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

Pramita Laksitarahmi Isrianto¹ & Putu Oky Ari Tania²

¹Educational Program of Biology, Faculty of Language and Science, Wijaya Kusuma University, Surabaya, East Java, Indonesia
²Biomedical Department and Biomolecular Research, Faculty of Medicine, Wijaya Kusuma University, Surabaya, East Java, Indonesia

Abstract
One of the efforts to optimize the utilization of natural materials that is used 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 dligu 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 dligu and single clove garlic contain IC50 values of 17.062 ppm, including a very strong class of antioxidants.

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 perialdehyde, 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 (Ikhtiarsyah, 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, distillated 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 Dlingu 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 µM. 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 (Kunterini & 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     |
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.

| 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 Phongpainchit 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 Dlingu 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 IC$_{50}$ 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        |                                          |                     |
| 150                       | 0.423        | 50.82        | 17,062 ppm                               | Very strong         |
| 200                       | 0.269        | 68.72        |                                          | (IC$_{50}$ < 50 ppm) |
| 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 IC$_{50}$ 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.

References

Azzahra, Velayaty L. 2015. Profile of Thin Layer Chromatography in Ethanol Extract of Mango’s Ginger (Curcuma mangga Val), (Acorus calamus) Rhizome, Garlic Tuber (Allium sativum) and Their Ingredients. Minithesis. Biology department, FST. Islamic state university of Maulana Malik Ibrahim.Malang

Harrizul, R., Ernita W.S. and Rusdi.2013. Ratio Influence of Ethanol-Water Solvent toward The Total Content of Phenolic Compound and Antioxidant Capacity of Soursop Leaf’s Extract (Annona muricata L.). *journal of sciences and pharmaceutical technology.* page 35-42.ISSN:1410-0177.

Hasan, Muhammad Nur. 2015. The Influence of Rhizome Extract (Acorus calamus L) in Some of Organic Solvents toward Antioxidant Activity and Antifungus in Vitro. https://www.researchgate.net/publication/308916822.

Ikhtiar, Yusrista Gilang, Inna A, Maria D.S., Endang Sulistyorini. 2014. *Bawang Putih/Garlic (Allium sativum L.).* http://ccrc.farmasi.ugm.ac.id/en/?page_id=441. It was accessed on 2nd march 2018.

Kuntorini, Evi Mintowati, Maria Dewi Astuti. 2010. Determining Antioxidant Activity in Ethanol Extract of *Eleutherine americana Merr.* *journal of science and applied chemistry, Vol.4, Number. 1:15 – 22.*

Kurniati, Ruth Indah. 2013. Antioxidant Activity Test of (Premna cordifolia Linn) Leaf by Using DPPH Method (2,2-difenil-1-pikrilhidrazil). *Publication document.* Pharmacy Program. Medical faculty. Tanjungpura University. Pontianak.

Nurhasan, Muhammad.2015. The Influence of Rhizome Extract (Acorus calamus L) in Some of Organic Solvents toward Antioxidant Activity and Antifungus in Vitro. *Minithesis.* UIN Syarif Hidayatullaah.Jakarta.

Prasonto,Djuned, Eriska Riyanti, Meirina Gartika. 2017. Antioxidant Activity Test of Garlic Extract (*Allium sativum*). *ODONTO Dental Journal. Volume 4(2).2017.*

Renhoran, Mawaddah. 2012. Antioxidant Activity and Antimicrobe of Extract *Sargassum polycystum. Minithesis.* IPB. Bogor.

Sayuti, Kesuma, Rina Yenrina. 2015. *Natural and Synthetic Antioxidant* Andalas University Press.Padang.
Sulistyorini, A. 2015. Antioxidant and Antifungus Potential of Garlic Tubers Extract (*Allium sativum* L.) in Some of Organic Solvents. *Minithesis*. Biology department, Science and Technology Faculty, Maulana Malik Ibrahim Islamic State University. Malang

Wadrianto, Glori K. 2017. 12 Amazing Use of Garlic. http://lifestyle.kompas.com/read/2017/11/01/110036020/12-menfaat-menakjubkan-dari-bawang-putih. it was accessed on 30th March 2018.