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Anthocyanin, Antioxidant and Metabolite Content of Butterfly Pea Flower (*Clitoria ternatea* L.) Based on Flowering Phase

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Abstract

The butterfly flowers (Clitoria ternatea L.) are used as natural dyes and herbal medicines because they contain metabolites, anthocyanins and antioxidants. The content of these metabolites is influenced by the phase of flower development. This study aims to determine the levels of anthocyanins, antioxidants and metabolites of butterfly pea flowers from different flowering phases. The Butterfly flower collection was taken from the village of Mulyaguna, Teluk Gelam, Ogan Komering Ilir, South Sumatra. The extraction was carried out with 70% ethanol solvent, and determination of the amount of anthocyanin content by spectrophotometry, antioxidant content by DPPH method, and metabolite compounds by GC-MS. Data on anthocyanin and antioxidant content were analyzed with averages and standard deviations, and GC-MS chromatograms were traced for compounds with reference to the PubChem, KEGG, ChEBI, PlantCyc, and Spectrabase websites, which then determined the dominant compound group. The results of the study on blooming butterfly pea flowers found that the antioxidant content was 6.58 ppm, higher than that of bud flowers, which were 2.55 ppm, and wither flowers, which were 1.74 ppm. The anthocyanin content of the blooming butterfly pea flower was 40.33 ppm, the withering flower was 4.36 ppm, and the bud flower was 3.60 ppm. The dominant metabolites were identified as fatty acids, organic acids, aromatics and flavanoids, followed by differences in antioxidant and anthocyanin content in the flowering phase of the butterfly pea flower.

Keywords: *Antioxidants, Anthocyanins, Butterfly Pea Flower (Clitoria ternatea L.), Flowering phase, Metabolites*

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INTRODUCTION

Butterfly pea flower (*Clitoria ternatea* L.) is used by Indonesian people as a natural dye because it contains anthocyanins. Anthocyanins are stable so they are often used as food and beverage coloring. Food and beverage products that use butterfly pea flowers include pudding, blue rice, tea, and syrup. Butterfly pea petals also function as antioxidants that can protect cells in the body from exposure to free

radicals (Suryana, 2021).

Antioxidants in butterfly pea flowers have the ability to counteract free radicals that are reactive and unstable in cells. Therefore, the use of the blue color of the butterfly pea flower is believed to help prevent several diseases. Processing of butterfly pea flowers into a functional drink can prevent atherosclerosis, which is a disease of blocked arteries. Consumption of butterfly pea flowers can also reduce symptoms of stress, depression, control obesity, and prevent cancer (Ngete & Mutiara, 2020).

Antioxidant compounds found in butterfly pea flowers can be used as herbal medicinal ingredients. The manufacture of herbal medicine from butterfly pea flowers is made in the form of packaged tea products. To brew this flower tea, simply boil the flowers until the boiled water is dark in color and then drink the boiled water. The simple process of processing butterfly pea tea makes it increasingly attractive to Indonesian people (Kristanti *et al.*, 2008).

Butterfly pea flowers contain secondary metabolites including flavonoids. Flavonoids have pigments in the form of anthocyanins which are antioxidants which can prevent clogging of blood vessels by oxidizing saturated fats so that they can protect blood vessels from being damaged. Anthocyanins can also protect and prevent the stomach from damage, prevent tumor cells, improve vision and anti-inflammation in the brain based on the phytochemical screening of butterfly pea calyx (Kurniati & Azizah, 2021).

Based on the research of Raihan & Dalimunthe (2015), the results of the phytochemical screening results of butterfly pea flowers showed positive for flavonoids, alkaloids, saponins, tannins, and steroids. Compounds from the class of alkaloids, flavonoids, and phenols make butterfly pea flowers have the potential for bioactivity because they contain anthocyanins which function as antioxidants and anti-bacterials. Antioxidants can inhibit the production of intracellular free radicals and increase the ability of defense enzymes against free radicals (Riyanto & Suhartati, 2019).

The butterfly pea flowers are known to contain phenolic compounds which act as antioxidants by donating hydrogen so as to stabilize the lack of electrons in free radicals. The total phenolic content of the ethanol extract of the butterfly pea flower was 19.43 ± 1.621 GAE (mg/g sample) from the flower, not based on the flowering phase (Andriani & Murtisiwi, 2020). The antioxidant content of phenolic compounds is believed to function as protection against oxidative stress (Sholekah, 2017).

The inflorescence phase (pre-anthesis) bgins with the bracts which develop into ever-enlarging buds. The large flower bud ends followed by an anthesis phase where the flower blooms during the first day. Flowers that bloom all day will wither, which can be seen from the petals that appear to close until they turn brown (Reformasintansari & Waluyo, 2021). During the flower development phase is also followed by changes in metabolite compounds. During the flower development

phase is also followed by changes in metabolite compounds. Therefore it is necessary to carry out research with the aim of determing the content of anthocyanins, antioxidants and metabolites of butterfly pea flowers in the flowering phase which includes buds, blooms and withering, so that the metabolites present in each phase of flower development are known to be utilized.

MATERIAL AND METHOD

Collection of plant samples and research sites

The research was carried out from September to December 2022. Butterfly pea flowers were collected from Mulyaguna Village, Teluk Gelam District, Ogan Komering Ilir Regency, South Sumatra Province, at the coordinates of the location 3°36'02.9"S 104°48'31.8"E (Fig.1.). The research was conducted at the Physiology and Development Laboratory, Genetics and Biotechnology Laboratory, Department of Biology. Determination of metabolites by GC-MS at the Laboratory of Instrumentation and Services, Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Sriwijaya.

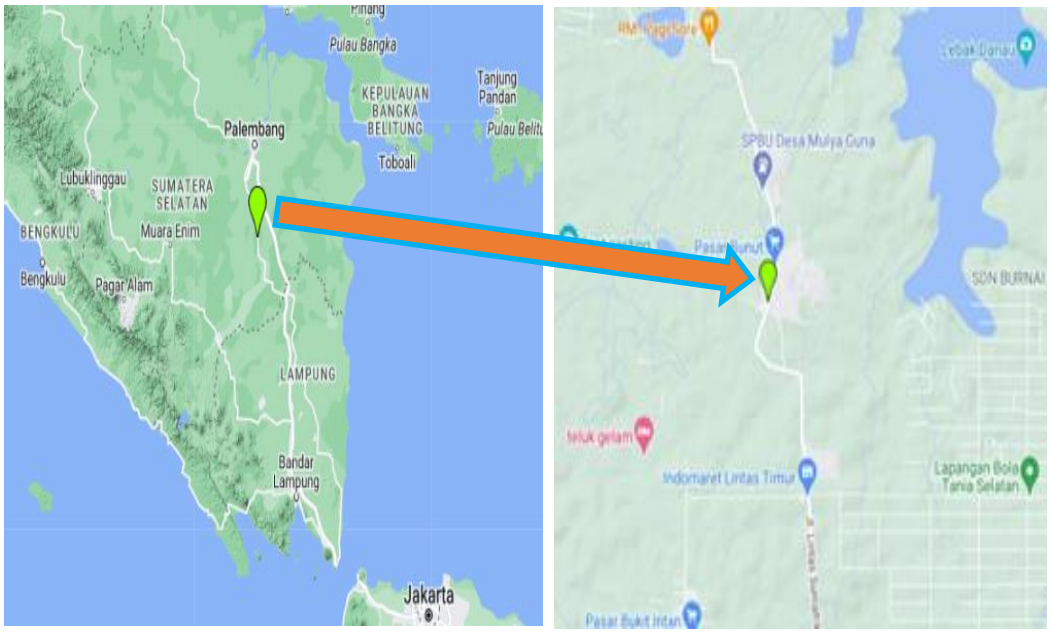

Figure 1. Location Map of the Bunga Telang sample collection in Mulyaguna village, Teluk Gelam, Ogan Komering Ilir, South Sumatra

Materials and tools

The materials needed are aquadest, butterfly pea flower based on bud flowering, blooming, and withering phases, DPPH (2,2-diphenyl-1-pikrihidrazil), and 70% ethanol solvent. The tools used in this study were a blender, centrifuge, Erlenmeyer, GC-MS TraceTM 1310 ISQ, measuring cups, beakers, quartz cuvettes, digital balances, ovens, refrigerators, UV-Vis spectrophotometers, vacuum rotary evaporators.

Procedure

Simplicia Preparation

The butterfly pea flower (*Clitoria teranatea* L.) is taken for the part of the flower that buds, blooms and withers. Furthermore, cleaned of impurities and dried with the sun's heat indirectly to prevent light oxidation. Dried butterfly pea flowers are mashed with a blender to obtain a homogeneous fine simplicia.

Extraction of Butterfly Pea Flower Metabolites

Butterfly pea flower simplicia powder weighing 10 grams was extracted by immersion maceration method using 70% ethanol solvent. Maceration is carried out within 2 x 24 hours. The extract was then filtered while the dregs were extracted by soaking again using 70% ethanol solvent. The soaked filtrate was then evaporated using a rotary evaporator to obtain a viscous ethanol extract of the butterfly pea flower.

Determination of Anthocyanin Content

A sample of 1 gram of fresh bud, blooming and wither butterfly pea flowers was crushed with a mortar in cold conditions, after being smooth, distilled water was added as a solvent with a ratio of 1:10 ml. The extract was then centrifuged for 15 minutes at 400g speed and the supernatant was collected. Absorbance with a UV-Vis spectrophotometer at a wavelength of 510 nm and 700 nm. This absorbance measurement used 70% ethanol as a blank. Total anthocyanin levels were calculated using references (Purwaniati *et al.*, 2020).

$$A \text{ (Final absorbansi)} = (A510 - A700)\ pH1 - (A510 - A700)\ pH\ 4{,}5$$

$$\text{Contens of anthocyanin} = \frac{A\,(absorbance\ of\ \ sampel)\,xMW\ (molecular\ weight)\,x\,DF\ (dilution\ \ factor)}{c\ (molar\ \ absorptivity = 26.900\ l\ (mol.cm)\,x\,L\ (cuvete\ \ width = 1cm)}$$

Determination of Antioxidant Contents with the DPPH KIT Method

Determination of antioxidant contents using the DPPH (2,2-diphenyl-1-picrihydrizyl) KIT, 0.5 ml of ethanol extract of butterfly pea flower samples were taken in different flowering phases, then mixed with 2.5 ml of 0.1 mM DPPH dissolved in ethanol and incubated for 30 minutes at 25°C and absorbance at a wavelength of 517 nm with a uv-vis spectrophotometer. The 70% ethanol was used as a blank and ascorbic acid was used as a standard antioxidant. Antioxidant contents are calculated by (AO, 2019) formula.

$$Anthocyanin\ content\ of\ sample = \frac{Absorbance\ of\ sample}{Absorbance\ of\ standard}\ x\ concetration\ of\ standard\ antioxidan$$

Determination of Metabolites

Determination of butterfly pea flower metabolites was carried out using

ethanol extract of butterfly pea flowers added with 10 ml of 70% ethanol and injected as much as 1 µl into the GC-MS according to the working instrument GC-MS TraceTM 1310 ISQ.

Data Analysis

Data analysis from the results of anthocyanin content and antioxidant content were analyzed with the mean and standard deviation. Chromatograms from CG-MS were traced for compounds with reference to the PubChem, KEGG, ChEBI, PlantCyc, and Spectrabase websites, then determined the class of the dominant compound.

RESULT AND DISCUSSION

Research on antioxidant content, anthocyanin content and metabolites in butterfly pea flowers based on the flowering phase.

Anthocyanin Content of Butterfly Pea Flower Based on Flowering Phase

Anthocyanin content in samples of fresh butterfly pea flowers that are still in bud flower, blooming and withered are presented in Figure 2.

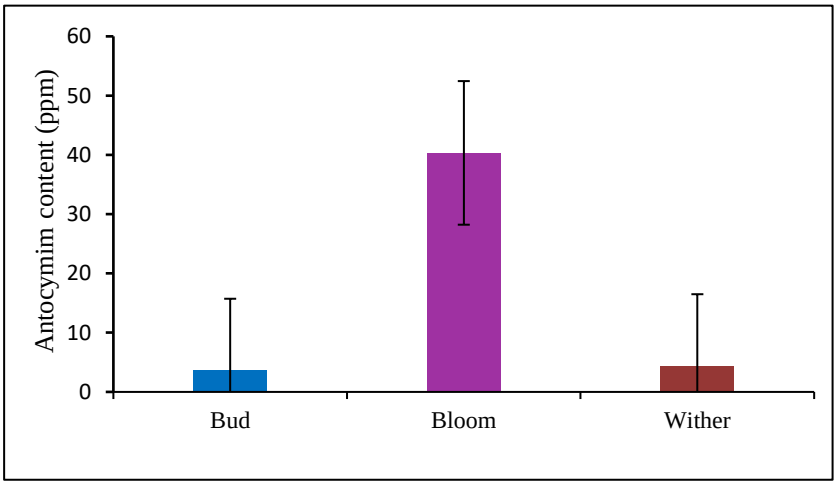

Figure 2. Anthocyanin levels in the 3 flower phases of *C. ternatea* bud, bloom, and wither.

Based on Figure 2. shows that the total anthocyanin content of the bud butterfly pea flower sample was 3.60 ± 2.47 ppm, in bloom was 40.33 ± 4.93 ppm and the anthocyanin content of wither butterfly pea flower was 4.36 ± 1.04 ppm. The anthocyanin content of the blooming butterfly pea flower was better than the bud and wither butterfly pea flower samples because the fresh blooming butterfly pea flower has entered an anthesis phase where the flower crown has fully opened and is dark blue in color. According to Purwaniati *et al.* (2020), fresh blue butterfly pea flowers produce greater total anthocyanins than dried anthocyanins because the anthocyanins in fresh butterfly pea flowers have not undergone any process that

could potentially damage the anthocyanins, such as drying or heating.

The flowering phase has a different total anthocyanin content, possibly due to differences in the flowering phase. According to Sangadji *et al.* (2017), butterfly pea flowers contain flavone compounds which function as important co-pigments to fully express the anthocyanin color in flower tissue which results in the highest anthocyanin content in the flower corolla while low anthocyanin levels are caused by a copigmentation reaction, and the extract still contains polyphenolase enzymes which catalyze browning reactions are found in dry and wither flowers.

Based on the results obtained, the butterfly pea flower has a high anthocyanin content. According to Suryana (2021), anthocyanins in butterfly pea flowers are organic compounds in the form of flavonoids which act as antioxidants. It was further explained that the natural pigment of butterfly pea flowers which shows a red-blue color is included in the anthocyanins of the delfinidin group (Mateus *et al.,* 2009). According to Palimbong & Pariama (2020), the blue dye contained in butterfly pea flowers contains anthocyanins which have the potential for the food industry, including being used as food coloring and traditional medicine. According to Lijon *et al.* (2017) & Marpaung (2020), butterfly pea flowers contain anthocyanins which show functional activity as an antidiabetic, control blood sugar, have anticancer, anti-inflammatory and analgesic effects, antiasthma, hepatoprotective activity and inhibit the growth of several pathogenic bacteria that produce extended-spectrum beta-enzymes, lactamase.

Antioxidant Content of Butterfly Pea Flowers Based on Flowering Phase

Based on the results of the analysis of the antioxidant content of samples of butterfly pea flowers that were still in bud, blooming, and withered, the total antioxidant content was presented in Figure 3.The total antioxidant content in the bud flower, blooming and withered butterfly pea flower samples was different for each flowering phase, where the total antioxidant content was better in the blooming butterfly pea flower sample of 6.58 ± 0.80 ppm. Butterfly pea flowers in bud have a total antioxidant content of 2.55 ± 0.50 ppm and withered butterfly pea flowers have an antioxidant content of 1.74 ± 0.44 ppm (Figure 3).

The blue butterfly pea flower contains antioxidants in the corolla of the flower. The total antioxidant content in the butterfly pea flower sample has a deep blue color indicating the presence of antioxidants in the form of flavonoids. According to research by Hassan and Laily (2014), the flavonoids contained in the hanging papaya flower extract when in bloom are more than the buds as potential antioxidants. Flavonoid compounds can neutralize free radicals thereby preventing cell damage.

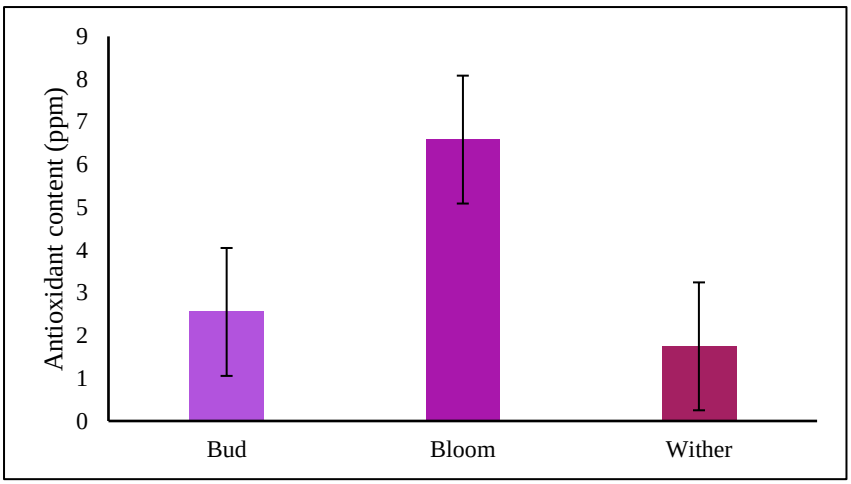

Figure 3. Antioxidant content in the 3 flower phases of *C. ternatea* bud, bloom, and wither.

The content of antioxidant compounds in fresh bloom butterfly pea flowers has a better antioxidant content. According to research by Cahyaningsih *et al.* (2019), fresh butterfly pea flowers that bloom perfectly contain tannins, carbohydrates, saponins, triterpenoids, phenols, flavonoids, flavonol glycosides, proteins, alkaloids, anthraquinones, anthocyanins, glycosides, stigmast-4-ene-3,6-dione, oil. essential oils and steroids which function as antioxidants. According to Al-snafi (2016), the content of antioxidant compounds is also useful as an antimicrobial, anthelmintic or antiparasitic and insecticidal agent, fever medicine and pain reliever, anticancer, antioxidant, lowering blood sugar levels, Alzheimer's disease, antiulcer, anticholesterol, hypoallergenic, immunomodulatory and can be used in the treatment of wounds.

The total antioxidant content in the butterfly pea flower sample was better because it contained phenolic compounds from the fully opened flower petals. According to Andriani & Murtisiwi (2020), butterfly pea flower extract has a very strong antioxidant activity because it contains phenolic content. The antioxidant mechanism of phenolic compounds is based on oxidation-reduction reactions, in which phenolic compounds will act as reducing agents so that they can reduce free radicals that are formed into non-reactive species.

Based on the research results obtained in the sample extracts of butterfly pea flowers that bloom and buds have a better antioxidant content found in butterfly pea flowers that bloom. According to Hassan and Laily (2014), the development of the generative organs (flowers) in plants, both when the flowers are budding and blooming, has different content of secondary metabolites, including one of which is the content of antioxidant compounds.

Secondary Metabolites of Butterfly Pea Flower Buds

Based on references to biosynthetic pathways of metabolites detected from GC-MS results, 13 classes of compounds were identified. Classes with more types of compounds can be seen in Figure 4.

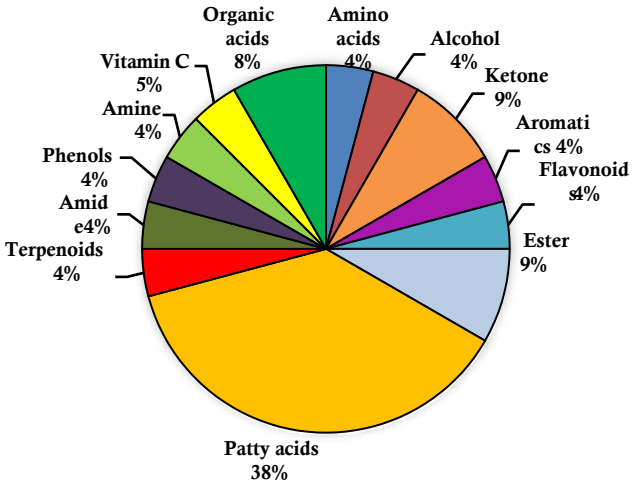

Figure 4. Abundance of metabolite classes in the bud flower phase of *C. ternatea*

In Figure 4, the components of the butterfly pea flower metabolite compound from the fatty acid class have a total percentage of 38%, followed by 8% organic acids, 4% aromatics and 4% flavonoids. Fatty acids were identified more than other classes due to their volatile nature. Based on research by Iqbal (2013), rosella flower simplicia (*Hibiscus sabdariffa* variety sabdariffa Linn.) analyzed by GC-MS contained 19 types of fatty acids. Most of the fatty acid compounds that have been formed when the butterfly pea flower buds will then decrease when the butterfly pea flower withers.

Secondary Metabolites Blooming Butterfly pea Flower

Based on the traceability of the biosynthetic pathway of the detected metabolites, 11 classes of compounds were identified in the blooming butterfly pea flower. Classes with more types of compounds can be seen in Figure 5.

Figure 5 shows the components of the butterfly pea blossom flower metabolite compound which has a higher total percentage than the other classes, consisting of 22% organic acids, 22% aromatics, 18% fatty acids and 4% flavonoids. According to Nurholis & Ismail (2019), the organic acid content reaches a maximum in the growth and development phase, then decreases in the maturation or maturation phase. According to Pertiwi *et al*. (2022), butterfly pea flowers have aromatic secondary metabolites which are part of the phytochemicals that act as antibacterial against gram-positive bacteria that are saprophytes on human skin.

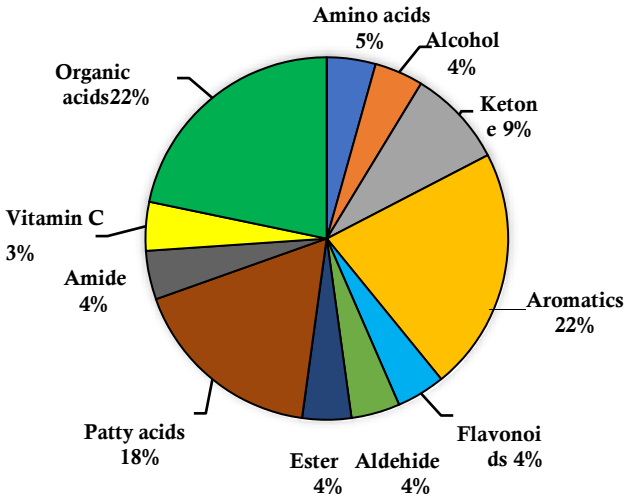

Figure 5. Abundance of metabolite classes in the blooming phase of *C. ternatea*

Secondary Metabolites of Butterfly pea Flowers Wither

Based on observations of the biosynthetic pathways of the detected metabolites, 12 classes of compounds were identified in the butterfly pea flower. Classes with more types of compounds can be seen in Figure 6. as follows.

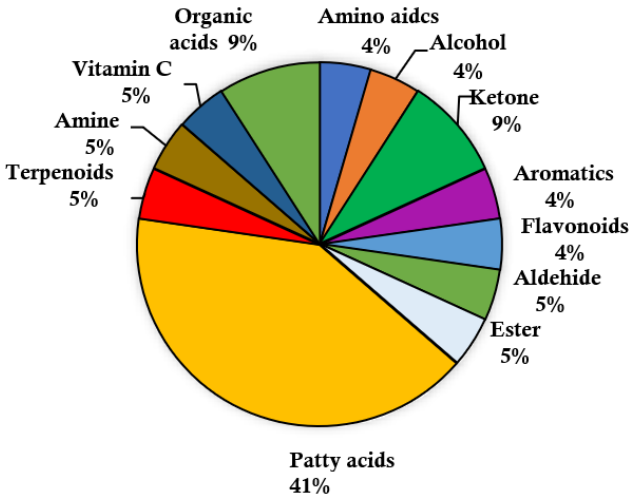

Figure 6. Abundance of metabolite classes in the wither phase of *C. ternatea*

In Figure 6. shows the components of butterfly pea wither flower metabolites have a class of compounds whose total percentage is greater than the other classes, consisting of 41% fatty acid class, 9% organic acids, and 4% aromatics and 4% flavonoids. According to Purwaniati *et al.* (2020), anthocyanins are group compounds Phenolic compounds synthesized via the flavonoid pathway. Flavonoid compounds are synthesized by plants as a defense system against infection by microorganisms so that they are effective as antimicrobials.

CONCLUSION

In the butterfly pea flower, dominant metabolites were found including the groups of fatty acids, organic acids, aromatics and flavonoids with different abundances of metabolites in the bud flowering, blooming and withering phases. The developmental phase of the blooming butterfly pea flower has anthocyanin content of 40.33 + 4.93 ppm which is more than the bud and wither flowers. The highest antioxidant content was also found in the blooming butterfly pea flower of 6.58 ± 0.80 ppm. It is necessary to conduct further research regarding the profile of butterfly pea flower metabolites based on the flowering phase, especially to look for bioactive compounds and their activity as well as metabolomics by fractionation.

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