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Research Article

Profile of Secondary Metabolites in Different Parts of the Butterfly Pea (*Clitoria ternatea*) Plant with Antioxidant Activity

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Abstract: The butterfly pea flower (*Clitoria ternatea*) is widely used in traditional medicine and has the potential to be an antioxidant. The study aimed to compare the antioxidant capacity of the ethanol extract of the butterfly pea flower and the metabolite profile of the n-hexane fraction in different parts of the plant. The butterfly pea flower was planted for 12 weeks, and as many as 30 plants were in the Tropical Biopharmaca Research Center Cikabayan experimental garden, Bogor, Indonesia. Plant measurements included plant height, number of leaves, stem diameter, and plant production, which always increased during the experiment. The root had the highest phenolic content of each part of the plant (roots, flowers, leaves, and stems), with a phenolic content of 83.45 mg GAE/g. At the same time, the highest flavonoid content was in the leaves, with a total flavonoid value of 5.96 mg QE/g. Flowers and leaves only have anthocyanin content. The root had the highest antioxidant activity (low IC_{50} value) of each part of the plant, with an IC_{50} value of 106.973 µg/mL. The GC-MS results from the roots showed 13 compounds identified: 12 in the flower parts, 11 in the leaf parts, and 9 in the stem parts.

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Footnote: This study is largely based on T. Muhammad Iqbal's BSC thesis, which was completed in 2019. Sulistiyani and Waras Nurcholis supervised this thesis.

1. Introduction

Traditional medicine using plants has been carried out for a long time by the community, so much research on the efficacy of medicinal plants presents a great opportunity as an alternative treatment for various diseases by making them traditional medicines (Rabeta and Nabil, 2013). The butterfly pea flower is one of the plants used by the community as traditional medicine by boiling it. The butterfly pea flower has the potential to be a raw material for medicine because it has many bioactive compounds and is efficacious in its use (Esmail, 2016; Lijon et al., 2017). The manufacture of medicinal raw materials uses solvents that can attract bioactive compounds in plants, such as ethanol. This solvent is

often used in the pharmaceutical raw material industry because it has the same polarity level as bioactive compounds in plants (El-Maati et al., 2016).

The butterfly pea flower (*Clitoria ternatea*), also known as the blue flower or butterfly pea, is a vine belonging to the Fabaceae family (also known as Leguminosae), which has light blue, white, pink, and blue flowers. Water extracted from butterfly pea flowers in the traditional way also has the potential to be a good antioxidant. The ethanol extract of butterfly pea flowers also has properties as an antioxidant, so it can prevent oxidative stress in the body (Adwas, 2019). In addition, ethanol extract is also thought to have efficacy as an antidepressant from the roots, which regulates the serotonergic system and acetylcholine (Parvathi et al., 2013). Parts of the butterfly pea plant can also be used as an anti-microbial, anti-inflammatory, anti-cancer, and antidiabetic (Esmail, 2016). Applying butterfly pea flowers as animal feed is also very beneficial for livestock businesses because it is suspected of increasing livestock production (Suarna & Wijaya, 2021). The efficacy of the butterfly pea flower is the activity of the content of various compounds present in the butterfly pea plant, one of which is anthocyanins (Thuy et al., 2021). The blue color in the butterfly pea flower is an anthocyanin compound that is owned by the butterfly pea flower. Anthocyanins can also treat cancer, diabetes mellitus, and heart attacks (Alam et al., 2021). This content is an accumulation of secondary metabolite compounds produced by plants.

Secondary metabolites are compounds produced by a plant's metabolism in order to defend itself, and they have a wide range of biological functions for human health (Thirumurugan, 2018). It is this biological activity that gives a plant its properties. Secondary metabolite compounds can be taken with certain solvents and identified using compound separation techniques using certain tools. Hexane is an efficient solvent that dissolves in samples to extract non-polar secondary metabolites in plants, even though they are classified as pollutant compounds (Yara-Varon, 2016). Hexane takes up non-polar compounds found in plants well because of its polarity (Ghazali and Yasin, 2016). Identify secondary metabolites using compound separation tools such as Gas Chromatograph – Mass Spectrophotometer (GC-MS) (Alp et al. 2022). In research by Neda et al. (2013), water and methanol extracts of butterfly pea flowers were identified as having the main compound, inositol, which is effectively used as an anticancer against several types of cancer.

The butterfly pea flower (*Clitoria ternatea*) is an ornamental plant and has the potential to be a medicinal plant. Butterfly pea plants can be used as an alternative source of antioxidants, that contain secondary metabolites. There is no scientifically based information about the antioxidant capacity of roots, flowers, stems, and leaves in ethanol solvents or comparisons of plant secondary metabolite profiles with n-hexane solvents. Information on secondary metabolites in the butterfly pea plant is needed so its potential can be seen. Secondary metabolites are very important in determining the biological activity of plants, so the identification and isolation of secondary metabolites are mostly done to determine the content and structure of secondary metabolites (Sholikhah, 2016). This study aims to compare the antioxidant capacity of each part of the ethanol extract of the butterfly pea plant and the content of secondary metabolites in the n-hexane fraction in each part of the butterfly pea plant (*Clitoria ternatea*), namely the roots, flowers, stems, and leaves. This research is expected to provide information about the content of secondary metabolites in the butterfly pea flower (*Clitoria ternatea*) through the analysis of the separation of metabolites. In addition, this study also provides information about the extract of the part of the butterfly pea plant that has the most potential as an antioxidant.

2. Material and Methods

2.1. Plant materials and preparations

The *Clitoria ternatea*, or butterfly pea, plant used in this study was grown at the Tropical Biopharmaca Research Center Experimental Garden, IPB University, Bogor, Indonesia. Thirty samples of butterfly pea flowers were planted, and the morphology of the plants was observed until the age of the plants reached 12 weeks. Observations were made on the leaves, stems, flowers, and roots. Parameters calculated on the leaves include the number of leaves and leaf shape, while the parameters on the stem include stem length, stem color, diameter at the base of the stem, and stem shape. Interest observations are calculated from the sixth week when the flowers have appeared. The calculated flowers

are bud flowers, blooming flowers, and overblown flowers. Furthermore, the roots were observed when the butterfly pea plant was 12 weeks old to determine the plant's root system.

The different parts of the plants were harvested and separated. Each plant part is weighed using an analytical balance (Kenko) to determine the fresh weight. Next, each plant part was dried in an oven (EYELA NDO-700) at 50°C for 3 × 24 hours. The dry samples were then weighed again to determine the dry weight of each plant part. Each plant part was then crushed to become simplicia powder (60 mesh).

2.2. Water content analysis (Depkes RI 2008)

The water content is measured by weighing a porcelain cup that has been desiccated at 105°C for 30 min using an oven (EYELA NDO-700) and a desiccator. A total of 1 gram of sample was put into a cup and weighed. The sample and cup were heated at 105°C for 5 hours using an oven, and the weight was again weighed. The moisture content was calculated using the following formula.

$$\% \text{ Water content} = \frac{A - B}{A} \times 100 \quad (1)$$

Note: A = Sample weight before drying (g)
B = Weight of sample after heating (g)

2.3. Sample extraction

Sample extraction was carried out by the maceration method based on Lee et al. (2011). As much as 20 g of simplicia powder was macerated with 200 mL of 96% ethanol (Merck KGaA Germany) at a ratio of 1:10. The mixture was macerated in a dark room using a water bath shaker (Memmert WNB 22 With a Shaking Device) for 2 × 24 hours at 110 rpm. The filtered sample filtrate was taken and concentrated using a rotary vacuum evaporator (Hahn-Shin, HS-2005 V) to obtain an extract paste. The extraction yield calculation uses the following formula.

$$\text{Yield (\%)} = \frac{\text{Extract weight (g)}}{\text{Simplicia weight (g)} \times (1 - \text{Water content})} \times 100 \quad (2)$$

2.4. Analysis of total anthocyanins (Tonutare et al., 2014)

A total of 10 mg of sample extract (in 10 mL of 96% ethanol) was added with 0.1 M HCl at a ratio of 85%:15% (v/v). The solution was centrifuged for 15 minutes at 5000 rpm. 3 mL of the centrifuged supernatant was diluted with 5 mL of buffer solution pH 1.0 (0.1864 g KCl in 95 mL distilled water and 5 mL concentrated HCl) and a buffer solution pH 4.5 (0.1864 g KCl in 95 mL distilled water and 5 mL concentrated HCl). Both solutions were measured at a wavelength of 520 nm and 700 nm using a UV-VIS spectrophotometer (Genesys 10 UV, Thermo Scientific). The absorbance calculation uses the following formula.

$$A = (A_{520}\text{-}A_{700})_{\text{pH 1.0}} - (A_{520}\text{-}A_{700})_{\text{pH 4.5}} \quad (3)$$

Meanwhile, monomeric anthocyanin levels (cyaniding-3-glucoside equivalent in mg/L) are calculated using the formula:

$$\text{Anthocyanin levels} = \frac{A \times MW \times DF \times 1000}{\varepsilon \times l} \quad (4)$$

Keterangan: A = Absorbance (A)
MW = Molecular Weight *cyaniding-3-glucoside* (449.2 g/mol)
DF = Dilution Factor

ε = Molar Absorptivity *cyanidin-3-glucoside* (26.900 molar)
L = Cuvette Width (cm)

2.5. Total phenolic analysis

The total phenolic content was measured based on the procedures of the Indonesian Ministry of Health (2011). A total of 15 mg of the extract was dissolved in 25 mL of methanol (pro analysis). As much as 1 mL of the extract solution was added to 5 mL of Folin-ciocialteu reagent (7.5% in water) in a test tube. Solution incubation was carried out for 8 minutes in a dark room (room temperature). Then, 4 mL of 1% NaOH was added to the solution and incubated again for 1 hour in a dark room (room temperature). The total phenolic content was measured using a UV-VIS spectrophotometer (Genesys 10 UV, Thermo Scientific) with a wavelength of 730 nm.

2.6. Analysis of total flavonoids

Measurement of total flavonoids refers to BPOM RI (2008) procedures. A total of 200 mg of the extract was added with 2 mL of 25% HCl, 1 mL of 0.5% w/v HMT, and 20 mL of acetone in an Erlenmeyer glass (50 mL PYREX). The solution was shaken and refluxed at 90°C for 30 minutes. The solution was added with a little acetone (pro analysis) and filtered into a measuring flask (PYREX 100 mL). A total of 20 mL of the filtrate was added with 20 mL of distilled water and 15 mL of ethyl acetate in a separatory funnel. The separated ethyl acetate fraction was collected in a measuring flask (50 mL PYREX). A total of 1 mL of the fractionated solution was added to 1 mL of 2% $AlCL_3$ and calibrated with 5% (v/v) glacial acetic acid to a volume of 25 mL in a measuring flask (25 mL PYREX). Measurement of total flavonoids using a UV-Vis spectrophotometer (Genesys 10 UV, Thermo Scientific) with a wavelength of 426 nm.

2.7. DPPH antioxidant activity analysis (Vats, 2014)

50 mg of the sample extract was dissolved in 50 mL of 96% (v/v) ethanol to obtain a stock solution of 1000 ppm. Stock solutions were diluted in stages to concentrations of 800, 600, 400, 200, and 100 ppm. A total of 1 mL of sample solution from each concentration was added to 1 mL of DPPH reagent and then incubated in a dark room for 30 minutes. The absorbance of the solution was measured at a wavelength of 517 nm using a UV-Vis spectrophotometer (Genesys 10 UV, Thermo Scientific).

2.8. GC-MS analysis

GC-MS analysis was carried out using the services of the Jakarta Regional Health Laboratory (Labkesda). A total of 10 mg of crude extract (in 10 mL of hexane solvent) was extracted in a sonicator for 30 minutes. The solution was filtered using a millipore, and the hexane fraction was taken for GC-MS analysis. GC-MS analysis used Agilent Technologies GC-MS 7890 and MS 5975 series equipped with HP INNOWAX capillary columns (internal diameter: 30 m x 0.25 mm, film thickness: 0.25 µm). The carrier gas flow rate (He) was 0.6 mL min^{-1}. 1 µL sample was injected into the GC-MS at an injection temperature of 250°C. The temperature of the GC-MS was set for the operating conditions, namely an initial temperature of 60°C (held for 0 minutes), increased at a rate of 2°C min^{-1} until the final temperature was 150°C (held for 1 minute), then increased at a rate of 20°C min^{-1} (hold for 10 minutes). The mass spectrophotometer was operated at 70 eV. GCMS analysis was compared using the Chemistation Data System to see the results.

2.9. Statistical analysis

ANOVA was carried out using statistical analysis based on a completely randomized design using the Minitab 16 program. The results of each plant part and the comparison of each tests were carried out using ANOVA and Tukey's follow-up test. The data is presented in terms of replication ± standard deviation, which is visualized as a bar chart.

3. Results

3.1. Morphology, growth and production of butterfly pea flower

3.1.1. Morphology of butterfly pea flower

The morphology of the butterfly pea plant can be seen in Table 1. The leaves of the butterfly pea flower are compound and have 3, 5, and 7 leaflets (Figure 1a), but they only have 2 leaflets when they are young. The roots of the butterfly pea plant have main roots, primary roots, secondary roots, and tertiary roots (Figure 1b). The stem of the butterfly pea plant has primary, secondary, and tertiary branches and tendrils. The plant has a brown stem base when it is 8 weeks old (Figure 1c).

The flower of the butterfly pea plant generally has three phases. The first is the initial phase when the flower is still in the form of a bud, which lasts 4-5 days. The second is the blooming phase (which lasts only 1 day) when the flowers fully bloom and have the perfect color (Figure 1d). The third stage is the late blooming phase, which will result in pods. This phase lasts for nine days. However, in this study, only 2 out of 6 flowers produced pods.

Table 1. The morphology of the butterfly pea plant

Sample	Morphology	Results
Stem	Stem Color	Green
	Stem Shape	Straight creeper (Round)
	Amount / plant	2 – 4 clumps
Leaf	Leaf Color	Green
	Leaf Shape	Oval (lanceolate when young)
	Bone leaves	Pinnate
	Leaf tip	Tapered
Root	Root Color	White
	Root Shape	Fiber
Flower	Flower Shape	Symmetry
	Flower Color	Purple blue gradation

(a) (b) (c) (d)

Figure 1. The parts of the butterfly pea plant (a) Leaves (b) Roots (c) Stems and (d) Flowers.

3.1.2. Flower production of butterfly pea flower plant

The calculated interest is the bud and bloom flowers counted daily (Figure 2). Flower blooms and buds increased every week but decreased to 345 flower buds and 374 flower blooms in the eighth week. Week 9 saw an increase in flower buds of 96.24% from before, but blooms still decreased to 298 blooms.

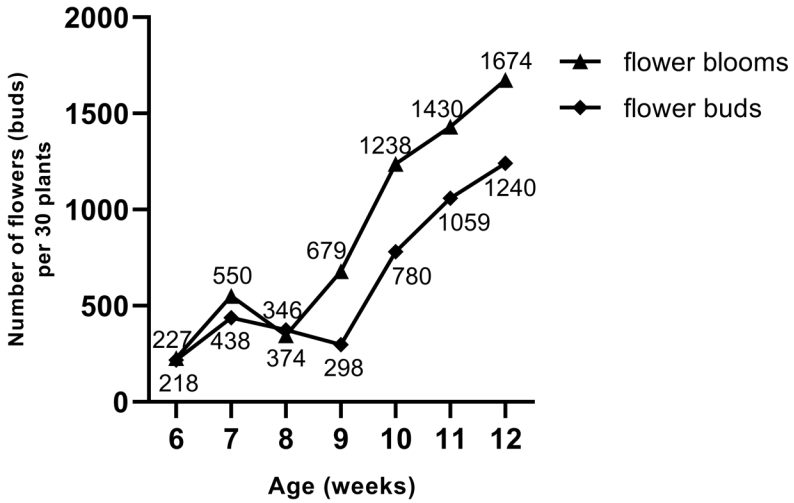

Figure 2. Graph of the number of flowers of the butterfly pea plant every week.

3.1.3. Growth of Butterfly Pea Flower

Agronomic measurements of the butterfly pea plant are carried out every week, including plant height, number of leaves, stem diameter, and number of flowers calculated every day, as well as the weight of each part of the plant, which will be calculated at the time of harvest. The results of the growth of butterfly pea plants that were measured included plant height, number of leaves, and stem diameter (Figure 3). Plant height was measured in the 2nd week using a measuring tape, and the results are shown in Figure 3a. Plant height reached its highest point in the 11th week with a height of 101.47 cm and decreased in the 12th week to 98.83 cm. Plant height in week 5 experienced a surge of 153.64% from the previous week.

On the leaves that blossomed, the number of leaves was counted to determine the photosynthesis capability of plants. The number of leaves every week has increased without a decrease in the number of leaves. Figure 3b shows the results of the number of leaves, which always increases every week. The highest number of leaves was found in week 12, with a yield of 679 leaves per plant. Plant stem diameter was measured at week 4 using a caliper. Stem diameter is measured to see the thickness of the stem supporting the plant. The stem diameter increased weekly and reached 7.21 mm in week 12 (Figure 3c).

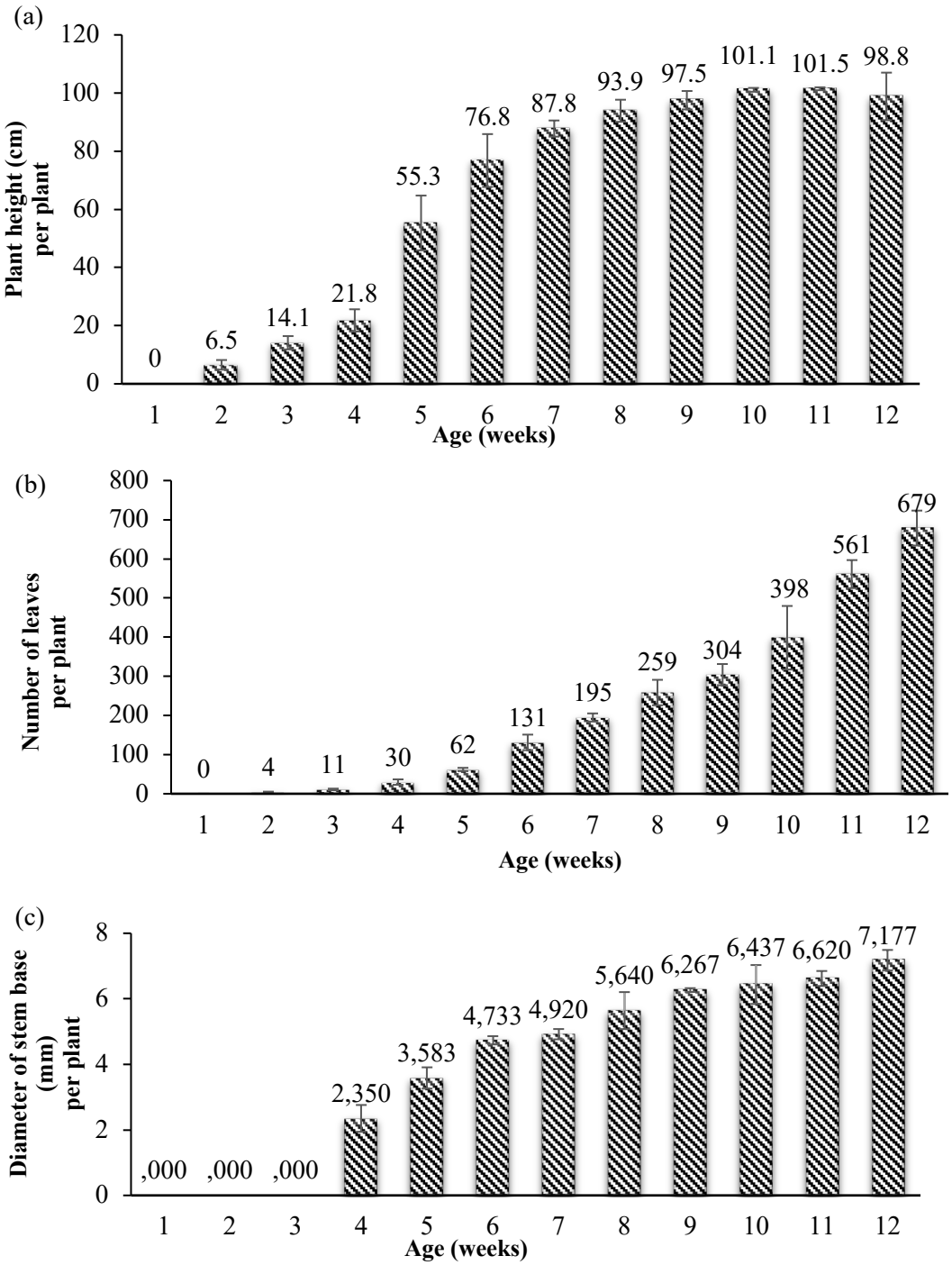

Figure 3. Growth of butterfly pea: (a) plant height, (b) number of leaves, and (c) stem diameter for 12 weeks.

3.1.4. Production of butterfly pea flowers

Yields consist of the fresh and dry weight of plant roots, stems, flowers, and leaves (Figure 4). The production yield is the total production of 30 butterfly pea plants. The highest dry weight obtained was in the stem sample, with a weight of 574.83 g, and the highest fresh weight was found in the leaves, 1425.64 g. The dry weight of the leaves is lower than that of the stems. The leaf and flower samples had significantly different wet and dry weight values.

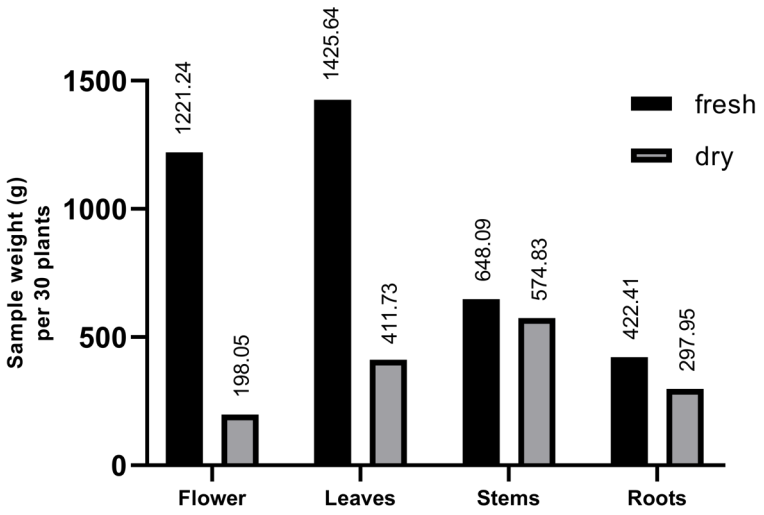

Figure 4. Fresh weight and dry weight of 12 weeks old butterfly pea.

3.2. Standardization of butterfly pea plant ethanol extract

Moisture content is one of the quality parameters of simplicia as a raw material for drugs used in treatment (Depkes RI, 2008). Water content was measured on all parts of the butterfly pea plant, namely the stems, roots, flowers, and leaves. The water content of the pea flower simplicia has a value between 3.52 – 8.98% (Figure 5). The results of the water content of all parts of the plant have a water content of <10%. These results are in accordance with the standard for simplicia as a medicinal ingredient, namely that the water content of simplicia is not more than 10% (BPOM RI, 2008).

The simplicia is then extracted using ethanol as a solvent to produce a yield. The yield comes from the process of concentrating simplicia using a solvent to obtain metabolites that match the polarity of the solvent (Erviani and Arif, 2017). The solvent used is 96% ethanol. The extract yield was calculated based on the ratio of the final weight of the extract produced to the weight of the simplicia used. The yield results showed that the butterfly pea flower sample had the highest yield, at 50.21% (Figure 6).

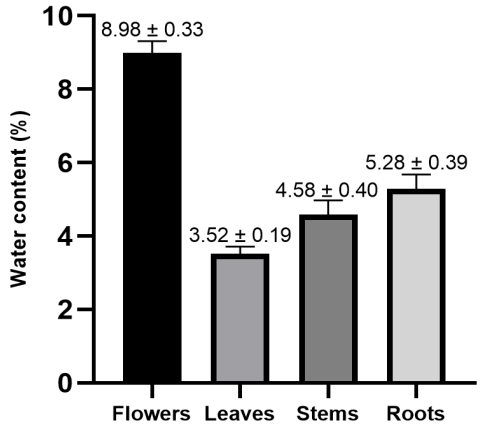

Figure 5. Water content of different parts of butterfly peat. Each value is presented as the mean of three replicates ± standard deviation (SD).

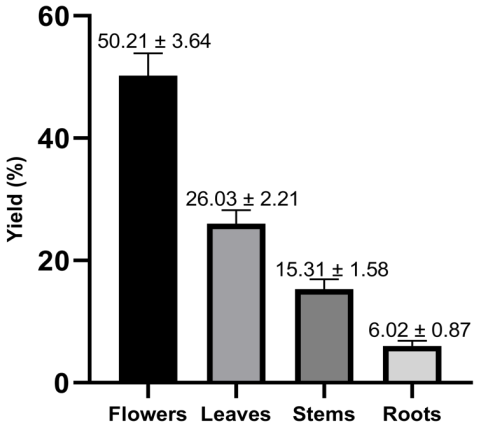

Figure 6. Yield of ethanol extract of different parts of butterfly pea. Each value is presented as the mean of three replicates ± standard deviation (SD).

3.3. Secondary metabolite content of butterfly pea flower plant

An analysis of secondary metabolite content was carried out to quantitatively determine the secondary metabolite content. The samples analyzed were ethanol extract to determine polar compounds

and the n-hexane fraction to determine non-polar compounds. The secondary metabolites measured in the ethanol extract samples were total phenolics, flavonoids, and anthocyanins. In contrast, the profile of secondary metabolites was identified using GC-MS for the n-hexane fraction.

3.3.1. Total phenolic and total flavonoids content

The highest total phenolic content was found in the root sample with a value of 83.45 mg GAE/g, and the lowest total phenolic content was found in the stem sample with a value of 37.09 mg GAE/g (Figure 7). The value of 83.45 mg GAE g^{-1} means that there is 83.45 mg of phenolic equivalent to gallic acid in one gram of sample. The phenolic content in flowers and stems is not significantly different ($p>0.05$), with values of 37.58 mg GAE g^{-1} and 37.09 mg GAE g^{-1}, respectively.

The analysis of the total flavonoid content was to determine the total flavonoid content in the sample in milligrams of quercetin equivalent per gram of sample. The highest total flavonoid content was found in leaf samples, with a value of 5.96 mg QE g^{-1}, and the lowest was in root samples, with a value of 0.27 mg QE g^{-1} (Figure 8). The value of 5.96 mg QE g^{-1}means there are 5.96 mg of flavonoids equivalent to quercetin in one gram of sample.

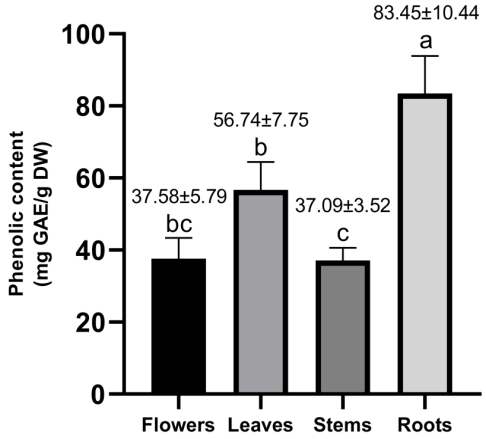

Figure 7. The total phenolic content of the butterfly pea. Each value is presented as the mean of three replicates ± standard deviation (SD). Different letters indicate significant differences at the 5% test level (Tukey test).

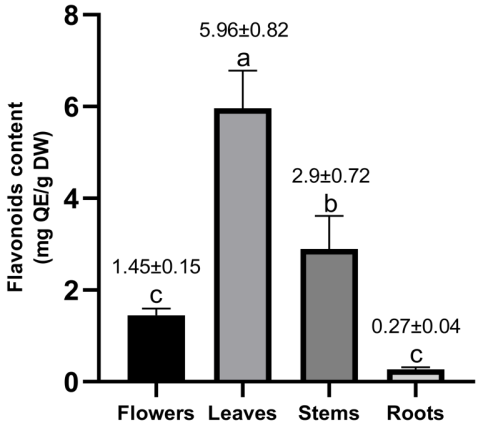

Figure 8. The total flavonoid content of the butterfly pea. Each value is presented as the mean of three replicates ± standard deviation (SD). Different letters indicate significant differences at the 5% test level (Tukey test).

3.3.2. Total Anthocyanin Content

The calculated anthocyanin levels are monomeric anthocyanin levels, namely cyaniding-3-glucoside equivalents in mg/L. The anthocyanin content of flower samples had the most anthocyanins compared to leaves. Stem and root samples obtained negative results in the calculation, so it can be said that the stem and root samples did not contain anthocyanins (Table 2). The flower anthocyanin level is 4.72 mg/L of cyaniding-3-glucoside equivalent.

Table 2. Anthocyanin content of butterfly pea

Sample	Total Absorbance	Total Anthocyanin (mg L^{-1})
Flowers	0.12 ± 0.01	4.72 ± 0.39
Leaves	0.07 ± 0.01	2.92 ± 0.33
Stems	-0.14 ± 0.03	N/A
Roots	-0.14 ± 0.02	N/A

Note: Each value is presented as the mean of three replicates ± standard deviation (SD); N/A: not available.

3.3.3. Identification of secondary metabolite compounds of n-hexane extract by GC-MS

A GC-MS analysis was carried out to determine the content of non-polar compounds in the sample. The analysis of the compound content of the flower samples showed that 12 compounds were identified (Table 3). In the chromatogram of the flower sample, there is one most dominant peak with a percent area of 31.95%, namely the citronellal compound, which belongs to the terpenoid compound group. The compounds identified in the flower samples contained many terpenoids such as sabinene, cymene, and alpha-terpinolene. Another compound identified is a vitamin K1 compound with a halogen element, fluoro.

Table 3. Non-polar compounds in the flowers of the butterfly pea

No.	Compounds	RT	MF (g mol^{-1})	MW	PP (%)
1	**2-methyl-4-pentenal**	3.639	$C_6H_{10}O$	98.145	8.99
2	**2-Pentanone-4-methyl (Methyl isobutyl ketone)**	3.832	$C_6H_{12}O$	100.161	8.99
3	**Citronellal**	7.645	$C_{10}H_{18}O$	154.253	31.95
4	**Sabinene**	4.094	$C_{10}H_{16}$	136.238	1.78
5	**Cymene**	4.866	$C_{10}H_{14}$	134.22	1.12
6	**Isodurene**	4.907	$C_{13}H_{20}$	134.22	2.19
7	**240α-terpinolen**	6.424	$C_{10}H_{16}$	136.238	1.35
8	**Tetradecamethylhexasiloxane**	30.889	$C_{14}H_{42}O_5Si_6$	458.995	3.61
9	**Heptacosane**	31.296	$C_{27}H_{56}$	380.745	1.03
10	**15-methoxymaysine**	39.853	$C_{29}H_{37}ClN_2O_8$	577.071	1.98
11	**Zinc, allyl-crotyl-**	42.183	$C_7H_{12}Zn$	161.553	3.35
12	**Vitamin K1 (20) Heptafluorobutyric**	48.934	$C_{35}H_{47}F_7O_3$	648.747	2.07

Note: RT: retention time (min); MF: molecular; MW: molecular weight; PP: peak percentage.

The analysis of the compound content of the stem samples showed that nine compounds were identified (Table 4). In the chromatogram of the stem sample, the dominant compound is the unsaturated fatty acid 9,12-Octadecadiynoic acid, with an area of 19.99%. Terpenoid group compounds were also identified in the stems: beta-humulene, squalene, and D-Friedoolean-14-en-3-ol(3.beta).

Table 4. Non-polar compounds in the stems of the butterfly pea

No.	Compounds	RT	MF (g mol^{-1})	MW	PP (%)
1	**Palmitic acid (hexadecanoic acid)**	29.392	$C_{16}H_{32}O_2$	256.42	12.79
2	**9.12-Octadecadiynoic acid**	30.372	$C_{18}H_{32}O_2$	276.42	19.99
3	**Squalene**	33.874	$C_{30}H_{50}$	410.73	1.08
4	**β-stigmasterol**	39.928	$C_{29}H_{48}O$	412.702	1.19
5	**γ-sitosterol**	41.073	$C_{29}H_{50}O$	414.718	4.37
6	**D-Friedoolean-14-en-3-ol,(3.beta.)-**	41.583	$C_{30}H_{50}O$	426.729	9.09
7	**α-(P-chlorobenzoyl)-P-chloroacetophenone**	41.949	C_8H_7ClO	154.593	1.00
8	**Isomultiflorenone**	42.480	$C_{30}H_{48}O$	424.713	12.10
9	**β -Humulene**	43.480	$C_{15}H_{24}$	204.357	10.45

Note: RT: retention time (min); MF: molecular; MW: molecular weight; PP: peak percentage.

The results of the chromatogram on the root sample (Figure 12) showed 13 compounds identified (Table 5) and 2 dominant compounds, namely 9.12-Octadecadiynoic acid and D-Friedoolean-14-en-3-ol(3.beta)-. D-Friedoolean-14-en-3-ol(3.beta)- belongs to the terpenoid group. Apart from D-Friedoolean-14-en-3-ol(3.beta), there are other terpenoid group compounds, namely citronellal, and taraxerone. Steroid group compounds were also identified in the root samples, namely Stigmasta-5.23-dien-3-beta-ol, and gamma-sitosterol.

Table 5. Non-polar compounds in the roots of the butterfly pea

No.	Compounds	RT	MF (g mol^{-1})	MW	PP (%)
1	**Heptane,2,4-dimetyhl**	3.832	C_9H_{20}	128.259	1.68
2	**Pyrrolidine,3-methyl-**	3.942	$C_{12}H_{17}N$	175.275	2.03
3	**Citronellal**	7.645	$C_{10}H_{18}O$	154.253	1.67
4	**Neophyadiene (Neophytadiene)**	27.896	$C_{20}H_{58}$	278.524	1.67
5	**Palmitic acid (hexadecanoic acid)**	29.392	$C_{16}H_{32}O_2$	256.42	9.17
6	**9.12-Octadecadiynoic acid**	30.372	$C_{18}H_{32}O_2$	276.42	27.16
7	**Stigmasta-5.23-dien-3-beta-ol**	39.866	$C_{29}H_{48}O$	412.702	1.23
8	**Delta14-taraxen3-3-one (Taraxerone)**	40.770	$C_{30}H_{48}O$	424.713	1.5
9	**γ -sitosterol**	41.073	$C_{29}H_{50}O$	414.718	4.96
10	**D-Friedoolean-14-en-3-ol,(3.beta.)-**	41.583	$C_{30}H_{50}O$	426.729	37.1
11	**Thunbergol**	42.204	$C_{20}H_{30}O$	290.491	1.51
12	**2,2,3,7-tetramethyltricyclo [5.2.2.01.6]**	42.852	$C_{12}H_{26}O$	246.38	6.03
13	**9,17-octadicadienal**	29.854	$C_{18}H_{32}O$	264.453	1.84

Note: RT: retention time (min); MF: molecular; MW: molecular weight; PP: peak percentage.

The leaf samples contained 11 compounds identified by GC-MS (Table 6). The most dominant compound was an unsaturated fatty acid compound, 9.12-Octadecadinoic, with an area of 31.22%. In addition, the leaf samples contained isoprenoid group compounds, namely vitamin E, with an area percent of 13.23%. The terpenoid compound group was also identified in the squalene leaf samples, with an area of 11.78%. In addition, there are also otochilone compounds that are included in the steroid class.

Table 6 Non-polar compounds in the leaves of the butterfly pea

No.	Compounds	RT	MF (g mol^{-1})	MW	PP (%)
1	**Heptane,2,4-dimetyhl**	3.832	C_9H_{20}	128.259	4.89
2	**Pyrrolidine,3-methyl-**	3.942	$C_{12}H_{17}N$	175.275	11.01
3	**Citronellal**	7.645	$C_{10}H_{18}O$	154.253	9.93
4	**Neophyadiene (Neophytadiene)**	27.896	$C_{20}H_{58}$	278.524	3.19
5	**Palmitic acid (hexadecanoic acid)**	29.392	$C_{16}H_{32}O_2$	256.42	4.35
6	**9.12-Octadecadiynoic acid**	30.372	$C_{18}H_{32}O_2$	276.42	31.22
7	**2.2 metilenebis**	32.061	$C_{25}H_{36}O_2$	368.561	1.78
8	**Squalene**	33.874	$C_{30}H_{50}$	410.73	11.78
9	**Vitamin E**	37.529	$C_{29}H_{50}O_2$	430.717	13.23
10	**Otochilone**	42.183	$C_{30}H_{48}O$	424.702	6.40
11	**Lanost-7-en-3-one**	42.797	$C_{30}H_{50}O$	426.729	5.16

Note: RT: retention time (min); MF: molecular; MW: molecular weight; PP: peak percentage.

3.3. Antioxidant activity (DPPH method)

With the DPPH method and vitamin C as a positive control, antioxidant activity was measured. Evaluation of antioxidant activity using ethanol extract samples from each butterfly pea plant part. Antioxidant activity can be measured by Inhibitory Concentration 50 (IC_{50}), the solution concentration required to inhibit 50% of free radicals. The lower the required IC_{50}, the stronger the antioxidant activity in the sample. Antioxidant activity was classified into very strong (IC_{50} < 50 µg mL^{-1}), strong (IC_{50} 50-100 µg/mL), moderate (IC_{50} 101-150 µg mL^{-1}), weak (IC_{50} > 150 µg mL^{-1}) (Fidrianny et al., 2015).

Vitamin C has an IC_{50} value of 3,888 µg/mL; the lowest IC50 value was obtained from a root sample of 106,973 µg mL^{-1} (Figure 9). The antioxidant activity of vitamin C is very strong, while the root samples are moderate antioxidants. Other plant parts, such as leaves, stems, and flowers, are classified as weak antioxidants. The results showed that the root has a stronger antioxidant content than other plant parts.

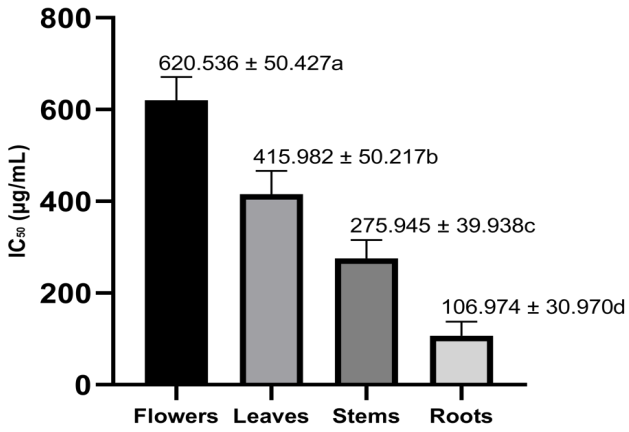

Figure 9. Antioxidant activity (IC_{50}) of the butterfly pea. Each value is presented as the mean of three replicates ± standard deviation (SD). Different letters indicate significantly different results at the 5% test level (Tukey test).

4. Discussion

4.1. Morphology, growth, and production of butterfly pea flower

The results of morphological observations are consistent with the literature of Suarna and Wijaya (2021), but the butterfly pea plant that was planted only produces blue flowers. The butterfly pea flower is a monocot plant, an annual shrub plant. Its main characteristic is that it has predominantly blue flowers, even though it also has white flowers (Artiyani et al., 2023). The butterfly pea plant has fibrous roots and deep, and woody roots. The stems of the butterfly pea plant are erect and slightly uphill, with a height of 20 cm to 90 cm, and have fine hairs. This plant can propagate with stems up to 0.5 to 3 meters long. The butterfly pea plant has oval leaflets with a hairless upper surface. The lower surface has scattered hairs and has a length of 1.5 cm to 7 cm and a width of 0.3 cm to 4 cm. Butterfly pea flowers have purple to almost white petals and an oval, funnel-shaped fruit 6 cm to 12 cm long and 0.7 to 1.2 cm wide. The egg-like structure of flower seeds is round.

4.2. Standardization of the butterfly pea flower plant simplicia

The standardization carried out in this study was the water content. The calculated water content is the total percentage of water in a sample. The standard for water content in plant samples dried is below 10% (BPOM RI, 2008). The water content in all samples is below 10%, so the Simplicia is good quality. The high-water content can affect the durability of the Simplicia. High water content can be damaged by microorganisms such as fungi or mold, so the water content of Simplicia cannot be too high (Taslim et al., 2021). Simplicia that complies with the standard is extracted with ethanol.

Extraction is the process of separating active compounds in samples, such as secondary metabolites. Extraction using ethanol as a solvent aims to obtain a more diverse compound due to its polarity. Ethanol is also a safe solvent for extracting samples as pharmaceuticals, especially phenolic compounds (El-Maati et al., 2016). The results of the extraction of the largest butterfly pea flower samples were flowers, leaves, stems, and roots. The yield of flower part has the highest yield compared to the other samples at 50.21%. This result is because flowers have compounds that have a polarity that matches the solvent. The yield of roots was lower compared to the study by Kadam and Ahire (2011), while the yield of stems and leaves had higher yield than in previous studies. According to Kadam and Ahira (2011), yields are influenced by several factors, such as the plant's environmental conditions, the yield processing, and the solvent used.

4.3. Secondary metabolite content of the butterfly pea flower plant

The total phenolic test used the folin-ciocalteu method with standard gallic acid, with the equation of the standard curve line $y = 0.008x + 0.0078$ and a value of $R^2 = 0.9968$. The plant parts with

the highest total phenolic yields were roots, leaves, flowers, and stems. These results indicate that roots have a higher phenolic content and are significantly different at 83.45 mg GAE g^{-1} compared to leaves, flowers, and stems. However, the total phenolic yield was not significantly different in flowers and stems. According to Setford et al. (2017), environmental factors, processing, and plant age resulted in differences in the content of phenolic compounds in different plants. The different phenolic content due to phenolic compounds in plants is a defense response carried out in plants, which can affect every part of the plant. The principle of the total phenolic test using folin-ciocalteu is that the reduction process of the folin-ciocalteu reagent, namely tungstate phosphomolybdate, reacts with phenolic compounds, which reduces the reagent to produce a blue complex. The absorbance of the color complex can be measured at a maximum wavelength of 730 nm, so the higher the absorbance value, the higher the total phenolic content in the sample. Gallic acid is used as a standard because it is stable and can be obtained easily and cheaply (Treml and Smejkal, 2016).

Testing for total flavonoids using the colorimetric method with $AlCl_3$ reagent and standard quercetin with the equation of the standard curve line $y = 0.0685x - 0.0071$ and the value of $R^2 = 0.9984$. The total flavonoid content results from the highest sources: leaves, stems, flowers, and roots. Leaves contain high levels of flavonoids and are significantly different at 5.96 mg QE g^{-1} compared to stems, flowers, and roots. The root contains very little flavonoids, at 0.27 mg QE g^{-1}, and is significantly different from other plant parts. This difference is due to the different functions of each part of the plant, environmental factors, and sample processing (Borges et al., 2013). Flavonoids are derivatives of phenolic compounds that have a defense function in plants and have antioxidant properties because they have hydroxyl groups (Gengaihi et al., 2014). The principle of measuring flavonoid levels using $AlCl_3$ reagent will form orange to red compounds with flavonoids in an alkaline state and then measured at a wavelength of 426 nm. Quercetin standard is a flavonol compound with very strong radical scavenging activity, so it can be a strong antioxidant compound because it has many hydroxyl groups. Quercetin is a flavonol group widely distributed in every part of the plant to be used as a standard (Kumar et al., 2017).

The total anthocyanin test used the pH comparison method with a buffer of pH 1 and pH 4.5. The principle of this test is to compare the results of the color change at pH 1 and pH 4.5 measured at 520 nm and 700 nm. Anthocyanins will change color from red to orange at an acidic pH, so the measured anthocyanin levels are at monomeric anthocyanin levels (Tonutare et al., 2014). The results of anthocyanin content showed that only flower and leaf parts had anthocyanin, while roots and stems did not contain anthocyanin. Flowers have a higher anthocyanin content than leaves. The butterfly pea flower plant has anthocyanin-type delphinidin in its flower parts, making the flowers' color turn bluish. Delphinidin has more OH groups than other types of anthocyanidins (Lijon et al., 2017).

Anthocyanins are included in the class of flavonoids, the largest group of phenolic compounds found in nature. Anthocyanins are known as natural dyes because these compounds are in the form of glycosides which cause colors ranging from red, blue, violet, and yellow found in plants (Kamiloglu et al., 2015). Anthocyanins are derivatives of flavylium or benzyl flavylium salts, which are easily soluble in water. There are eighteen types of anthocyanidins, but only six play an important role in food coloring, namely pelargonidin, cyanidin, delphinidin, peonidin, petunidin, and malvidin (Šulc et al., 2017).

Identification of metabolite compounds using GC-MS was carried out to determine the content of metabolite compounds which are included in volatile compounds, non-polar compounds, and have a relatively low molecular weight (Wijit et al., 2017). Identification of compounds using GC-MS (Table 7) shows that the flower parts have a high content of citronellal compounds. The leaves and stems contain the dominant compound, linoleic acid. The root contains the dominant compound of taraxerol.

The compounds in the ethanol extract of butterfly pea flower identified by GC-MS have useful biological activity. Linoleic acid (Figure 10a) is an unsaturated fatty acid compound that functions as a precursor metabolite for forming EPA (eicosapentaenoic acid) in plants and is found in many parts of the leaves (Rajram, 2014). Citronellal (Figure 10c) is an aldehyde monoterpenoid with an aldehyde group abundant in eucalyptus trees. Citronellal compounds are thought to have biological activity as antifungal and antibacterial (Ho et al., 2020). According to Mus et al. (2022), taraxerol (Figure 10b) is a steroid-derived terpenoid with anti-cancer, antioxidant, and antimicrobial activity. Taraxerol is produced in plants via the mevalonate pathway in the cell cytoplasm with its precursor, squalene. Taraxerol is thought to have high toxicity and effectiveness in inducing apoptosis (Surapaneni and Prakash, 2018).

Table 7. Metabolites identified by GC-MS in flower of butterfly pea

No.	Compounds	Class	Biological Activity
1.	**Citronellal**	Terpenoids	Anti- inflammatory, Anti- microbial (Ho et al., 2020)
2.	**9.12-Octadecadiynoic acid (asam linoleat)**	Unsaturated fatty acid	Antidiabetic, Anti-inflammatory (Ferraz et al. 2018)
3.	**D-Friedoolean-14-en-3-ol,(3beta) (Taraxerol)**	Terpenoids	Anti-tumor, anti-microbial, anti-inflammatory agent (Mus et al., 2022)

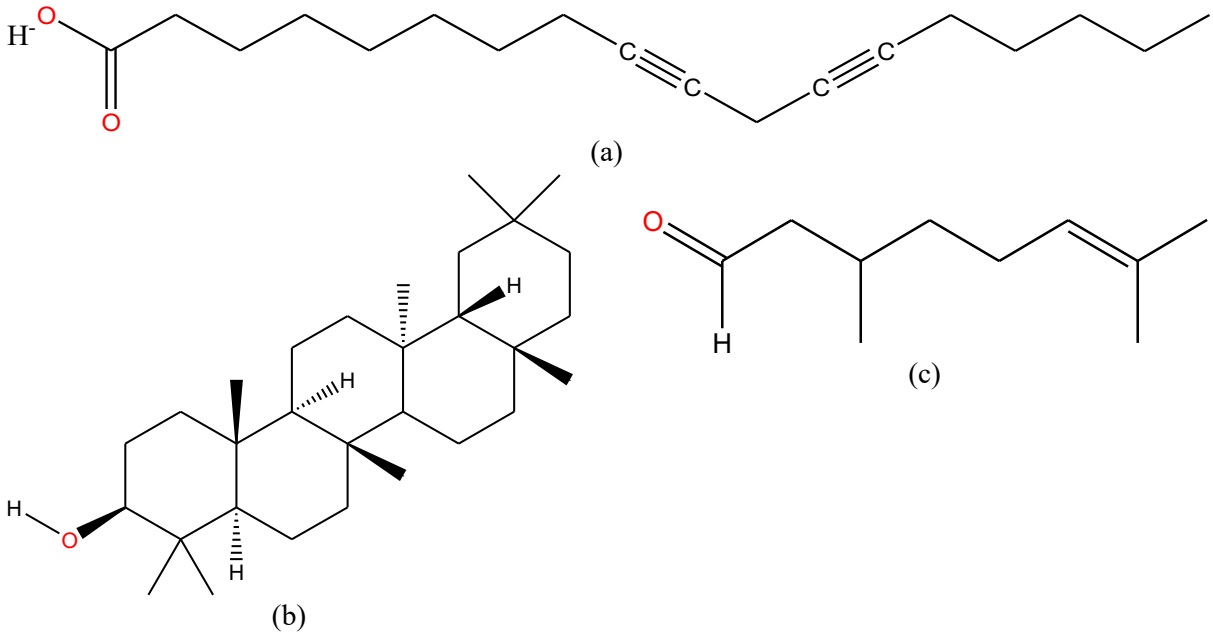

Figure 10. Structures of compounds identified by GC-MS (a) linoleic acid, (b) taraxerol and (c) citronellal.

4.4. Antioxidant activity of butterfly pea plant

According to Gengaihi et al. (2014), the DPPH method is easy and fast for determining non-enzymatic antioxidant activity. DPPH is a purple free radical with maximum absorption at a wavelength of 517-520 nm. DPPH will change color to yellow when antioxidants are added by donating electrons and transferring H atoms from antioxidants to DPPH. The results of the highest antioxidant activity were roots, stems, leaves, and flowers. The part that belongs to the root is a moderate antioxidant because it has an IC_{50} value between 100-150 µg mL^{-1}, while the leaves, stems, and flowers are included in a weak antioxidant because it has an IC_{50} value of more than 150 µg mL^{-1}. The IC_{50} value is inversely proportional to the antioxidant activity. The lower the IC_{50} value, the higher the antioxidant activity (Kaur et al., 2019).

The results of the antioxidant activity show the same thing as the research by Desmukh et al. (2013). The highest antioxidant activity was found in the root sample with an inhibition percentage of 82.87% at a concentration of 500 µg mL^{-1}, then the stem sample, and finally, the leaf sample. The antioxidant activity of a sample can be determined by knowing the compounds that can scavenge free radicals, such as phenolics, flavonoids, and anthocyanins. The highest analysis of phenolic compounds was found in the roots, but the roots had the lowest flavonoid content compared to other parts, and the highest antioxidants were found in the roots. The results of the content of secondary metabolites in the roots, which have properties as antioxidants, are thought to be non-flavonoid phenolic compounds. The phenolic mechanism inhibits antioxidants by directly transferring hydrogen atoms and producing more stable compounds due to the resonance of aromatic rings (Mohandas and Kumaraswamy, 2018). The GC-MS results showed that the compound that could act as an antioxidant was taraxerol (Table 7). Terpenoid compounds can inhibit antioxidants by capturing different antioxidant activities, which can

be caused by synergistic or antagonistic effects between the active components in a sample (Gengaihi et al., 2014).

Conclusion

The profiles of volatile compounds differed between different parts of the butterfly pea. Significant constituents of the flower and root were citronellal and taraxerol, respectively, while linoleic acid was found in the leaves and stems. The root had the greatest total phenolic content and antioxidant activity, whereas the leaves contained the highest total flavonoid content. In flowers and leaves, anthocyanins have been identified.

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