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The Antioxidant Content and Activity of Various Plant Organs of Kitolod (*Isotoma longiflora)*

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Abstract. Antioxidants play a crucial role in human health owing to their ability to neutralize oxidative reactions. Kitolod (*Isotoma longiflora*) is one of the medicinal plants that can be used as a source of antioxidant. The aim of study was to compare the content of antioxidant compounds (flavonoids, phenolics, chlorophylls, carotenoids, and ascorbic acid) in various organs of the kitolod plant. The plant organs studied including roots, stems, leaves, flowers, and fruits. The extraction of each plant organ was conducted by maceration technique using ethanol as a solvent at the room temperature for 48 hours. The analysis of total flavonoids, phenolics, and ascorbic acid was performed by colorimetry method using $AlCl_3$, Folin, and sulfosalicyclic acid reagent respectively, while chlorophylls and carotenoids was determined by direct colorimetry method with methanol as a solvent. The antioxidant activity of various plant organs of kitolod was analyzed using the DPPH method. The highest total flavonoid, phenolic, chlorophyll, and carotenoid compounds were found in leaves, respectively by 10.48, 1.46, 7.25, and 56.98 ppm. The highest ascorbic acid content and antioxidant activity were obtained from fruits.The research findings provide new and important information aboutthe contents and antioxidant activity of the secondary metabolites (flavonoid, phenolic, chlorophyll, carotenoid, and ascorbic acid) in each organ of kitolod plant. The information from the results of this study can be used to increase the medicinal value of kitolod plants.

Key words: Antioxidant Activity; Ethanol Extract; Organs; *Isotoma longiflora;* Secondary Metabolite

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INTRODUCTION

Weeds are defined as unwanted wild plants and tend to be harmful. Weed is considered detrimental because it competes with plants that are cultivated in fighting over growing space, nutrients, water, and air. However, there are several types of weeds that have positive function, one of which is as a medicinal plant (Fernandes & Agency, 2016). According to Badrunasar & Santoso (2017), there were 48 identified types of weed vegetation from 27 families of wild plants (weeds) that have potential as medicinal plants because they can produce secondary metabolites.

Secondary metabolites are synthesized in order to attract pollinators and as a signaling moleculs. Moreover, stressed plants also synthesize this compound to survive from their habitat. Secondary metabolites are synthesized in the form of compounds in certain phases such as in the growth phase to attract pollinators, as signaling molecules, and if there are certain stresses plants can defend themselves from their habitat. Production of secondary metabolites in plants is influenced by the composition of growth media as well physical (temperature, light, humidity, etc.), genetic (cell genotypes), and environmental stress factors (heavy metals, elicitors, UV rays) (Mariska, 2013). According to Abotaleb et al.(2019), secondary metabolites are divided into 5 major groups, including alkaloids, carotenoids, phenolics (phenolics, flavonoids, tannins, coumarin, stilbenoid), organic sulfur, and N compounds. Secondary metabolites are commonly used in the development of drugs because they have antioxidant molecules that can prevent and neutralize oxidation reactions involving free radicals (Parwata, 2016)

Isotoma longiflora Kitolod is a weed included in the Campanulaceae family. This plant is often found in damp and open places such as culvert/rivers, paddy fields, and around the fences. Kitolod plants have herbaceous stems, branching from the base, white sap, lanceolate-shaped leaves, white star-shaped flowers, and square-shaped fruit with a lot of seeds (Badrunasar & Santoso, 2017). Research on the kitolod has been carried out, especially regarding its ability of antibacterial and anticancer. Based on research by Hapsari et al. (2016), it was showed that the entire kitolodorgan contains secondary metabolit compounds such as alkaloids, saponins, polyphenols, flavonoids, tannins, and steroids that can inhibit the growth of HeLa cells (cells that cause cervical cancer) with IC_{50}value of 227 ppm. Hapsari (2016)also

reported that kitolod herbal extract had cytotoxic activity against MCF-7 cells (cells that cause breast cancer) with IC_{50} value of 239.4 ppm. The leaves and flowers of kitolodwere able to inhibit the growth of bacteria that cause dental caries, such as *Streptococcus mutans*, and *Enterococcus faecalis* (Fazil et al., 2017)

Built upon the previous research, this research is to develop it further and compare flavonoid, fenolic, & ascorbic acid and to analyze antioxidant activity in some plant organs such as, in flowers, leaves, stems, roots, and plant seeds. This study also examined the potential of the chlorophyll pigment and the carotenoid of the plants to be use as natural coloring agent. The content of the components analyzed was the total content of flavonoids, phenolics, ascorbic acid. Those compounds that not only related to the potential of the plant stood but have the ability as a source of antioxidants. This research also analyzed the natural pigments chlorophyll and carotenoids, so that it can be used as natural coloring agents. A piece of information about the content of antioxidant compound such as flavonoids, phenolics, chlorophylls, carotenoids, and ascorbic acid of kitolod's organ can develop the research about the medicinal plant about founding the alternative antioxidant source.

METHOD

Isotoma longiflora (kitolod) plant was taken from Pabelan paddy Field, Salatiga on November, 2019. The leaves used were the fresh green leaves with perfect shape and no disease. The stems used were the main stem taken from the tip to the base of the stem. The flowers used were the fresh flowers with complete crowns (5 petals The roots used were the fresh roots from the base of the stem to the tip of the root and for the fruits, they were the fresh fruits with a perfect bell shape and seeds contained inside it.

Sample Extraction

The 20 grams of fresh sample was extracted by the maceration technique using 96% ethanol in dark conditions and room temperature for 48 hours. The maceration processes were repeated 3 times. Samples were stored in a plastic bottle and put in the refrigerator

Determination of chlorophyll and carotenoid contents

Determination of chlorophyll and carotenoid contents was conducted based on a study by Sumanta et al. (2014). The total chlorophyll and carotenoid contents were measured using direct spectrophotometer methods. The samples were measured using spectrophotometer ultraviolet at wavelengths of 649, 664, and 470 nm. The calculation of chlorophyll and carotenoid content was conducted with the following formula:

Chlorophyll a (ppm) = $(13.36A_{664} – 5.19A_{649})$

Chlorophyllb (ppm) = $(27.43A_{649} – 8.12A_{664})$

Chlorophylltotal = a + b

Carotenoid (ppm)= $(1000A_{470} –2.13$Chlorophyll a $– 97.63$Chlorophyllb$)/209$

Determination of total flavonoid contents

Determination of total flavonoid contents was conducted based on a study by John et al. (2014). The totalflavonoid contents were measured using the $AlCl_3$ method. As much as 1 ml of each sample was placedinto the reaction tube and added with 0.3 ml of $AlCl_3$ 10% (followed by incubation for 5 minutes), 0,3 ml $NaNO_2$5% (followed by incubation for 5 minutes), and 2 ml of NaOH 1M. The absorbance value of samples were measured at 510 nm wavelength. The flavonoid concentrations were calculated using the linear regression equation of the standard quercetin. The standard quercetin used was at range of 0-100 ppm.

Determination of total phenolic contents

Determination of total phenolic contents was conducted based on a study by Almey et al. (2010). The total phenolic contents were measured using the Folin Ciocalteau method. The phenolic compound standard used was gallic acid. The amount of 1 ml of sample was added with 1 ml of Folin Calteau reagent (followed by incubation for 5 minutes), and 10 ml of Na_2CO_3 7%. Each sample solution was then incubated for 90 minutes and measured at 550 nm wave length.

Determination of ascorbic acid contents

Determination of total ascorbic acid contents was conducted based on a study by Omaye (1979) (as cited in Balogh & Szarka, 2016). The ascorbic acid contents were measured using the Sulfosalicylic acid method. As much as 1 ml of the each samplewas added with 3 ml of Sulfosalicylic acid, 2 ml Na-molybdate, 2 ml 0.15 N H_2SO_4, and 1 ml of Na_2HPO_4 1.5 mM. The samples were measured at a wavelength of 660 nm.

Determination of antioxidant activity

Determination of antioxidant activity was conducted based on a study by Chan et al. (2007). The antioxidant activity was measured using the DPPH method. A total of 6 mg DPPH solution was dissolved in 100 ml of absolute methanol (50 ppm). The antioxidant activiy standard compoundused wasascorbic acid. Samples were made with concentrations ranged from 0.001 mg/ml-0.1 mg/ml.

1 ml samples and 1 ml methanol (for control) was added with 2 ml DPPH. Each sample was taken 1 ml (for control take 1 ml methanol) and then 2 ml of DPPH. They were incubated in dark for 30 minutes and measured at 517 nm wavelength.

Data analysis

The data was analyzed using SPSS version 22. The tests conducted were included homogenity test, normality test, One Way ANOVA, Pearson Correlation and Tukey Test.

RESULTS AND DISCUSSION

Secondary MetaboliteContents in Plant Organs of Kitolod

Secondary metabolites are important compounds in the health field related to their function that can inhibit and neutralize the oxidation reaction involving free radicals. One of the medical plants that has antioxidants properly is kitolod.Based on research that has been done on the content of antioxidants in kitolod, the following results are obtained (Table 1).

The secondary metabolite compounds containedin various plant organ of kitolod areshown in Table 1. The plant and various plant organs of kitolod that were used in thisstudy are showed in Figure 1. The highest flavonoids (10.48 ppm), phenolics (1.46 ppm), chlorophylls (72.5 ppm), and carotenoids (56.98 ppm) were obtained from leaves, while the highest ascorbic acid content (0.14 mM) was obtained from fruit.

Flavonoids are secondary metabolite compounds that have a basic structure in the form of two aromatic rings with three C atoms between the rings (C_6-H_3-C_6). This research proved that flavonoids were found in all organs of kitolod with different concentration. The leaves have the highest amount of flavonoids (10.48 ppm) while the root has the lower content of flavonoid (0.53 ppm). This condition is in accordance with Raharjo (2013) statement that flavonoids are distributed in plant parts such as fruit, leaves, seeds, roots, bark, stems, and flowers. The high concentration of flavonoid in the leaves seems to be related to the photosynthesis process that occurs in the leaves. The results of the CO_2 photosynthesis reaction will form carbon compounds that are used in the process of primary metabolites (Novitasari, 2017). The compounds produced by primary metabolites are the basic part of secondary metabolite compounds, including flavonoids. Flavonoids are synthesized through two metabolic pathways, there are shikimic acid and polyketide pathway. Shikimic acid pathway produces phenylpropanoids in the form of 4-hydroxycinnamoyl's-CoA (p-coumarin-CoA) which functions as a starting compound in the polyketide pathway (Raharjo, 2013). The relative low flavonoid content in the root is related to other functions of flavonoid as a source pigments for flowers, leaves, and fruits. The pale white color of Kitolod roots indicates the low flavonoid content in the roots.

Table 1. Secondary metabolite contents in various plant organs of kitolod

Organs	Secondary metabolite contents				
	Flavonoids (ppm)	Phenolics(ppm)	Chlorophylls (ppm)	Carotenoids (ppm)	Ascorbic acid (mM)
Root	$0.53^{d} \pm 0.13$	$0.22^{c} \pm 0.01$	$2.72^{c} \pm 0.48$	$1.59^{c} \pm 0.05$	$0.08^{c} \pm 0.02$
Stem	$0.72^{d} \pm 0.12$	$0.30^{c} \pm 0.02$	$2.99^{c} \pm 1.47$	$2.73^{bc} \pm 0.03$	$0.09^{bc} \pm 0.01$
Flower	$1.10^{c} \pm 0.11$	$0.67^{b} \pm 0.05$	$8.88^{b} \pm 1.17$	$5.68^{b} \pm 0.14$	$0.11^{b} \pm 0.01$
Leaves	$10.48^{a} \pm 0.10$	$1.46^{a} \pm 0.12$	$72.5^{a} \pm 12.91$	$56.98^{a} \pm 1.04$	$0.10^{bc} \pm 0.01$
Fruit	$2.27^{b} \pm 0.23$	$0.89^{ab} \pm 0.02$	$6.21^{bc} \pm 2.68$	$3.48^{bc} \pm 0.18$	$0.14^{a} \pm 0.01$

Note: The numbers followed by the same superscript letterin the same column show no significantly different values based on analysis using Tukey test ($p < 0.05$)

Figure 1. The plant and various plant organs of kitolod (a. whole plant in field and plant sampel, b. roots, c. stem, d. leaves, e. flowers, and f. fruits)

The analysis of phenolic contents in various plant organs of kitolod was determined by using the Folin-Ciocalteu method. The basic principle of this method is the oxidation reaction and colorimetric reduction to measure all phenolic compounds in the tested sample. Phenolic compounds will react with phosphomolybdic oxidizers (from folin reagents) under alkaline conditions (pH greater than 7) (Adawiah et al., 2015). The content of total phenols in Kitolod showed different values in various organs. The highest phenolic content was found in leaves by 1.46 ppm. This result has a similirity with flavonoid content. The total phenolic contents has the similar result with flavonoids, which are leaves that have the highest phenolic and lowest in the roots. This is because flavonoids are included in phenolic compounds. This compound has the chemical formula of C_6H_5OH with the structure of a hydroxyl group (-OH) that binds to the phenyl ring (Raharjo, 2013). Based on statistical analysis, differences in phenolic content were found in all organs.

The chlorophyll and carotenoid contents in various plant organs of kitolod showed different values. The highest chlorophylls and carotenoids were found in leaves by 72.5 and 56.98 ppm respectively. Based on statistical analysis, differences in chlorophyll contents were found in flowers and leaves. The chlorophyll content of flowers was significantly different from roots, stems, and leaves. In the case of carotenoid, the different content was found in flowers and leaves. Flowers have significantly different carotenoid content with roots and leaves, also with whole organs (roots, stems, flowers, and fruit). Chlorophylls and carotenoids are classified as potential antioxidants and can be converted to vitamin (Maliya et al., 2019). Chlorophyll pigments produce green color in plants. The ability of leaves in photosynthesis is affected by the content of chlorophyll pigments, specifically,chlorophyll a and b. Chlorophyll b acts as an antenna to collect light which will be transferred to the reaction center which is composed of chlorophyll a (Herlina, 2013). Both chlorophyll types are able to absorb solar radiation and release electrons in the photochemical process, so that they can convert light energy into chemical energy (Herlina, 2013).Other pigments studied werecarotenoids. Carotenoids are included in terpenoid compounds which color ranged from colorless to yellow, orange, and red (Nisar et al., 2015). Carotenoids color on the leaves are not present due to high chlorophylls accumulation in that organ. Carotenoid pigment levels are directly proportional to chlorophyll pigments. This relates to the function of carotenoids which helps chlorophyll in the absorption of light. Carotenoids absorb light at different wavelengths than those absorbed by chlorophyll (Maliya et al., 2019).

Ascorbic acid contents in plant organ of kitolod showed different values. The highest value of ascorbic acid was shown in fruit organ, which was equal to 0.14 mM or 26.118 mg. The level of ascorbic acid is almost similar to the lime which has 25 mg of ascorbic acid. However, the ascorbic acid contents in fruit of kitolodis still small compared to the other fruits with the highascorbic acid content, such as guava (230-300 mg per fruit) and oranges (50 mg) (Ullah et al., 2012). The sour smell of kitolod fruit indicates the higher levels of ascorbic acid. The higher levels of ascorbic acid in the fruit of kitolod due to the sour smell that exists in these organs. The acidic odor of ascorbic acid is affected by the high acidity of the compound, which is between pH 2-4. Formation of ascorbic acid was affected by genetic factors, odor, chemical content, and productivity (Napitupulu, 2014). Ascorbic acid is a powerful antioxidant compound that plays an active role in the capture of free radicals (Adawiah, et al., 2015). Ascorbic acidin plants is made from sucrose which is hydrolyzed into glucose and fructose from a mono saccharide. Glucose and fructose will then enter the biosynthesis pathway of ascorbic acid through the D-glucoronic acid and L-gluconate pathways (Noviati et al., 2012). Fenech et al.(2019) reported that high allocation of ascorbic acid in fruit organs is because the

biosynthesis of ascorbic acid in fruit through Smirnoff Wheeler pathway. Emmanuel et al.(2016) also reported that ascorbic acid is formed in mature leaves, but leaves just use ascorbic acid in a lower consumption, therefore, ascorbic acid is always translocated to the other organs, such as to young leaves (Maliya et al., 2019) and fruit (via Smirnoff Wheeler pathway) (Fenech et al., 2019).

Antioxidant activity / strength in IC_{50} values

IC_{50} (The Inhibition Concentration) values in various plant organs of kitolodhave been measured using the DPPH method and showed different values. IC_{50} value describes the level of a compound that can deactivate 50% of the strength of DPPH free radicals. The results of the determination ofantioxidant activity of various kitolod plant organs are shown in Table 2.

When testing DPPH, free radicals color change from purple to yellow was noticed which was due to the transfer of hydrogen atom in reaction caused by free antioxidant compounds and this results in producing was stable free radical DPPH. In the DPPH assay, free radicals has color purple to yellow is caused by the reaction of hydrogen atom transfer by antioxidant compounds so that DPPH free radicals become stable (Nur & Lukitaningsih, 2017). The strength of fruit organs in capturing free radicals is directlyproportional to the high content of ascorbic acid in theorgans. Basically, IC_{50} is not only affected by one/two compounds, but several compounds that are likely to form strong antioxidant compounds.

Table 2. IC_{50} values and antioxidant activity of various kitolod plant organs

Organ	IC_{50} (ppm)	Strength of Antioxidant*
Roots	$254.32^{a} \pm 7.63$	Weak
Stems	$224.19^{b} \pm 6.56$	Moderate
Flowers	$32.06^{d} \pm 2.39$	Very Strong
Leaves	$52.70^{c} \pm 1.83$	Strong
Fruits	$20.56^{d} \pm 1.04$	Very Strong

Note: The numbers followed by the same superscript letterin the same column showed no significantly different values based on analysisusing Tukey test ($p< 0.05$). * Determination of the strength of antioxidant (Molyneux, 2004): very strong (IC_{50}<50 ppm), strong (50-100 ppm), moderate (101- 250 ppm), weak (251-500), and IC_{50} >500 ppm are not considered as antioxidant.

Table 3. Correlation between total chlorophylls, phenolics, carotenoids, ascorbic acid, flavonoids, and antioxidant activity

AntioxidantCompound	r					
	Chl	Phe	Car	Asc	Flv	IC_{50}
Chl	1	0.831**	0.960**		0.913**	-0.612*
Phe	0.831**	1	0.789**	0.625*	0.906**	-0.871**
Car	0.960**	0.789**	1		0.901**	-0.544*
Asc		0.625*		1		-0.783**
Flv	0.913**	0.906**	0.901**		1	-0.668**
IC_{50}	-0.612*	-0.817**	-0.544*	-0.783**	-0.668**	1

Note: r from N=15, Chl = chlorophylls, Phe = phenolics, Car = carotenoids, Asc = ascorbic acid, Flv = flavonoids, IC_{50} = antioxidant activity. **. Correlation is significant at the 0.01 level (2-tailed); *. Correlation is significant at the 0.05 level (2-tailed).

Based on the r count (Pearson correlation) showed in Table 3, the relationship between antioxidant activity and the content of flavonoid , phenolic, chlorophyll, carotenoids, and ascorbic acid is negatively correlated (indicated by a minus sign), it means that the higher contents of these compounds, the smaller the value of IC_{50} antioxidant, which means the stronger of antioxidant ability.

Phenolic or flavonoid components are the main compounds in the role of antioxidants (Tungmunnithum et al., 2018). The antioxidant ability of flavonoid and phenolic was very dependent on the number and location of the -OH group. This group related to their ability to donate electrons to suppress free radicals (Tungmunnithum et al., 2018) so the free radicals neutralized. This is what causes the relationship between the total content of phenolics, included flavonoids with antioxidant activity.This is what causes the relationship between the total content of phenolic with antioxidant activity. The higher content of phenolic and flavonoids, the higher the ability to donate electrons, the higher antioxidant ability.

In the case of chlorophyll as antioxidant parameter, the r count between antioxidant activity and chlorophyll is (value of 0.612) more than r table (value of 0.514). About carotenoid, the r count value between antioxidant activity and carotenoids (value

of 0.544) more than r table (value of 0.514). As same as at ascorbic acid, the r count (value of 0.783) more than r table (value of 0.514). That means that there was a strong correlation between antioxidant activity and the content chlorophyll, carotenoid, also ascorbic acid. This shows that antioxidant activity is not only affected by one or two compounds, but by many compounds that are equally able to ward off free radicals. Other compounds could affect the antioxidant activity such as pentacyclic triterpene compounds, ascorbic acid, chlorophyll, sulfur compounds, or nitrogen (Al-Hajj et al., 2014), β-carotene (Mueller & Boehm, 2011) and tocopherol (Jiang, 2014).

This research is the first to provide an information about the antioxidant content of flavonoids, phenolics, chlorophylls, carotenoids, and ascorbic acid in each organ of kitolod (leaves, flowers, stems, fruit, and roots). The results obtained from this studycan help the development of research about medicinal plant in phytopharmaca to found an alternative plant that containsantioxidant.

CONCLUSION

Based on the research, there are a significance different between each organ of kitolod plant. Leaves hadthe highest content of flavonoids, phenolics, chlorophylls, and carotenoids, which were10.48, 1.46, 72.5, and 56.98 ppm respectively, while fruit hadthe highest content of ascorbicacid and very strong category of antioxidant activity(0.14 mM and 20.56 ppm respectively). Root had the lowest content of flavonoids, phenolics, chlorophylls, carotenoids, and ascorbic acid, which were 0.53 ppm, 0.22 ppm, 2.72 ppm, 1.59 ppm, and 0.08 mM respectively. Root also hadthe weakest antioxidant activity by 254.32 ppm. The strongest correlations between type of compound and its antioxidant activity were found on phenolics and ascorbic acid with r count of 0.871 and 0.783 respectively.

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