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(RESEARCH ARTICLE)

Metabolite profiling of butterfly pea flower (*Clitoria ternatea* L.) in the flowering development phase

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Abstract

The butter pea flower (*Clitoria ternatea* L.) has long been known as a medicinal plant because it contains various metabolite compounds, the content of metabolite compounds that are influenced by plant development. So it is necessary to conduct metabolomics profiling research to determine the metabolite compounds found in butterfly pea flowers in the flower bud, blooming, and wilting phases. Metabolite profiling from ethanol extract in the development phase of butterfly pea flowers in the bud, blooming, and wilting phases using GC-MS analysis identified 20 metabolite compounds each with butterfly pea flowers in the bud phase having the dominant compound *Dihydroxyacetone*, blooming flowers found the compound *3-O-Methyl-d-glucose*, and *n-Hexadecanoic acid* compound withered flowers. Three dominant compounds were found in all development phases, namely *4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl; Dihydroxyacetone*; *Benzoic acid, ethyl ester*; and *Dehydroxyacetone*. The unique metabolite compound *4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl* is included in the flavonoid group, especially anthocyanin which has the potential to be an antioxidant, antibiotic, antidiabetic, anti-inflammatory, and immunomodulatory so it can be used as a natural coloring agent for functional foods or drinks and herbal medicines.

Keywords: Butterfly pea flower; *Clitoria ternatea* L.; GC-MS; Metabolite profiling; Flowering development

1. Introduction

Butterfly pea flowers (*Clitoria ternatea* L.) are used as a natural coloring for drinks and food because of their anthocyanin content. Anthocyanin a type of flavonoid, which are stable and function as antioxidants that protect cells in the body from exposure to free radicals [1]. Therefore, using the blue color of butterfly pea flowers is believed to help prevent several diseases. Processing butterfly pea flowers into a functional drink can prevent atherosclerosis, which is a blood vessel blockage disease. Consuming butterfly pea flowers can also reduce symptoms of stress and depression, control obesity, treat asthma, and prevent cancer [2].

In butterfly pea flowers there are secondary metabolites such as flavonoids which have pigments in the form of anthocyanin which function as antioxidants and can prevent blockage of blood vessels by oxidizing bad fats. Anthocyanin also protect and prevent the stomach from damage, prevent tumor cells, improve vision, and are anti-inflammatory [3]. Furthermore, according to [4], butterfly pea flowers also contain flavonoids, alkaloids, saponins, tannins, and steroids. Compounds from the alkaloid and flavonoid groups make butterfly pea flowers have the potential for bioactivity because they contain anthocyanin which function as antioxidants and antibacterial [5].

The composition of metabolite compounds can be determined using a metabolomics analysis approach [6]. Metabolomics analysis is a study that studies the components of bioactive compounds in plant extracts [7]. The method that can be used for metabolomics analysis is metabolite profiling. The metabolite profiling method is an initial

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metabolomics approach that describes the profile of metabolite compounds in plant samples [8]. Non-target metabolomics analysis can be used to identify metabolite compounds in samples using GC-MS (Gas Chromatography-Mass Spectrometry). Based on research by [9], 8 compounds were obtained from butterfly pea flower metabolites using GC-MS which were successfully extracted from methanol solvent, and 18 compounds were obtained from n-hexane extract solvent.

Flowering (phenophase) begins after flower induction and continues with the differentiation process of flower organs, pre-anthesis, anthesis, and pollination. The inflorescence phase (pre-anthesis) begins with the bracts developing into increasingly larger buds. The large flower buds end followed by the anthesis phase where the flowers bloom for 1 day. Flowers that bloom all day will wilt, which can be seen from the flower petals which appear to close until the color turns brown [10].

The growth and development of flowers influences the abundance of metabolite compounds. The development of flowers when entering the flowering phase is thought to be a factor that influences the abundance of metabolite compounds, so research was carried out to study non-target metabolite profiling of butterfly pea flowers based on the phase of flowering bud, blooming and wilted.

2. Materials and Methods

2.1. Sampling

The butterfly pea flowers were taken from Mulyaguna Village, Teluk Gelam District, Ogan Komering Ilir Regency, South Sumatra Province, Indonesia, at the location coordinates 3°36'02.9"S 104°48'31.8"E.

The butterfly pea flowers used as samples were flower buds with criteria starting from the small and large bud phases. Butterfly pea blooms are taken with the characteristics of a flower corolla that has completely emerged from the flower petals. Samples of wilted butterfly pea flowers with the criterion that the flower petals will appear closed in a curled position with a dry brown color (Figure 1.).

(a) (b) (c)

(a)Flower buds, (b) Flowers bloom, (c) Flowers wilt

Figure 1 Butterfly pea flowers based on flowering phase

2.2. Instruments and Chemicals

The instrument used in this research is GC-MS Trace TM 1310 ISQ, vacuum rotary evaporator. The materials needed for this research include distilled water and 70% ethanol.

2.3. Preparation of simplicia

Sampling of butterfly pea flowers was carried out in the morning at 07.00 local time. The butterfly pea flower was taken from the part of the flower that buds, blooms, and withers. Next, it is cleaned from impurities and dried in direct sunlight. Then 200 g of dried butterfly pea flowers were ground with a blender.

2.4. Extraction of butterfly pea flower metabolites

Simplicia powder from butterfly pea flowers was extracted using the immersion maceration method using 70% ethanol solvent, within 2x24 hours. The extract is then filtered while the dregs are extracted by soaking again using 70% ethanol solvent. Extraction using maceration extraction was repeated until the filtrate was clear. The filtrate resulting from soaking is then evaporated using a rotary evaporator to obtain a thick, concentrated ethanol extract of butterfly pea flower.

2.5. Metabolite Profile Analysis with GC-MS instruments

GC-MS analysis of butterfly pea flowers was carried out by adding ethanol extract of butterfly pea flowers to 10 ml of 70% ethanol and injecting 1 µl into the GC-MS according to the method instrument based on the GC-MS Trace TM 1310 ISQ work protocols.

2.6. Analysis of GC-MS Data

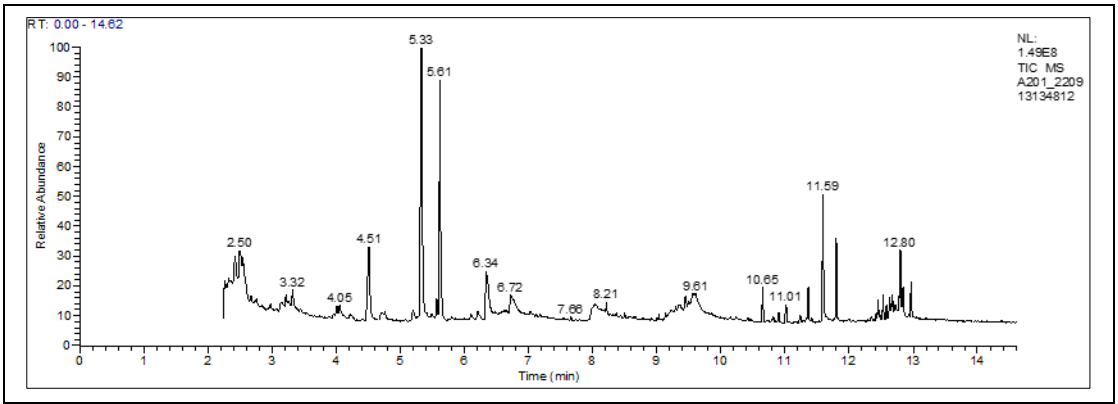
Metabolite profile and identification of metabolite compounds from the GC-MS chromatogram equipped with a list of detected chemical components, chemical formula, retention time, and area. Then a search was carried out for metabolite compounds using the PubChem, KEGG, ChEBI, PlantCyc, and Spectrabase websites

3. Results and Discussion

Research on the analysis of metabolite profiles in butterfly pea flowers based on the flowering phase, namely flowering butterfly pea buds, blooming, and wilting, obtained the following results:

Chromatograms on mass spectrometry (MS) of butterfly pea flowers from the three flowering phases showed a diversity of metabolite compounds. The metabolite profiles detected from the GC-MS results of butterfly pea flowers fall into the classes of fatty acids, carbohydrates, aromatics, organic acids, amino acids, ketones, flavonoids, phenols, alcohols, esters, aldehydes, terpenoids, amides, amines, and ascorbic acid. The class groupings are adjusted based on official websites such as ChEBI, PubChem, PlantCyc, KEGG, and Spectrabase.

Metabolite profiles resulting from GC-MS analysis of butterfly pea flower samples in the bud, blooming, and wilting phases showed changes in chemical compound components both qualitatively and quantitatively which were influenced by differences in the bud, blooming, and wilting phases. The compound components of each butterfly pea flower are displayed in the form of each peak in the chromatogram. The chromatogram obtained from the results of GC-MS analysis of each phase of the butterfly pea flower is shown in Figure 2.



(a)

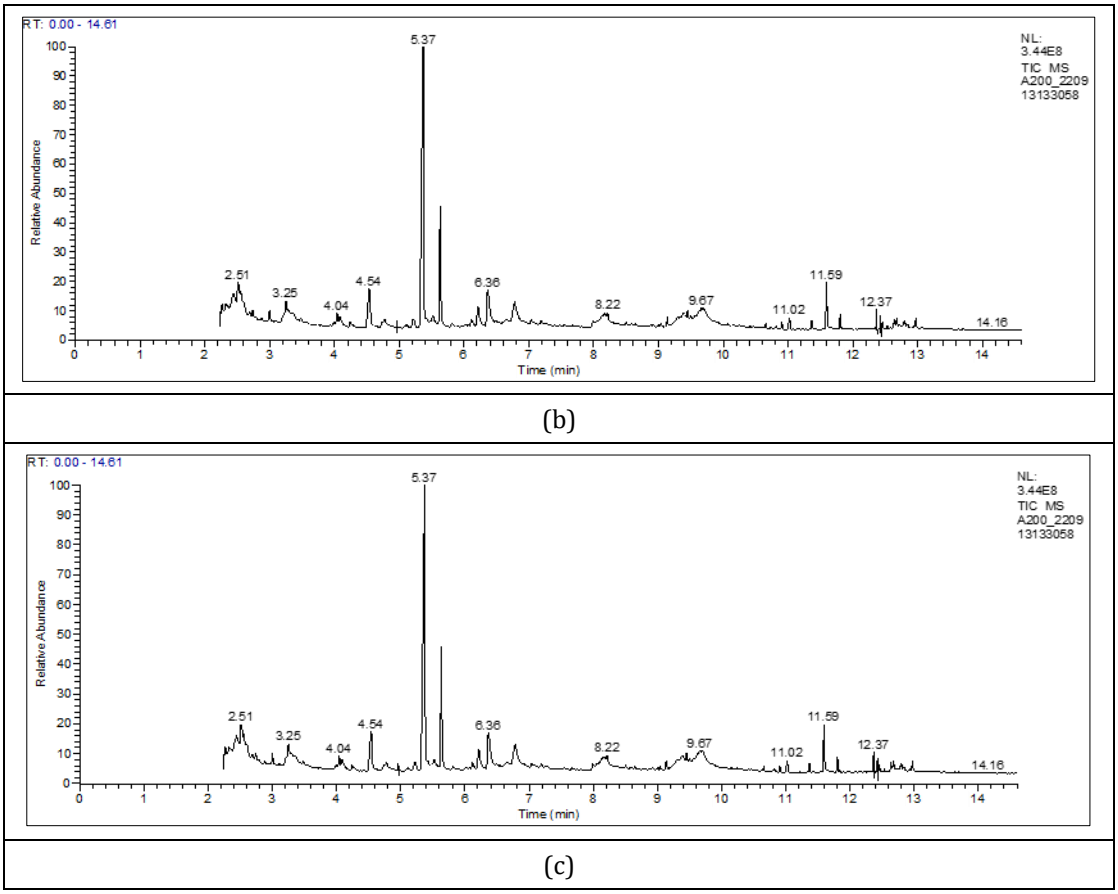


Figure 2 Chromatogram from GC-MS of butterfly pea flower ethanolic extract in the flowering phase (a) bud flowers (b) blooming flowers, and (c) wilting flowers

The chromatogram in Figure 2 shows 20 main peaks detected for each eluted sample. Each peak represents a different compound with a different retention time. Domain peaks in Figure 2 represent peaks with a higher percent area than other peaks. The percent area expresses the abundance of a detected compound. Qualitative analysis, the number of compounds in the ethanol extract of butterfly pea flowers using GC-MS can be seen from the number of chromatogram peaks. The peaks of this chromatogram will have a number proportional to the number of compounds present in the sample being tested. The number of peaks in the chromatogram will indicate the number of components that make up the mixture so that the metabolite profile of each sample can be known. According to [11], the relative amount of each compound detected can be determined based on the percentage of peak area relative to the total peak.

Based on Figure 2, shows a higher abundance of metabolite compounds in the relative area percent which is called the dominant compound. The dominant compounds from the three flowering phases of butterfly pea flowers fall into the aromatic classes of flavonoids, fatty acids, organic acids, alcohols, and carbohydrates. Metabolites identified from GC-MS analysis of ethanol extract of butterfly pea flowers can be seen in Table 1. (Table 1a. bud lower phase, 1b. booming flower phase, and 1c. wilted flower phase)

Table 1a Metabolite compounds, chemical formula, RT, and area of butterfly pea flower ethanol extract in the bud flower phase

(a) Metabolite compounds from the ethanol extract of butterfly pea in the bud flower phase			
Metabolite compounds	**Chemical formula**	**RT (min)**	**Area (%)**
1,2-Butanediol, 1-(2-furyl)-	$C_8H_{12}O_3$	2.27	1.42
1-Hydroxy-2-pentanone	$C_5H_{10}O_2$	2.33	2.63
o-Acetyl-L-serine	$C_5H_9NO_4$	2.44	5.10

Dihydroxyacetone	$C_3H_6O_3$	2.51	8.70
2,4-Dihydroxy-2,5-dimethyl-3(2H)-fur an-3-one	$C_6H_8O_4$	3.25	5.66
Benzeneacetaldehyde	C_8H_8O	4.04	2.68
3H-Pyrazol-3-one, 2,4-dihydro-2,4,5-trimethyl2-Pyrazolin-5-one, 1,3,4-trimethyl	$C_6H_6O_3$	4.54	4.46
l-Gala-l-ido-octose	$C_8H_{16}O_8$	4.78	2.00
Glucosamine, N-acetyl-N-benzoyl	$C_{15}H_{19}NO_7$	5.52	1.91
Benzoic acid, ethyl ester	$C_9H_{10}O_2$	5.63	6.31
5-Hydroxymethylfurfural	$C_6H_6O_3$	6.22	1.78
Methyl 6-oxoheptanoate	$C_8H_{14}O_3$	6.78	4.95
1,2,3-Propanetriol, 1-acetate	$C_5H_{10}O_4$	6.37	5.27
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	$C_6H_8O_4$	5.36	24.28
d-Mannose	$C_6H_{12}O_6$	9.45	5.16
Desulphosinigrin	$C_{10}H_{17}NO_6S$	9.67	4.46
ç-Thionodecalactone	$C_{10}H_{18}OS$	11.59	1.91
d-Gala-l-ido-octonic amide	$C_8H_{17}NO_8$	9.31	3.51
n-Hexadecanoic acid	$C_{16}H_3O_2$	11.59	2.68
9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	$C_{21}H_{36}O_4$	12.68	1.76
Total abundance of the compound (%) **93.38**			

Table 1b Metabolite compounds, chemical formula, RT, and area of butterfly pea flower ethanol extract in blooming flowers

(b) Metabolite compounds from the ethanol extract of butterfly pea in the booming flower phase			
Metabolite compounds	**Chemical formula**	**RT (min)**	**Area (%)**
1,3-Butadiene-1-carboxylic acid	$C_5H_6O_2$	2.26	1.77
2-Nitro-1-buten-3-ol 3-Nitro-3-buten-2-ol	$C_4H_7NO_3$	2.32	3.66
o-Acetyl-L-serine	$C_5H_9NO_4$	2.42	4.73
Dihydroxyacetone	$C_3H_6O_3$	2.50	8.86
2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	3.22	1.77
Isosorbide Dinitrate	$C_6H_8N_2O_8$	3.22	1.94
3-Thiazolidinecarboxamidine, 2-imino	$C_4H_8N_4S$	4.05	2.15
3H-Pyrazol-3-one, 2,4-dihydro-2,4,5-trimethyl2-Pyrazolin-5-one, 1,3,4-trimethyl	$C_6H_6O_3$	4.51	5.92
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	$C_6H_8O_4$	5.33	16.25
Benzoic acid, ethyl ester	$C_9H_{10}O_2$	5.61	10.6
1,2,3-Propanetriol, 1-acetate	$C_5H_{10}O_4$	6.35	4.81
Methyl 6-oxoheptanoate	$C_8H_{14}O_3$	6.73	4.77

L-Glucose	$C_6H_{12}O_6$	8.04	4.22
Desulphosinigrin	$C_{10}H_{17}NO_6S$	9.35	2.77
Cyclopropanetetradecanoic acid, 2-octyl-, methyl ester	-	9.45	1.73
3-O-Methyl-d-glucose	$C_7H_{14}O_6$	9.58	8.76
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	11.59	5.42
Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	11.80	2.87
9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	$C_{21}H_{36}O_4$	12.67	2.02
Ethyl 9.cis.,11.trans.-octadecadienoate	$C_{20}H_{36}O_2$	12.80	4.97
Total abundance of the compound (%) **95.18**			

Table 1c Metabolite compounds, chemical formula, RT, and area of butterfly pea flower ethanol extract in wilted flower

(c) Metabolite compounds from the ethanol extract of butterfly pea in the wilted flower phase			
Metabolite compound	**Chemical formula**	**RT (min)**	**Area (%)**
1,3-Butadiene-1-carboxylic acid	$C_5H_6O_2$	2.28	2.01
2-Nitro-1-buten-3-ol 3-Nitro-3-buten-2-ol	$C_4H_7NO_3$	2.35	2.92
o-Acetyl-L-serine	$C_5H_9NO_4$	2.43	4.53
Dihydroxyacetone	$C_3H_6O_3$	2.50	8.48
2,4-Dihydroxy-2,5-dimethyl-3(2H)-fur an-3-one	$C_6H_8O_4$	3.22	2.21
DL-Arabinose	$C_5H_{10}O_5$	3.32	2.07
Propanoic acid,3-(acetylthio)-2-methylS-Acetyl-2-methyl-3-mercaptopropionic acid	$C_6H_{10}O_3S$	4.06	2.54
3H-Pyrazol-3-one, 2,4-dihydro-2,4,5-trimethyl2-Pyrazolin-5-one, 1,3,4-trimethyl	$C_6H_6O_3$	4.52	5.47
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	$C_6H_8O_4$	5.34	18.88
Benzoic acid, ethyl ester	$C_9H_{10}O_2$	5.62	8.61
1,2,3-Propanetriol, 1-acetate	$C_5H_{10}O_4$	6.35	3.97
Methyl 6-oxoheptanoate	$C_8H_{14}O_3$	6.75	4.67
L-Glucose	$C_6H_{12}O_6$	8.05	2.82
9-Octadecenoic acid, (2-Phenyl-1,3-dioxolan-4-yl)methyl 9-octadecenoate, cis	$C_{28}H_{46}O_4$	9.29	2.06
Desulphosinigrin	$C_{10}H_{17}NO_6S$	9.36	1.71
á-D-Glucopyranose, 4-O-á-D-galactopyranosyl		9.61	2.22
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	11.59	7.10
Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	11.80	2.02
cis-Vaccenic acid	$C_{18}H_{34}O_2$	12.67	5.62
Ethyl 9.cis.,11.trans.-octadecadienoate	$C_{20}H_{36}O_2$	12.80	4.08
Total abundance of the compound (%) **93.39**			

Table 1a. Butterfly pea flower ethanol extract in bud phase has the dominant compounds *4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl* (24.28%). *Dihydroxyacetone* (8.7%). and *Benzoic acid. ethyl ester* (6.31%). Blooming flowers have the dominant compounds *4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl* (16.25%). *Benzoic acid, ethyl ester* (10.6%). and *Dihydroxyacetone* (8.86%) %) (Table 1b). Meanwhile. withered flowers found the dominant compounds *4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl* (18.88%). *Dihydroxyacetone* (8.48%). *Benzoic acid ethyl ester* (8.61 %) (Table 1c). According to research [9]. the largest group of flavonoids has a pyran ring that connects three-carbon chains which are often found in the seeds. The compound *4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl* is more abundant in the buds flower because it has just entered the initial flowering phase, followed by different changes. According [12], *Benzoic acid, ethyl ester* is a *benzoic acid* derivative that has activity as an antioxidant, antifungal, analgesic, anticancer, anti-inflammatory, and anti-Herpes Simplex Virus.

The metabolite compounds detected were the same in all three phases of butterfly pea flower development, total 16 compound (Tabel 1a, 1b and 1c.). Metabolite compounds namely, *1-(Methoxymethoxy)-3-methyl-3-hydroxybutane*; *o-Acetyl-L-serine*; *Dihydroxyacetone*; *2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one*; *4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl*; *Benzoic acid, ethyl ester*; *1,2,3-Propanetriol, 1-acetate*; *Methyl 6-oxoheptanoate*; *L-Glucose*; *d-Mannose*; *n-Hexadecanoic acid*; *Methanol. oxo-, benzoate*; *Isosorbide Dinitrate*; *Propanoic acid, 2-nitro-, methyl ester*; *3H-Pyrazol-3-one, 2,4-dihydro-2,4,5-trimethyl2-Pyrazolin-5-one, 1,3,44-trimethyl*; *Desulphosinigrin*; and *l-(+)-Ascorbic acid 2,6-dihexadecanoate*. Some of these compounds have bioactivity as antioxidants [12], [13], [14], [15], [16], antibacterial [17], [18], antidiabetic [14], [15],[19], [20], antimicrobial [21], antitumor [20], anti-inflammatory [12], immunomodulatory [22], antifungal [18], and there are several compounds whose bioactivity is unknown.

Butterfly pea flowers have a large area percentage of the compound *4H-Pyran-4-one 2,3-dihydro-3,5-dihydroxy-6-methyl* which belongs to the flavonoid pigment class (including anthocyanins). According to [23], anthocyanins are a group of phenolic compounds that are synthesized via the flavonoid pathway. Flavonoid compounds are synthesized by plants as a defense system against infection by microorganisms so they are effective as antimicrobials. According to [24], flavonoids are a polyphenolic compound that has various bioactivities including antioxidant, antitumor, anti-inflammatory, antibacterial, and antiviral effects.

In butterfly pea flowers. there is also a higher class of carbohydrate compounds, namely *d-Mannose*. The *d-Mannose* compound is a simple carbohydrate that is often found in plants. Simple carbohydrates. especially sugar, are the main source of energy stored as starch in plants. According to [25], *d-Mannose* is a simple carbohydrate that has antibacterial activity by clumping *Saccharomyces cerevisiae* so that it can increase antibacterial activity.

4. Conclusion

Metabolite profiling from ethanol extract in the development phase of butterfly pea flowers in the bud, Blooming, and wilting phases using GC-MS analysis identified 20 metabolite compounds each with butterfly pea flowers in the bud phase having the dominant compound *Dihydroxyacetone*, blooming flowers found the compound *3-O-Methyl-d-glucose*, and *n-Hexadecanoic acid* compound withered flowers. Three dominant compounds were found in all development phases. namely *4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl; Dihydroxyacetone*; *Benzoic acid, ethyl ester*; and *Dehydroxyacetone*. The unique metabolite compound *4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl* is included in the flavonoid group, especially anthocyanin which has the potential to be an antioxidant, antidiabetic, antibiotic, and antiaggregation so it can be used as a natural coloring agent for functional foods or drinks and herbal medicines.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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