International Journal on Advanced Science Engineering Information Technology

Vol.13 (2023) No. 4
ISSN: 2088-5334

Characteristics of Anthocyanin as Natural Dyes from Butterfly Pea (*Clitoria ternatea* L.) on Regions Growing in North Maluku

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***Abstract*—Butterfly pea has the potential to be a natural dye. The identification of anthocyanin compounds and anthocyanin content and antioxidant activity of butterfly pea flowers that grow in several regions in North Maluku need to be traced to identify the quality of anthocyanin content from late flowers. The research samples for the butterfly pea flower came from seven growing regions in North Maluku, and this study aimed to identify the phytochemical components of anthocyanins using the GC-MS method and to determine antioxidant activity using the DPPH method in ethanol extracts defined using the spectrophotometric method. The data obtained revealed the results of GC-MS phytochemicals and confectionery was identified as approximately 4-7 anthocyanin compounds with varying retention time peaks, with the best growth area for the highest anthocyanin content in Fitu Ternate with 35.38%, the Tidore region 33.92% and Maliaro 33.22%. Gas Chromatography-Mass Spectrometric (GC-MS) analysis results are intended to filter phytochemical, functional component groups presented by the Fourier Transform Infrared Spectrophotometer (FT-IR) in ethanol extract of *Clitoria ternatea* flowers. The results showed that the antioxidant activity test obtained IC50 values on dried eagle flower extracts from several growing areas in North Maluku, which ranged from 39,009 – 74,481 µg / ml. IC_{50} values are best produced in the growing area of Fitu Ternate and Tidore of 39,01 and 39,37 criteria for oxidant activity with powerful free radical inhibition.**

***Keywords*— *Clitoria ternatea*; anthocyanins; natural dyes, antioxidants, growing regions.**

Manuscript received 20 Aug. 2022; revised 21 May 2023; accepted 26 Jun. 2023. Date of publication 31 Aug. 2023.

I. INTRODUCTION

Among the countries that have plasma resources *nutfah* high crop diversity is Indonesia. Many plants native to Indonesia are helpful and have not been excavated. *Clitoria ternatea*, or butterfly pea, is a plant native to Indonesia with a tropical climate. The first discovery of this flower was in the Ternate region, North Maluku Province, so the local people called it the local name blue flower because the flower is predominantly blue and functions as a natural dye. *Clitoria ternatea*, found in some growing areas in North Maluku, from this growing area, can provide the same or different anthocyanin content. The plant's growing area can also cause a high content of anthocyanins because agroclimatic and nutrient content in different soils can provide differences in anthocyanin content in flowers. *Clitoria ternatea* is a type of legume plant with purple or bluish-purple flowers. Blue or bluish-purple color on the flower can be used for coloring in food. Factory-made dyes (chemical) can be substituted with dyes derived from plants obtained from *Clitoria ternatea*; the flower parts are blue or bluish-purple. These late petals produce anthocyanin color substances and are a group of flavonoid compounds used as natural dyes [1].

In *Clitoria ternatea*, there are components of anthocyanin compounds, so they have the potential as natural dyes in foodstuffs. The anthocyanin content of butterfly peas will be decisive in the color of the extraction results. Specifically, pelargonidin is a marker of orange to red, cyanidin indicates red, and delphinidin marks purple to blue. Likewise, Anthocyanin can function as a natural supplement, especially in boosting antibody (inflammatory), anti-microbial, anti-carcinogenic, and antioxidant, and includes anti-candida activity [2].

The color of the flower is blue; this color comes from anthocyanin compounds. The content of phytochemical compounds anthocyanins is stable and resistant to changes in acid-base colors that can be used as natural dyes for the culinary and food industries. The content of other phytochemicals in late flowers, such as flavonoids. The

flavonoid content of the flower can act as a source of warding off free radicals in the body because it acts as an antioxidant.

Given the huge potential of anthocyanins and the abundant content found in butterfly peas, *Antosian* is a compound derived from flavonoid glycosides, consisting of a sugar group (*glycon*), a non-glucose group, namely anthocyanidins (*glycocons*), and some anthocyanins containing acyl groups. But anthocyanins consist of *polyethoxy glycosylation* and p*olyhydroxy* salt flavylium in their structure. Meanwhile, the anthocyanin form is in the form of *aglicon*, namely anthocyanins. However, the most widely distributed anthocyanins within the plant consist of delphinidin, pelargonidin, peonidin, petunidin, and cyaniding [3].

The potential of butterfly peas as a natural dye then the identification of anthocyanin compounds and anthocyanin content and antioxidant activity of butterfly peas that grow in several regions in North Maluku need to be traced to be identified the quality of anthocyanin content from butterfly pea flowers that grow in the North Maluku region.

II. Material and Method

A. Antosianin Analysis

Samples of butterfly peas were taken from the regions of Bastiong Ternate, Tabona Ternate, Fitu Ternate, Maliaro Ternate, Kalaodi Tidore, Bibinoi Halmahera Selatan, Dehegila Morotai. The flowers are then cleaned of the dirt attached and continued with the process of drying flower samples using a cabinet drying at 40 °C. Then the sample that has been dried is mashethentil it becomes a powder. The powder is sipped to produce a fine powder using 100 mesh for smooth and homogeneous powder. Solution maceration is done by making extracts of butterfly pea using the immersion method; the solution is then put into a *microtube*, then *vortexed* and *checkedrifuge*. The supernatant formed is used for GC-MS analysis methods. The analysis time is 60 minutes with an injector temperature of 260 ^{0}C, a detector of 250 ^{0}C, and a column of 325 ^{0}C. Furthermore, the flow rate using a 1 ml/min speed using a carrier media in helium gas. The identification process using the GC-MS method contained in the laboratory produces a list of compounds in which a mixture of gases and the molecular weight of each compound is presented as a chromatogram.

B. Antioxidant Analysis

Antioxidant activity with DPPH [4], the anthocyanins obtained by extracting are used to analyze DPPH compounds using a maceration technique ±24 hours, with the addition of a 96% ethanol solvent (1: 10), then centrifuged at 500 rpm, ± 14 minutes, the supernatant is obtained, and vacuum filtering is carried out. The filtrate is concentrated with an evaporator at 35 ^{0}C to obtain 1/10 of the initial filtrate as a concentrated extract. DPPH method can detect antioxidant activity and measurements using a UV-Vis spectrophotometer with ƛ 515 nm, where antioxidants are determined based on how much there is an absorption barrier in free radicals. Furthermore, calculate the percentage of inhibition of absorption of *1,1-diphenyl-2-picrilhydrazyl* (DPPH) using the formula:

$$\%of\ inhibition = \frac{AO-Asample}{AO} x100 \qquad (1)$$

The percentage of inhibition obtained is a control absorbance (A0) or blank, while (A sample) is an absorbance of the test compound. In addition, 50% inhibition is a concentration of compounds that shows the calculation of the plot of the percentage of each inhibition to the concentration in the sample. For determining IC_{50} (inhibition concentration), concentration variations range from 20% (b/v), 40% (b/v), 80% (b/v), 160% (b/v), Vitamin C as a positive control and extract that has the greatest percentage of inhibition at the initial treatment.

III. Result and Discussion

A. Anthocyanin

The anthocyanin content contained in the butterfly pea is characterized by a physical appearance in the form of blue pigments, and the results of previous studies have shown that this flower has benefits for human health. Anthocyanins in the pigment acid condition are reddish, while the pigment will show a blue hue in the alkaline condition. In addition, Anthocyanin is stable to high heat conditions and different pH, especially in coffee and acylation, which can maintain its structure well. The blue pigment of the flower is obtained by extracting the flower of the butterfly pea using a maceration method that produces a deep blue extract. Anthocyanins from flower flowers can be used as an acid-base indicator because these pigments change color when dripped with acids or bases and natural color substances.

Anthocyanin blue flower color will change color along with the change in pH value. At high pH, anthocyanins tend to be blue or colorless, then lead to red color at low pH. Most anthocyanins produce a color at a pH of less than 4. This discoloration is related to the hydroxy or methoxy group in the structure of the anthocyanin ring, thus affecting the color of anthocyanins. The dominant number of hydroxy groups causes the color to lead blue and is generally less stable. While the number of dominant methoxy groups compared to hydroxy groups in the structure of anthocyanins causes the color to be reddish.

Thus, in the GC-MS results, it can be seen that in the samples of butterfly peas from seven regions grown in North Maluku in ethanol extract, namely the *Bastiong*, *Tabona*, *Fitu*, *Maliaro*, *Tidore*, *Bacan,* and *Morotai* growing areas. From each area identified, approximately 4-7 anthocyanin compounds; the best growth area for the highest anthocyanin content is in Fitu Ternate with 35.38%, Tidore region with 33.92%, and Maliaro 33.22% and the rest in 4 growing regions with anthocyanin content ranging from 17.84% - 24.35%. The high content of anthocyanins in the three growing regions is likely because the region is a mountainous area, and the soil results from volcanic soil deposits with a lot of high enough nutrient content that can support the metabolism of anthocyanin formation in late flowers.

The study produced the number of monomeric anthocyanins expressed in mg equivalent *sianidin-3-glucoside* because Malvidin is one of the anthocyanins in the butterfly pea. The total content of Anthocyanin monomeric extracts from various regions grown in North Maluku consecutively is 21.55; 19.82; 35.38; 33.22; 33.92; 24.35; 17.84 (%) (bk). Total monomeric anthocyanins differed between samples of late confectionery from the area of origin

of growth studied. This difference is due to differences in the growing place and the environment. The biosynthesis process of anthocyanin compounds in each plant is strongly influenced by environmental conditions, especially air temperature, rainfall conditions, sunlight, and nutrients in the soil in each region where the plant grows [5].

It is reported that secondary metabolite compounds in phytochemical content, such as beta carotene and flavonoid components contained in plants, have different concentrations in which the plant grows. The components of such compounds are influenced by acidity (pH), environmental conditions, humidity, and nutritional elements contained in each soil nutrient [6]. The research report suggests cold environmental conditions are better suited for producing soybean anthocyanins and isoflavones [7]. According to [8], the higher the topographic plain where plants grow, the higher the anthocyanin content. [9] Reports of climate and growing places affect the virgin tread's bioactive components and antioxidant activity (Catharanthus roseus). It is also recommended that there are differences in the anthocyanin content of potatoes because they are influenced by the location where the plant grows. Still, the factor that has the most significant influence is the genotype factor of the potato plant on its anthocyanin content. It is also recommended that there are differences in the anthocyanin content of potatoes because they are influenced by the location where the plant grows. Still, the factor that has the most significant influence is the genotype factor of the potato plant on its anthocyanin content. [10], Moreover, the determining factors for the growth of plants with the best anthocyanin content are influenced by the biosynthesis process, mainly by the intensity of sunlight, good rainfall conditions, environmental conditions, and good nutrients in the soil or the area where the plant grows [5]. Thus, it can be concluded that the place of growth affects the monomeric anthocyanin production of butterfly peas. To better know the environmental influence on anthocyanin biosynthesis, further research is needed to pay close attention to the composition of nutrients, the intensity of exposure to ultraviolet light, the temperature of day and night, as well as rainfall in the location and topography of the growth of late plants.

In addition, the stability of anthocyanins depends on light, pH, temperature, enzymes, metal ions, oxygen, anthocyanin pigments, antioxidants, and coffee. Anthocyanin compounds with good stability are dominated by a B ring contained in the anthocyanin content, and there is a *hydroxyl* or *methoxyl* group. Even lowering the stability of anthocyanins present in one solution has groups that can be known. Therefore, under different pH conditions, there will be a change in the color of several types of anthocyanin stability. Therefore, under different pH conditions, there will be a change in the color of various kinds of anthocyanin stability.

Furthermore, the pH condition of the sample greatly affects the anthocyanin color. Therefore, pH of the solution greatly affects the discoloration of the anthocyanin components. In addition, anthocyanins have a molecular structure with ionic properties [15]. Likewise, in acidic conditions, anthocyanin compounds appear to show a red color. Even anthocyanins show a purple hue with a neutral pH; on the contrary, under alkaline pH conditions, it turns blue. A large anthocyanin pigment consists of a red flavylium cation. Therefore, a lower pH of the solution will stabilize the anthocyanin compounds. In addition, flavylium cations with a low pH will affect anthocyanin compounds and be water-soluble. A decrease in water concentration increases the rate of deprotonation of flavylium cations, thereby lowering the stability of the color. In addition to pH, the polymerization of anthocyanins can also improve color stability at lower pH.

The extraction results using *ethanol* on *Clitoria* ternate using GC-MS analysis will obtain the appearance of phytochemical component profiles. The results of FT-IR analysis also show functional groups with their grouping. Ethanol extract with GC-MS reveals that *Clitoria t*ernate has many components of the active compound (Table 1 and Figure 1).

TABLE I
THE COMPOUND WAS IDENTIFIED BY GC-MS ANALYSIS USING ETHANOL EXTRACT *C. TERNATEA* FROM NORTH MALUKU

Areas Sample	Retention Times	Name of the Compounds	Formula Molecular	Molecular weight (kDa)	Area Peak	Anthocyanin Content (%)
	12,241	Melezitose	$C_{18}H_{32}O_{16}$	504	0.38	
	15,513	Melezitose	$C_{18}H_{32}O_{16}$	504	0.37	
Bastiong	18,087	Melezitose	$C_{18}H_{32}O_{16}$	504	11.15	
	21,604	Melezitose	$C_{18}H_{32}O_{16}$	504	5.84	
	29,668	L- (+)- ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652	3.57	21,55
	32,260	9-octadecenoic acid (Z)-2-hydroxyl-1(hidroxymethl) ethl ester	$C_{21}H_{40}O_4$	356	0.24	
	15,512	Melezitose	$C_{18}H_{32}O_{16}$	504	0.42	
	17,478	Eicosanoic acid	$C_{20}H_{40}O_2$	312	0.51	
Tabona	18,009	Melezitose	$C_{18}H_{32}O_{16}$	504	10.93	19,82
	22,318	Melezitose	$C_{18}H_{32}O_{16}$	504	0.96	
	29,671	L- (+)- ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652	7.00	
	10,060	Melezitose	$C_{18}H_{32}O_{16}$	504	1.11	
	15,509	Melezitose	$C_{18}H_{32}O_{16}$	504	0.36	
	18,022	Melezitose	$C_{18}H_{32}O_{16}$	504	13.29	
Fitu	22,325	Melezitose	$C_{18}H_{32}O_{16}$	504	2.31	35.38
	29,671		$C_{38}H_{68}O_8$	652	6.29	

Areas Sample	Retention Times	Name of the Compounds	Formula Molecular	Molecular weight (kDa)	Area Peak	Anthocyanin Content (%)
		L- (+)- ascorbit acid 2,6-dihexadecanoatee				
	32,980	Oleiic acid	$C_{18}H_{34}O_2$	282	12.02	
	12,237	1- Decanol, 2- ethyl	$C_{12}H_{26}O$	186	0.58	
		Melezitose				
	15,516	Melezitose	$C_{18}H_{32}O_{16}$	504	0.55	
	16,125	Melezitose	$C_{18}H_{32}O_{16}$	504	0.21	
Maliaro	17,505	Melezitose	$C_{18}H_{32}O_{16}$	504	0.65	33.22
	18,104	L- (+)- ascorbic acid 2,6-	$C_{18}H_{32}O_{16}$	504	13.46	
	29,678	dihexadecanoate	$C_{38}H_{68}O_8$	652	8.64	
		Oleic acid				
	32,980		$C_{18}H_{34}O_2$	282	9.13	
Bacan	15,516	Melezitose	$C_{18}H_{32}O_{16}$	504	0.49	
	18,104	Melezitose	$C_{18}H_{32}O_{16}$	504	13.32	
	19,655	Melezitose	$C_{18}H_{32}O_{16}$	504	0.65	
	22,372	Melezitose	$C_{18}H_{32}O_{16}$	504	0.92	
	29,678	L- (+)- ascorbic acid 2,6-dihexadecanoat	$C_{38}H_{68}O_8$	652	8.55	24.35
	39,221	9-octaadecenoic acid (Z)-2-hydroxyl-1(hidroxymethl) ethil esteer	$C_{21}H_{40}O_4$	356	0.42	
Morotai	1,816	Eicosanoic acid	$C_{18}H_{32}O_{16}$	504	0.14	
	15,509	Melezitose	$C_{18}H_{32}O_{16}$	504	0.28	
	17,961	Melezitose	$C_{18}H_{32}O_{16}$	504	6.67	
	21,876	Melezitose	$C_{18}H_{32}O_{16}$	504	1.28	
	29,678	L- (+)- ascorbic acid 2,6-dihexadecanoat	$C_{38}H_{68}O_8$	652	9.17	17.84
	32,283	9-octadecenoic acid (Z)-2-hydroxyl-1(hidroxymethl) ethl ester	$C_{21}H_{40}O_4$	356	0.30	

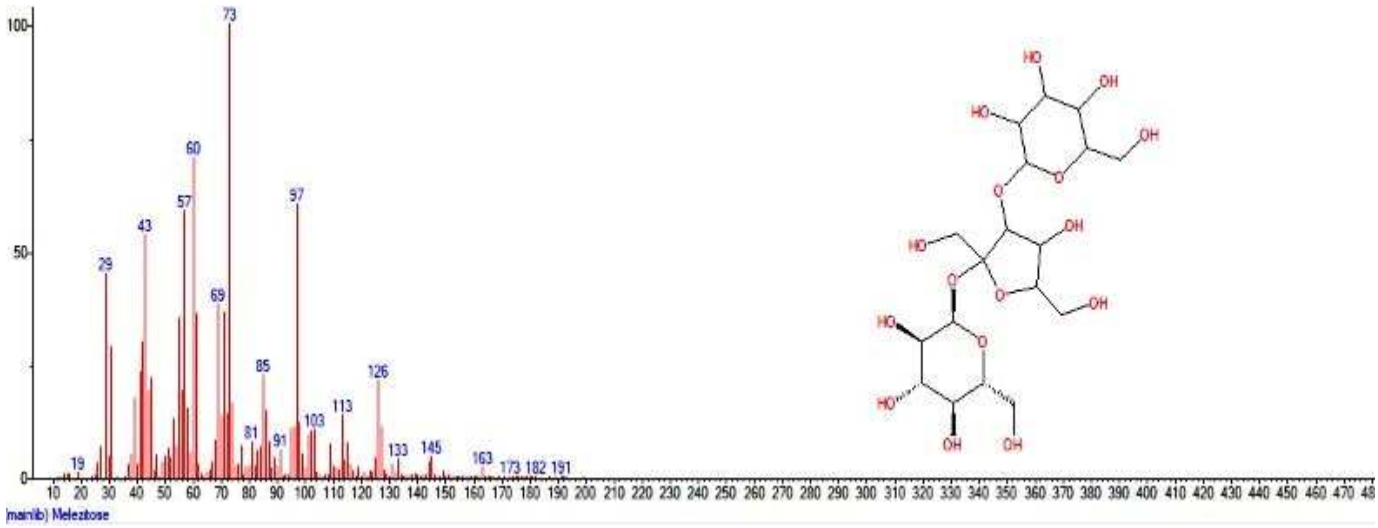

Fig. 1 GC-MS chromatogram of anthocyanin compounds present from ethanol extract *Clitoria ternatea*

B. Antioxidant Activity

Bars Research results to find out the results of extraction of dried flowers using ethanol solvent; the use of the DPPH method in analyzing antioxidant activity based on the ability to use ethanol extract and can reduce free radical compounds, especially DPPH compounds. This study used samples of butterfly peas taken from growing regions in four districts in North Maluku. In the extraction process, the flower sample is cleaned of dirt, dried, cut into small pieces, and put into a bottle containing 96% ethanol solvent. The solvents' solubility and the dissolved components are the basis of adding solvents to a material. In this study, ethanol solvents were used. This solvent was chosen because, according to [12], [13], ethanol can extract more active compounds than other types of organic solvents, besides the utilization of this confectionery is intended for natural dyes for food, so it is expected not to cause toxins that can cause poisoning when used for food and relatively safer.

TABLE II
CLITORIA TERNATEA FLOWER EXTRACT ON ANTIOXIDANT ACTIVITY FROM NORTH MALUKU

Region	IC_{50} (µg/ml)		Average IC_{50} (µg/ml) ±SD	Antioxidant Category
	Rep 1	Rep 2		
Bastiong	60.86	62.83	61.84 ± 1.39	Strong
Tabona	73.68	75.29	74.48 ± 1.14	Strong
Fitu	39.62	39.11	39.37 ± 0.36	Very Powerful
Maliaro	59.07	53.98	56.51 ± 3.62	Strong
Tidore	38.91	39.11	39.01 ± 0.15	Very Powerful
Bacan	63.47	52.46	57.96 ± 7.79	Strong
Morotai	59.00	59.58	59.29 ± 0.40	Strong

Based on data from the analysis of antioxidant activity, IC_{50} values were obtained in dried confectionery extracts from several growing regions in North Maluku, which ranged from 39,01 to 74,48 µg/ml. IC_{50} values are best produced in the growing area of Fitu Ternate and Tidore of 39.01, and 39.37 criteria for oxidant activity are very strong. This shows that samples of butterfly peas that grow in the North Maluku region have a strong to very strong level of antioxidant activity. The concentration contained in the sample solution has an IC_{50} value by reducing DPPH activity by 50%, where the smaller the IC_{50} value, the higher the reading of antioxidant activity in the sample [14], [15], and the more antioxidant activity to reduce the stronger. Table 3 presents antioxidant levels using the DPPH method at IC_{50} values.

TABLE III
LEVELS OF ANTIOXIDANT POWER WITH THE DPPH METHOD

Value IC_{50} (ppm)	Level of Antioxidant Activity
151-200	Weak
100-150	Is
50-100	Strong
<50	Very Strong

Source [31]

In the test results using dry *Clitoria ternatea* extract obtained, the results of the antioxidant activity test in Table 3 show that the susceptibility of IC_{50} values < 100 ppm is a strong and very strong activity. This is due to the analysis of the low water content of the butterfly pea sample. Water content is low enough when analyzing the activity of the ingredients causing antioxidant compounds contained in the ingredients will be higher in value the water content is low, then the content of antioxidant compounds can be detected better, meaning that antioxidant activity will be stronger if the water content of the ingredient is reduced or low [16], [23]-[24]. When the water content of the material is high, it can cause flavonoid compounds to oxidize, thereby decreasing antioxidant activity.

Using the DPPH method in butterfly pea extract will show results where the antioxidant activity is very strong, likely due to the presence of phenolic compounds in it. The antioxidant mechanism of phenolic compounds is based on oxidation-reduction reactions. Due to reducing agents contained in this phenolic compound, there is a reduction in reactive free radical compounds and reactions between DPPH radicals and phenolic compounds in late flowers as radical capture compounds [25], [26].

Meanwhile, to interpret the results of the DPPH method, it was recently introduced, namely efficient concentration (EC_{50}), or called the IC_{50} value, as one of the parameters in calculating antioxidants. IC_{50} can be interpreted as a substrate concentration that can eliminate 50% of DPPH activity through discoloration. Therefore, several research results show that phenolic compounds strongly correlate with antioxidant activity [17], [18], [27], [28].

Phenolics in ethanol extract will release H •, one of the free radicals. H• will bind to the DPPH radical to form a new compound, the stable *diphenyl picrilhydrazin*. Phenolic compounds contained in ethanol extract as a free radical catcher that loses H will become new radicals that are relatively more stable and harmless to the body because of the resonance effect of antiaromatic so that free radicals are not formed and can prevent or repair tissue damage that is the effect of free radical attacks. Phenolic compounds from heterogeneous chemical groups containing phenol groups (functional hydroxyl groups in aromatic rings) in their basic structure. In addition, phenolic compounds can induce antioxidant protein synthesis and increase the activity of antioxidant enzymes [19], [20].

Antioxidant activity in the butterfly pea (*Clitoria ternatea*) calculation of IC_{50} value with linear regression curve, so that a concentration with the percentage of damping in the test sample is obtained and then made in a linear regression curve, $y = bx + a$, where x is the concentration (ppm), and y is the percentage of IC_{50}, where the relationship between the concentration of the test solution with percent damping, obtained regression equation with $R^2 = 0.8216$ from the Bastiong Ternate region. From the value of R^2, it can be known that there is a significant relationship between the concentration of the solvent and the percentage of immersion observed with a degree of intensity of 0.8216; this indicates an 82% degree of inhibition (Figure 2). The highest inhibition power in the growing area Fitu Ternate amounted to $R^2 = 0.9525$ degrees inhibition by 0.95%, and the growing area Tidore $R^2 = 0.9499$ degrees inhibition 94% is powerful. Other growing regions obtained R2 values ranging from 0.7877 - 0.8343 with degrees of inhibition of 78 % - 83% criteria for strong inhibitory antioxidant activity.

These results align with previous researchers' findings that found that the reducing power of anthocyanins of purple sweet potato extract [21], [22], and lychee skin anthocyanin was greater than that of ascorbic acid [29]. For this reason, it can be concluded that the growing condition of *Clitoria Ternatea* does not affect the anthocyanin content in the form of antioxidant activity. The degree of similarity in carrying out anthocyanin extraction affects the antioxidant activity produced. Although the reducing force and free radical capture activity are at pH 1 because it is the more dominant *flavilium cation* compound in the anthocyanin structure, it can

provide hydrogen cations that easily reduce $Fe3^{+}$ to $Fe2^{+}$ and stabilize DPPH electrons [30].

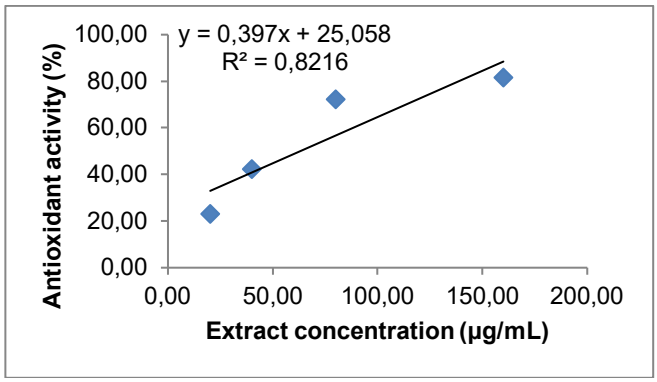

Fig. 2 Ethanol Extract of *Clitoria Ternatea* with Antioxidant Activity from Ternate Bastiong

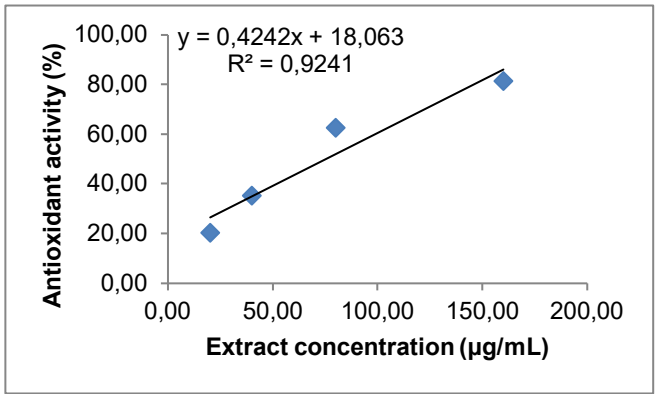

Fig. 3 Ethanol Extract of *Clitoria Ternatea* with Antioxidant Activity from Ternate Tabona

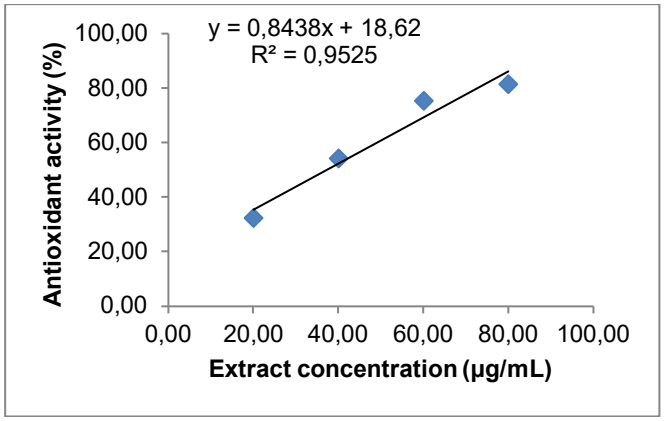

Fig. 4 Ethanol Extract of *Clitoria Ternatea* with Antioxidant Activity from Ternate Fitu

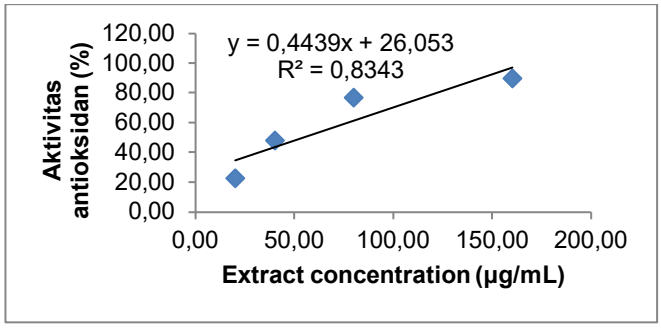

Fig. 5 Ethanol Extract of *Clitoria Ternatea* with Antioxidant Activity from Ternate Maliaro

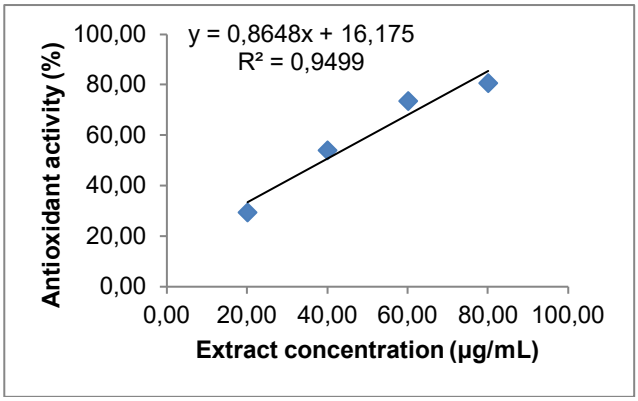

Fig. 6 Ethanol Extract of *Clitoria Ternatea* with Antioxidant Activity from Tidore

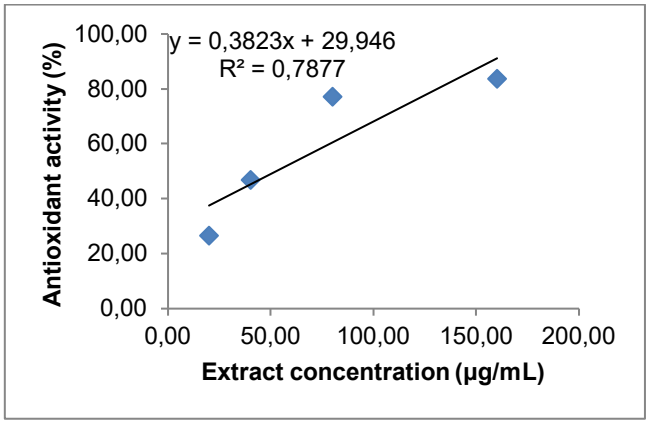

Fig.7 Ethanol Extract of *Clitoria Ternatea* with Antioxidant Activity from Bacan

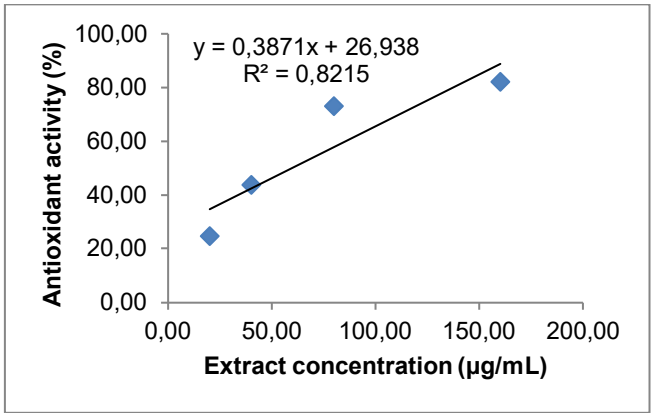

Fig. 8 Ethanol Extract of *Clitoria Ternatea* with Antioxidant Activity from Morotai

IV. Conclusion

Analysis of GC-MS phytochemicals was identified as approximately 4-7 anthocyanin compounds; the best growth area for the highest anthocyanin content is in Fitu Ternate with 35.38%, Tidore region 33.92% and Maliaro 33.22%. Therefore, in this study, the results of testing antioxidant activity obtained IC_{50} values from dried butterfly pea extract from several growing areas in North Maluku, which ranged from 39,01 – 74,48 µg/ml. IC_{50} values are best produced in the growing area of Fitu Ternate and Tidore of 39,01 and 39,37 criteria for oxidant activity with very strong free radical inhibition.

ACKNOWLEDGEMENT

This research is fully supported by the Funding of Competitive Research Excellence of Universities (PKUPT) Khairun University in 2021 No:016 / PEN-PKUPT / PG.12 / 2021.

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