

antioxidant activity in combinantion extract of Acorus Calamus and Alium Sativum

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4 Antioxidant Activity In Combination Extract of *Acorus Calamus L.* (Dlingu) and *Allium Sativum* (Garlic)

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1 Abstract

One of the efforts to optimize the utilization of natural materials that is used them as medicinal plants, including Dlingu (*Acorus calamus L.*) and Garlic (*Allium sativum*) which have the potential as antioxidants. This study aims to determine the effectiveness of a combination extract of *Acorus calamus L.* (Dlingu) and *Allium sativum* (Garlic) on antioxidant activity. This research is an experimental research which is the test of antioxidant content and antioxidant activity test with 5 series of concentrations and 4 times of repetition. The research materials, namely dlingu stem and single clove garlic tubers were extracted, phytochemical screening was carried out and then antiradical free IC₅₀ was determined. Based on the test results of the chemical extract of dlingu and single clove garlic, the highest content was alkaloid 5.16%, tannin 4.05%, saponin 3.01%, and flavonoid 2.18%. The test results of the chemical antioxidant activity of extracts dlingu and single clove garlic contain IC₅₀ values of 17.062 ppm, including a very strong class of antioxidants.

7 Article History

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Keyword

Antioxidant,
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Single Clove (Male)
Garlic.

Introduction

The development efforts toward natural materials as traditional medicine is conducted immediately to find out the newest medicines in overcoming the various of health problems which is caused by the modern medicine contain chemicals that have side effects either direct effects or indirect accumulated effects (Nurhasan, 2015). The utilization of natural materials which is used as medicine rarely causes the side effects.

One of the efforts is to optimize the utilization of natural materials which have the potential as medicine, including Dlingu (*Acorus calamus L.*) from Araceae family. Dlingu (*Acorus calamus L.*) is a water plant which is found excessively as the wild plant at the bank, marsh and the flooded material during the year. Biological activity that is resulted by Dlingu plant is able to produce the compounds particularly from the class of Terpen, Flavonoid,

alcohol saponin, aldehyde, and phenol such as, karvakrol, eugenol, timol, sinamaldehyde, sinamat acid, and perilaldehyde, volatile oil (Nurhasan, 2015). The high and low quality of volatile oil depends on the area of Dlingu rhizome itself.

According to Afifah (2015), combination extract of *Acorus calamus* (L.), *Curcuma mangga* Val, and *Allium sativum* (Linn) is able to keep down *Candida albicans*. Antioxidant can be found from natural materials that were dominated by land plant as medicinal plant such as Dlingu and garlic. Chemical substances of garlic which have biological activity and the benefits in treatment, namely; the compound of organosulfur (the compound of S-ak(en)-il-L-Sistein sulfoxide (ACSOs) such as allicin. The active substances that contain in garlic have the special utility as the medicine of high blood pressure, to assuage a headache, to reduce the cholesterol, as the medicine of stomach disorder, as anti-cancer, and as anti-inflammation (Ikhtiarisyah, *et al.*, 2014). Moreover, the male species of garlic (*Allium sativum*) is besides as herbal antibiotic, also able to treat the wound, reduce the inflammation and does not cause the scar tissue, accelerate the decreasing of inflammation symptom (florid symptom) at the wound. It is caused by the content of active substance -Allicin which has the function as anti-inflammation. Garlic is enriched by vitamine B1, B2, B3, folate, vitamine C, calcium, iron, magnesium, mangan, phosphor, calium, natrium and zinc (Wadrianto, 2017).

Based on the background above, the utilization of combination extract of Dlingu and garlic has not yet been observed excessively, if it is compared to the only individual plant. Therefore, the combination of extract is conducted because on each of plants has a potential as antioxidant, thus it is expected to prevent and treat infection that is caused by the activity of normal floral bacteria, such as *S.aureus* and *Candida albicans*. Later, from the combination extract of both plants is also expected to make the supply like as embrocating oil for anti-inflammation in increasing the value of product. Based on some previous researches, both plants Dlingu and single clove garlic have the potential as antioxidant. The main function of antioxidant is to minimize the occurrence of oxidation process either in the foods or the bodies. Besides antioxidant is used in industry of pharmacy, it is also used widely in foods' industry, petroleum's industry, rubber's industry, and so on (Sayuti & Rina, 2015). In the bodies, antioxidant is also expected able to obstruct the oxidation process. Oxidation process which occurs continuously can affect the degenerative disease and aging. This research aims to know the antioxidants' activity in combination extract of *Acorus calamus* L. (Dlingu) and *Allium sativum* (Garlic).

Materials and Methods

The research was done in laboratory of biology FBS Wijaya Kusuma University of Surabaya and laboratory of research association and industrial consultation (BPKI). It was conducted on June until September 2018. The sample that was used is the extract of single clove of garlic (*Allium sativum*) and the extract of Dlingu rhizome (*Acorus calamus* L.) by using descriptive qualitative method.

Tools that were used during the research are the cutter, grinder, oven, big tray, analytical balance, watch glass, Erlenmeyer 500 mL, 250 mL, aluminium foil, shaker, graduated glass 100 mL, beaker glass 100 mL, distilled paper, filter Buchner, rotary evaporator, Spectrophotometer UV-VIS, incubator, vortex, autoclaf, disk paper, ose needle, petri cup, reaction tube, spatula. The steps that were conducted in this research are:

Preparation of Sample

The sample that was used in this research is Dlingu rhizome and single clove garlic or is usually called the male garlic which obtained from traditional market Buduran Sidoarjo. The sample of Dligu rhizome and single clove garlic were washed previously until clean, then, dried with the wind. Furthermore, was conducted the process of sorting, washing, cutting into the small pieces, drying, grinding and filtering thus dry powder of each plants was obtained.

Maceration Method

The sample as much 100 gr was macerated by 70% ethanol solvent and was waited during 1x24 hours, after that, the obtaining extract was filtered by using vacuum filter Buchner. Then, the obtaining filtrate was putted into the rotary vacuum evaporator during 24 hours until thick extract was obtained.

Determining photochemistry analysis of alkaloid, tannin, saponin and flavonoid

The extract was done in laboratory of research association and industrial consultation (BPKI).

Test of antioxidant's content

For determining the antioxidant's activity of each concentration, solvent sample was taken 1 mL by using micro pipette, putted into reaction tube, then added 4 mL DPPH solvent 50 μ M. The mixture was homogenized and left during 30 minutes in the dark place, the absorption was measured by using Spectrophotometer UV-VIS in wave length 517 nm. The antioxidant's activity of sample was determined by the large of radical absorption resistance DPPH through percentage calculation (%) inhibition of absorption DPPH (Renhoran, 2012). IC₅₀ value was calculated from regression linear curve between the concentration of various concentration toward percentage (%) of antioxidant's activity (Kuntorini & Maria, 2010).

Results and Discussion

On this research, the aim is to determine the existence of antioxidant's activity in combination extract of Dlingu and single clove garlic. The sample of this research is Dlingu rhizome and single clove garlic or is called the male garlic. Previously, the sample was sorted, then, prepared the sample of Dlingu stems as much 350 gram and male garlic tubers as much 200 gram. After both sorted samples which were cut into small pieces were blown to bits by using grinder to expand the surface thus to ease the extraction process and to produce the excessive extraction. The method that was used in extracting the sample is maceration method by using ethanol solvent because its characteristics of high polarity, low boiling point, and able to extract the material more excessive than the other organic solvent. Based on the result of organoleptic on the table 1, was found the result as follows:

Table 1. Organoleptic of combination extract of Dlingu and male garlic

No.	Organoleptic	The information of Maceration Result		
		Dlingu	Male garlic	Combination
1	Form	Liquid	Liquid	Liquid
2	Color	Deep brown (sepia)	Jet deep brown	Brown

3	Scent	Exclusive	Exclusive smell of garlic	Exclusive smell
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The test of photochemistry compound became the first base in detecting the existence of a secondary metabolic compound in the sample. Based on the test result, the compound in combination extract of Dlingu and garlic can be seen on the table 2.

Table 2. Test of compound in combination extract's sample of Dlingu and male garlic.

No.	Contents	Percentage (%)
1	Alkaloid	5,16
2	Tannin	4,05
3	Saponin	3,01
4	Flavonoid	2,18

The test result of compound in combination extract's sample of Dlingu and single clove garlic on this research showed the excessive contents were alkaloid 5,16 %, tannin 4,05%, saponin 3,01%, and Flavonoid 2,18%.

The compound of Flavonoid, xenolic, tannin are the responsible compound toward the antioxidant activity. The research result of Prasanto et al (2017) showed that the single clove garlic has the stronger antioxidant ability rather than the other variety of garlic.

Alkaloid compound has the great role in antioxidant, particularly indol group because it has the ability to stop the free-radical chained reaction efficiently. Indeed, the other alkaloid compounds which have the characteristic as antioxidant are quinolone, caffeine which acted as the reducer of radical hydroxide; and melatonin for radiation and toxicity of medicine (Harrizul *et al.*, 2013).

Based on the observed research, it also showed that the extract of ethanol and chloroform of the garlic extract contained much compounds of alkaloid and triterpenoid (Azzahra, 2015). This fact was also showed on Silistyorini's research (2015), suggested that the result test of higher potential antioxidant in ethanol extract of garlic (*Allium sativum* Linn) has IC₅₀ value as much 151,1 ppm.

The result of activity test in combination extract of Dlingu and single clove garlic on this research was categorized strong 17,062 ppm (table 3). From the table 3, it also can be seen that the inhibition % of concentration 50 ppm was as much 14,66, concentration 100 ppm as much 37,45, concentration 150 ppm as much 50,82, concentration 200 ppm as much 68,72, and concentration 250 ppm was as much 83,14. The resulted average was highest on the concentration 250 ppm. On the table 4.4 above, the result of antioxidant activity in combination extract of dlingu and male garlic showed that the strongest antioxidant activity was caused by IC₅₀ value < 50 µg/mL, as cited on Phongpachit *et al* research (2007) that the antioxidant activity was very strong if IC₅₀ value ≤ 50 µg/mL, it was strong if IC₅₀ value 50-100 µg/mL, it was medium if IC₅₀ value 100-150 µg/mL, it was low if IC₅₀ value 151-200 ppm, meanwhile if IC₅₀ value ≥ 200 ppm then its antioxidant's activity was very low. Based on the research which was conducted by Kurniati (2013) explained that the compound of alkaloid, saponin, flavonoid, and phenolic in 70% ethanol fraction of the leaf *Premna cordifolia* Linn

was responsible compound toward antioxidant activity. Some of Dligu rhizomes (*Acorus calamu L.*) also emphasized that the rough extract of *Acorus calamu L.* By using DPPH method with ethanol solvent showed the medium antioxidant activity with IC₅₀ value 137,7 mg/L (Hasan, 2015).

Table 3. Test result of antioxidant's activity in combination extract of Dlingu and male garlic

Sample concentration (ppm)	Abs. Average	% Inhibition	IC ₅₀ (inhibition concentration 50%)	Antioxidant activity
50	0,734	14,66		
100	0,538	37,45		Very strong
150	0,423	50,82	17,062 ppm	(IC ₅₀ < 50 ppm)
200	0,269	68,72		
250	0,145	83,14		

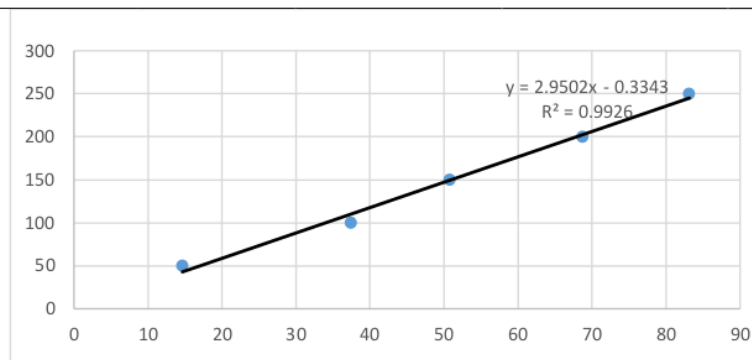


Figure 1. Linear regression curve of antioxidant test in combination extract of Dlingu and single clove garlic by using DPPH method

Based on figure 1 above, it showed that the higher of ppm concentration then % value of inhibition was more increasing, and it showed the existence of relationship between the concentration and % inhibition. Antioxidant activity was influenced by compound structure on sample, thus the occurrence of inhibition (%) can be influenced by compound structure on sample. Electron relocation of secondary metabolic compound such as alkaloid and phenol occurred through the resonance in radical structure of antioxidant, thus prevented the forming of new radical and obstructed the chained reaction of free radical (Cholisoh and Utami (2008) as cited by Sulistyorini (2015). From the result test above, combination extract of dligu rhizome and single clove garlic tuber contained alkaloid, tannin, saponin and flavonoid (table 2) which quite high thus, these compounds were expected in having the potential as anti-inflammation compound. Combination extract of both can catch radical with higher extract concentration, thus the result of IC₅₀ value was categorized strong.

Conclusions

Combination extract of *Acorus calamus* L. (Dlingu) rhizome and *Allium sativum* (single clove garlic) tuber had the very strong influence toward antioxidant activity with antioxidant value as much 17,062µg/mL.

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