoids extract of *Cyclosorus parasiticus* (Linn.) Farwell ferns

The average level of flavonoids and alkaloids leaves *C. parasiticus* was higher than those in stems of all areas (table 2). The high average level of flavonoids and alkaloids in the leaves indicates the uneven

distribution of secondary metabolites in plant organs (Ahmad et al., 2015). In addition, the secondary metabolites, flavonoids and alkaloids in certain organs were affected by these secondary metabolites plant physiology.

Table 2 Average levels of flavonoids methanol extract of leaves and stems fern *Cyclosorus parasiticus* (Linn) Farwell at three different locations

part of plant	locations	Average levels of flavonoids Total (%)	Average levels of alkaloids Total (%)
Leaf	Tancak	.3241	.0728
	Garahan	.4462	.1102
	Gumitir	.5732	.1662
Stem	Tancak	.0306	.0263
	Garahan	.1370	.0318
	Gumitir	.5838	.1413

Cyclosorus parasiticus leaf is part of the plant that is susceptible to insect attack so that the accumulation of flavonoids and alkaloids was higher in the leaves than in the stems. The high level of flavonoids and alkaloids in the leaves intended for defense for the plants. It is supported by Gunawan et al. (2016) who said that the function of flavonoids for the plant defense against pests, microbes, and viruses, as well as protection against UV radiation. Secondary

metabolite alkaloid served as toxins to protect plants from herbivores (pests and diseases), and as alkaline minerals to maintain ion balance.

This study was conducted in three plantations in Jember that had different heights. Based on Table 3, Gumitir had the highest altitude among Tancak and Garahan.

Table 3. The average measurement of abiotic factors in three sampling sites of ferns *Cyclosorus parasiticus* (Linn) Farwell.

Abiotic factors	locations		
	Gumitir	Garahan	Tancak
Altitude (masl)	707	538.46	416.33
temperature (°C)	29.51	30.25	28.54
Air humidity (% rh)	66.02	64.34	77.16
soil pH	5.75	5.48	4.28
Soil moisture (%)	41.55	50.44	75.55
The light intensity (lux)	155.55	211.11	222.22

Nuryanto et al. (2014) states that altitude divided into lowland (0-200 masl), plains (200-600 masl) and highlands > 600 masl. Based on this statement, Gumitir was included in the class of plateau for the height is 707 meters above sea level, while Garahan and Tancak included in class of mid plains at a height of 538.66 meters above sea level and 416.33 meters above sea level. Altitude somewhere related to abiotic factors such as soil acidity (pH), light intensity, temperature, humidity, soil moisture and others. Soil pH in Gumitir (5.75) was higher than Garahan (5.48) and Tancak (4.28). pH tends to increase with altitude in line with increased soil organic content. Supriya et al. (2016) said that the pH is positively correlated with altitude.

The highest of flavonoids and alkaloids leaves was found in Gumitir. The high content of flavonoids and alkaloids in the leaves in Gumitir was supported by the growth *C. parasiticus* environmental factors such as pH, light intensity, soil moisture and others. Generally, environmental

factors such as pH affects the formation of both secondary metabolites. The Gumitir soil pH was 5.75. The soil pH is optimum for average growth of plants that is from 5.6 to 6.0 (Supriya et al., 2016). The pH of the soil is important for the plant because the soil solvent contains nutrients like N, K, P and others that the plants urgently need a certain amount to grow, thrive and survive against pest attack.

At the optimal pH conditions, the macro nutrients (N, P, K, Mg, Ca and S) were dissolved in the soil solvent and in available form so that its presence was high and absorbed easily by plants (Jovita, 2018). Macronutrient N is one of the important nutrients for plants. Since a plant fulfills the need for nitrogen then its growth will be better because besides to help in the process of photosynthesis it is also to affect the result of flavonoid synthesis (Aristyanti, 2014; Pratiwi, 2017). Based on Salim et al. (2016), macro soil nutrients such as nitrogen (N), potassium (K), organic matter (BO) and carbon (C)

have a linear relationship with the organic formation of secondary metabolites. By increasing availability of the macro nutrients in the soil, the establishment of flavonoids and alkaloids will also increase.

The existence of nitrogen are also able to increase the number of total chlorophyll, because nitrogen is an essential component of constituent porphyrin ring of chlorophyll which is a basic framework. It is supported by Jovita's statement (2018) that the benefits of nitrogen play a role in the formation of chlorophyll, amino acids, lipids, enzymes, and other compounds. The growth of plant with sufficient nitrogen showed an increased expression of the flavonoid biosynthetic genes (Coronado et al., 1995).

The optimal pH condition also affected the high content of alkaloids *C. Parasiticus* in Gunitir. Alkaloids were secondary metabolites compounds containing nitrogen derived from the amino acid biosynthesis. The statement was supported by Wink (2008) that the alkaloids are secondary metabolites, the most that have a nitrogen atom. Therefore, increasing the formation of amino acids will affect the increased alkaloid. It is why the alkaloid content in Gunitir was higher than Tancak.

Another factor supporting high flavonoid and alkaloid in Gunitir was light intensity. Light intensity in Gunitir was

155.55 lux (Table 3). The light intensity is suitable to *Cyclosorus parasiticus*. growth Based on Mukti et al. (2016) who states that the appropriate intensity of light with the growth of ferns that is in the range of 128.3 lux - 3000 lux. The appropriate light intensity will support the optimal process of photosynthesis of a plant. The results of photosynthesis such carbohydrates will go through glycolysis to form phosphoenolpyruvate and pyruvate. Flavonoids are secondary metabolites formed from phosphoenolpyruvate and pyruvate from glycolysis stages in the process of respiration of plants. Phosphoenolpyruvate will be formed into phenolic compounds by the shikimic acid pathway while pyruvate by the malonic acid pathway (Taiz and Zieger, 2002), Flavonoids are secondary metabolites that are included in a large group of phenolic compounds (Vickery and Vickery, 1981 in Ekawati, 2018). Thus the optimal photosynthesis will indirectly increase the secondary metabolites of flavonoids. While alkaloids formed from the results of photosynthesis were carbohydrates which undergo glycolysis into acetyl CoA. Acetyl CoA through the tricarboxylic acid cycle will form amino acid that is a precursor aliphatic alkaloid formation. Alkaloids can also be generated from the aromatic amino acid produced from phosphoenolpyruvate through the shikimic acid pathway (Taiz

and Zeiger, 2002 in Setyorini and Eriyanto, 2016).

The low intensity of light in Gunitir allegedly also affected the increasing of auxin hormone. Based on Rahayu et al. (2003) environmental conditions with low intensity will increase the concentration of auxin in plants. In flavonoid synthesis, auxin has function to improve the action of the enzyme phenylalanine ammonia lyase (PAL), which produces cinnamic (via the shikimic acid pathway) of phenylalanine. Thus, if the content of auxin increases, the formation of flavonoids also increases.

The lowest level of flavonoids and alkaloids of leaves was found in Tancak. The low levels of flavonoids and alkaloids in these locations can not be separated from the environment in which the growth factor *Cyclosorus parasiticus*. Tancak had Low pH (4.28) that is classified as sour. The pH value of Tancak was lower than that of Gunitir and Garahan. Tancak pH was not optimal for plant growth. The optimal pH for growth is 5.6 to 6.0 (Supriya et al., 2016). The condition of soil pH that is relatively acidic can affect the availability of nutrients that can be absorbed by plants. At acidic pH the plant was poisoning Al and Fe because the solubility of the two metals were high. Rosmarkam and Yuwono (2002) states that the pH affects the availability of nutrients, at the acid pH, the solubility of some nutrients decreased, but the

solubility of Al and Fe were high consequently the plant growth was stunted. The plant growth was inhibited among them are cause of Al and Fe toxicity, reduced chlorophyll biosynthesis, amino acids and proteins (enzymes).

The reduction of chlorophyll content caused the photosynthetic process inhibition. Inhibited photosynthesis resulted in impaired respiration consequently the formation of flavonoids decreases. Inhibition of respiration process caused phosphoenolpyruvate and pyruvate decrease. Both of these compounds will be formed into a phenolic compound through the shikimic acid pathway while pyruvate through malonic acid (Lincoln and Zieger, 2002), Based on Aristyanti's research (2014) which states that the flavonoids in the tabatbarito leaves (*Ficus deltoidea* Jack) were less in locations with low soil pH. It is due to the land and waters that have lower pH levels in general are often encountered in conditions of nutrient deficiency, followed by a low level of productivity as well. Similarly to alkaloid biosynthesis was inhibited at low pH. Alkaloid is a nitrogen-containing secondary metabolites of amino acid biosynthesis. Nitrogen is an important organic compounds such constituent amino acids, proteins and nucleic acids (Goh and Hardter, 2010). Absorption N by plants was reduced so that the amino acid biosynthesis process reduced and

influenced the reduction in alkaloid produced.

The low content of flavonoids in Tancak was caused by the effect of pH and also the light intensity. The light intensity in Tancak is 222.22 lux. The light intensity was actually suitable for the growth *C.parasiticus*. The appropriate light intensity for the growth of ferns that is the range of 128.3 lux - 3000 lux (Mukti et al., 2016). The appropriate light intensity make the photosynthesis process go well. The light intensity in Tancak was higher than that in Gunitir but still classified in the low light intensity. The higher light intensity in Tancak than in Gunitir likely affect the lower the auxin hormone in Tancak location. The low auxin hormone also affected the flavonoid. It is supported by Rahayu et al.(2003) statement that when auxin is reduced, flavonoid formation is also reduced.

CONCLUSION

Stems and leaves of *Cyclosorus parasiticus* (Linn) Farwell ferns in Gunitir, Garahan and Tancak contains secondary metabolite compounds contain flavonoids and alkaloids. The content of flavonoids and alkaloids in leaves was higher than that of in stems. Leaves *Cyclosorus parasiticus* (Linn) Farwell in Gunitir contains higher flavonoids and alkaloids compared to that in Garahan and Tancak. The highest

flavonoid and alkaloid was found in the leaves *Cyclosorus parasiticus* in Gunitir is 0.5732% and 0.1662%, while the lowest content (0.0306% and 0.0263%) of flavonoids and alkaloids in the stem located in Tancak.

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