Investigation of Antioxidant Properties on Some Garlic Varieties and Influence of Farming Inputs on the Quality of Garlic

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***Abstract*—the antioxidant activity of garlic influenced by its variety and farming practices. The objectives of this study were to determine the phenolic compounds and antioxidant activity of garlic varieties which were marketed in Indonesia. The phenolic compounds were determined with Folin-Ciocalteu and antioxidant activity was examined using 2,2-diphenyl-2-phicrylhydrazyl (DPPH). Ascorbic acid was used as antioxidant references. In this experiment nine garlic varieties from different region in the world, as well as in Indonesia were investigated. Those varietis were Chinese, Egyptian, Indian, Sangga Sembalun (purple), Sangga Sembalun (white), Sangga Nunggal, Lumbu Kuning, Lumbu Hijau, and Ncuna Sambori. The results showed that antioxidant activities of Lumbu hijau, Sangga Sembalun (white) and Ncuna Sambori were among the highest but not different statistically ($p<0.05$). Moreover, Sangga Sembalun (purple), Lumbu Kuning, Indian and Sangga Nunggal were in the second level and not different statistically ($p<0.05$), while Chinese and Egyptian varieties were the lowest. Sangga Sembalun (white) variety was further cultivated with different inputs (manure, dolomit, NPK, SP-36, ZA and KCl). Results showed that the antioxidant activities of Sangga Sembalun (white) which cultivated with different inputs were not different statistically ($p<0.05$). In general, different inputs in farming practices were not changed the antioxidant activities of garlic variety.**

***Index Terms*—antioxidant activity, garlic varieties, phenolic compounds**

I. Introduction

Garlic (*Allium sativum* L.) is one of the horticultural commodities with a very high economic value. It is a very important horticultural product for Indonesian people because it is used as a cooking spice, medicine and cosmetic ingredients [1]. The national demand of garlic in Indonesia is currently estimated at 500,000 tons per year, and can only be fulfilled by domestic production of 20,000 tons [2]. This caused the number of imported garlic to reach 480,000 tons (around 96%). This condition resulted that many varieties of garlic circulating in the market both local markets and supermarkets. Imported varieties such as from China, Egypt and India, while local varieties such as Sangga Sembalun (white), Sangga Sembalun (purple), Sangga Nunggal, Lumbu Kuning, Lumbu Hijau and Ncuna Sambori can be found at the markets in West Nusa Tenggara Province of Indonesia.

Garlic has been cultivated in various regions in Indonesia. The type of garlic that is often found in Indonesia is Lumbu Hijau and Lumbu Kuning. Several varieties of garlic can also be found in various regions in Indonesia. However, other types are thought to originate from the same source due to modifications from existing sources. Because garlic is propagated with tubers continuously, a very large mutation may occur which will change the properties of garlic. The Santong variety in Lombok, for example, is similar to the Lumbu Hijau variety, and the Tawangmangu and Cirebon varieties are similar to the Lumbu Kuning variety [3].

Garlic is known to contribute greatly to health and many benefits for a long time. This is because garlic contains bioactive compounds that can act as antioxidants and have a strong aroma that is widely used in various cuisines [4]. The volatile components found in garlic are mostly sulfur compounds. The sulfur component contained in garlic not only gives a distinctive flavor but also has several properties as bioactive compounds. The amount of sulfur compounds in garlic is influenced by varieties, maturity, cultivation techniques, and environmental conditions [5]. There is no definitive information on varieties that have high antioxidant properties yet on garlic in Indonesia.

Sembalun Highland in Lombok Island, West Nusa Tenggara Province is one of the centers of garlic in Indonesia (Figure 1and 2). Since 1997 until 2017

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stretching of garlic cultivation in the region has declined due to imported garlic at very cheap prices therefore garlic which produced in Sembalun cannot compete. In the last few years, the cultivation of garlic was revived by the Indonesian government.

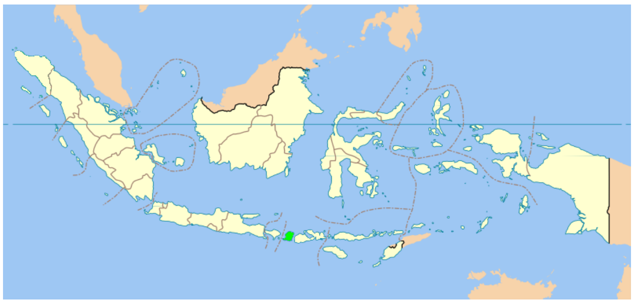

Figure 1. Map of Indonesia where Lombok Island is in the green colour below the map

Figure 2. Map of East Lombok District where Sembalun is one of the sub-districts on the top of the map with yellow colour

The objectives of this research were to determine the antioxidant activities and phenolic compounds of garlic varieties which are marketed in Indonesia as well as to investigate antioxidant activities of garlic harvested from different farming inputs.

II. MATERIALS AND METHODS

A. Materials and Reagents

Local and imported garlic varieties which were purchased from local market in West Nusa Tenggara Province of Indonesia; Folin Ciocalteu's reagent, ascorbic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Co.-Aldrich (St. Louis. MO. USA); local cow manure; dolomit; fertilizers (NPK, SP-36, ZA, KCl); and pesticides.

B. Preparation and extraction of fresh garlic extract

Extraction of fresh garlic according to [6] with slight modification. The fresh garlic cloves were peeled from the bulb and ground by a hugh-speed blender. The ground BG was mixed with deionized water for 1 h at room temperature in a shaker (CR300, Fine PCR, Seoul, Korea). The extracts were centrifuged (400 rpm, 10 min) and supernatant were collected. Finally the supernatant was analysed as soon as possible (Figure 3).

Figure 3. Process of extraction of fresh garlic at the IAAT Laboratory

C. DPPH radical scavenging activity

DPPH radical scavenging activity was determined according to [7]. DPPH solution in methanol (1.5 mM) was prepared, and then 3 ml DPPH solution was mixed with 1 ml crude extract of fresh garlic. The mixtures were incubated for 1 h in the dark room. Shimadzu UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan) was used to measure the absorbance at λ 524 nm. The increasing of DPPH radical scavenging activity was shown by decreasing of the DPPH solution absorbance. The equation to calculate antioxidant activity was given as per cent DPPH radical scavenging activity.

$$RSA(\%) = \left[\frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}}\right] \text{X } 100\%$$

The control contained 3 ml of 1.5 mM DPPH solution and 1 ml of methanol. Ascorbic acid was used as positive controls. Data were reported as means ± SD for three replications.

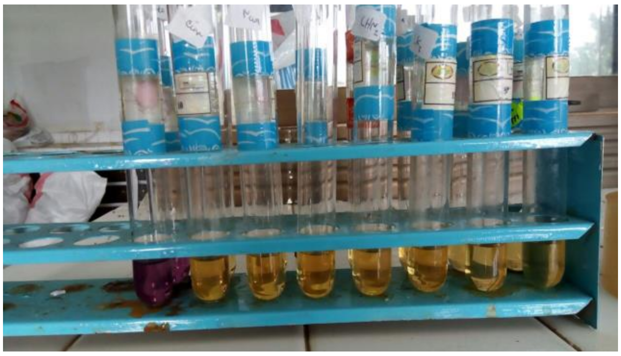

Figure 4. Samples absorbance compared with control absorbance

D. Determination of total phenolic content

The crude extract (2 ml) was placed in a tube, and 1 ml Folin-Ciocalteu reagents was added, mixed, and allow to stand for 1 min. Then 4 ml of 15% of sodium carbonate (Na_2CO_3) solution were added, mixed and placed in a dark room for 2 h at room temperature [8]. Absorbance of the resulting blue complex was then measured at 760 nm using a shimadzu UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan). Methanol was used as the blank and gallic acid used as standard. The used of gallic acid based on its stability and purity. The results were expressed as mg gallic acid equivalents (GAE)/g of garlic. Data were reported as means ± SD for three replications.

All of laboratory experiments were conducted at the laboratory of IAAT, West Nusa Tenggara Province of Indonesia from February to August 2018.

E. Field experiment

Field experiment was conducted at Sembalun Higland (1200 m asl) in Lombok Island of Indonesia from February to June 2018 to investigate the influence of farming inputs toward antioxidant activities of harvested garlic. The experimental design was used Randomized Completely Block Design (RCBD) consisting of four blocks of experiment. Each block consisted of four treatments, namely: 1) Exsisting technology which were used by farmers (P1); 2) Technological Package I (P2); 3) Technological Package II (P3); and 4) Technological Package III (P4). Each treatment was repeated four times therefore there were sixteen plots of treatment (Table 1 and 2).

Table 1. Technological packages which were applied in field experiment during the growing season in 2018.

Inputs	Existing Techno-logy (P1)	Technological Packages (P2)	Technological Packages (P3)	Technological Packages (P4)
Dolomit (kg/ha)	0	1000	750	500
Cow manure (ton/ha)	3	10	7,5	5
NPK 16:16:16 (kg/ha)	500	400	300	200
SP-36 (kg/ha)	400	300	250	200
ZA (kg/ha)	400	300	250	200
KCl (kg/ha)	300	250	200	150
Pest control	Existing technology (mixing pesticides as usual used by farmers)	Spraying pesticides based on rotation strategies	Spraying pesticides based on rotation strategies	Spraying pesticides based on rotation strategies

Table 2. The experimental plot design in the field experiment at Sembalun Highland in Lombok Island of Indonesia during the growing season in 2018.

BLOCK			
I	**II**	**III**	**IV**
P3	P1	P4	P2
P1	P2	P3	P4
P4	P3	P2	P1
P2	P4	P1	P3

The land area of 0.4 ha was divided into four replication blocks of 0.1 ha each, in each replication block there are four treatment packages (P1-P4). The details of technological packages which were applied are shown in Table 1. Furthermore, the position of each technological packages inside the blocks is shown in Table 2.

Garlic variety which were used for field experiment was local variety namely Sangga Sembalun (white). Figure 5-7 showed some photos taken from field experiment during growing season 2018 in Sembalun Village, Sub District of Sembalun, District of East Lombok, Province of West Nusa Tenggara, Indonesia.

F. Statistical Analysis

Data which were collected from laboratory and field experiments were analysed with SPSS version 12.0 using one way ANOVA followed by least significant different (LSD) test at the 95% confident level ($p<0.05$).

Figure 5. The selected site for field experiment 2018 at Sembalun Highland of Lombok Island, Indonesia. Photo taken after soil tillage and during installation of plastic mulch in February 2018.

Figure 6. The sowing time during field experiment 2018 at four blocks of experiment.

Figure 7. Performance of garlic crop during field experiment 2018.

III. RESULTS AND DISCUSSION

The diversity of % radical scavenging activity is thought to be influenced by the genetic traits of each plant that are influenced by the conditions of the niche of cultivation. One example of Lumbu Hijau garlic leaves has a darker color than Lumbu Kuning garlic leaves. This causes the Lumbu Hijau garlic to have a higher radical scavenging activity than the Lumbu Kuning variety.

The antioxidant properties of nine garlic varieties is shown in Table 3. It is showed that three local varieties namely Local Ncuna Sambori, Local Sangga Sembalun (white) and Local Lumbu Hijau were among the highest % radical scavenging activities, while Egyptian variety was the lowest.

Based on the data in Table 3, garlic which has a high % radical scavenging activity tends to contain a greater total phenolic. These causes the phenolic compounds to be oxidized by the Folin Ciocalteu reagent to become a phenolic ion to be large, therefore the phenolic ion formed will increase. The ion will reduce heteropoly acid and form a more concentrated blue compound complex. Further explains the technical reason in the laboratory that the blue color is more concentrated will result in a higher absorbance value so the phenolic number will be greater. High phenolic compounds is high radical scavenging activity because the oxidation of folin reagents results in blue.

Table 3. The antioxidant properties of nine garlic varieties which were purchased from local market in West Nusa Tenggara Province of Indonesia in 2018.

No	Varieties	% Radical Scavenging Activity	Phenolic content (mg GAE/ g of garlic)
1	Chinese (imported)	66.62 b	8.15 a
2	Egyptian (imported)	50.26 a	8.05 a
3	Indian (imported)	81.44 c	9.07 bc
4	Local Sangga Sembalun (purple)	77.12 c	8.19 a
5	Local Sangga Sembalun (white)	92.02 d	10.23 c
6	Local Sangga Nunggal	83.28 c	8.99 ab
7	Local Lumbu Kuning	79.78 c	8.22 a
8	Local Lumbu Hijau	91.55 d	10.22 c
9	Local Ncuna Sambori	92.24 d	10.36 c

Moreover, the influence of farming inputs on the antioxidant properties of garlic (Sangga Sembalun white variety) is shown in Table 4.

Table 4 showed that different farming inputs (P1, P2, P3, and P4) were not significantly influence the % RSA and total phenolic compounds of harvested garlic. This is indicated that low input in garlic farming sistem would not directly decrease the quality of garlic both percentage of radical scavenging activity and total phenolic. Unfortunately, the field research accomodate only one variety out of nine. Further investigation is needed for other varieties in order to gain general conclusion. Therefore cultivation of all varieties is needed in the future in order to gain valid conclusion on the influence of different input farming toward quality of each garlic variety.

Table 4. The % RSA and total phenolic on garlic (Sangga Sembalun white variety) with different farming inputs harvested from field experiment during the growing season in 2018

No	Tretaments	% Radical Scavenging Activity	Total Phenolic
1	P1	85.5 ab	9.88 a
2	P2	92.28 b	10.09 a
3	P3	95.40 b	10.21 a
4	P4	96.36 b	10.22 a

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