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Exploration Antioxidant Properties of Indonesian Local Single-Bulb Garlic Extract (Var. Temanggung) in a Mixed Solvent

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ABSTRACT

Single-bulb garlic is known for its high antioxidant activities. Single-bulb garlic (var. Temanggung) has a benefit potency, considering that Temanggung is one of Indonesia's most preeminent garlic cultivation areas. However, the quality of its antioxidant activities is yet to be explored widely. This research aims to carry out antioxidant characterization in single-bulb garlic (var. Temanggung) under the conditions of different solvent mixtures (ethanol and water). The Completely Randomized Design (CRD) was used with different ethanol-water mixture treatments (F1 = 100%:0%, F2 = 80%:20%, F3 = 60%;40%, F4 = 40%:60%, F5 = 20%:80%, and F6 = 0%:100% v/v). Extraction results were analyzed for total phenolic content, antioxidant activities using two methods (%RSA, IC_{50}, and FRAP), and chemical compound identification using GC-MS. The Results demonstrated that different solvent treatments produced antioxidant properties which also differed, either qualitatively or quantitatively. The best treatment was F2 (ethanol:water = 20%:80% v/v) at the highest antioxidant activity values (IC_{50} DPPH of 10.35 ppm and a FRAP value of 9.41 µM equivalent Fe(II)/g), and a TPC value of 61.02 mg GAE/g.

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1. Intoduction

Four favorite synthetic antioxidants according to consumers, i.e., butylated hydroxyanisole (BHA), tert-butylhydroquinone (TBHQ), propyl gallate (PG), and butylated hydroxytoluene (BHT), are also reportedly to come with adverse effects [1]. Flavonoids, polyphenols, and antioxidants are verified to exist in garlic [2]. Overall, studies have unveiled that we can find a high number of organic sulfur compounds in garlic, 33 in total, explaining where it gets its distinctive flavor and therapeutic properties [3]. Earlier research shows 1.3 g of ash and amino acid, 0.7 g of crude fiber, 4.4 g of protein, 0.2 g of fat, and 23 g of carbohydrates in 100 g of fresh garlic [4]; [5]; [6]. Currently, researchers have also found a substantial number of bioactive compounds in the essential oil of garlic, also adding that the oil has a high level of saponin ligands, phenolic compounds, essential amino acids, steroid saponins, and many different non-sulfur compounds, in addition to organic sulfides like diallyl disulfide (DADS) and DATS [7].

Additionally, garlic contains organosulfur components, two of which are S-allyl mercapto cysteine and S-allyl cysteine with a potent antioxidant activity [8]. Wilujeng & Anggarani [9] also report that imported garlic extract using ethanol contains lower antioxidant activity, total phenolics, and total flavonoid levels than local ones. Growing locations are closely associated with how secondary metabolites and antioxidant activity are produced as they impact plants' biochemical processes and environmental temperature, generating different levels of secondary metabolites, e.g., phenolic Growing environmental conditions influence the chemical compounds in garlic, including the quality and quantity of antioxidants. Temanggung, a district in Central Java, Indonesia, has a suitable agroclimatic requirement for garlic cultivation, giving the single-bulb garlic (var. Temanggung) distinguished antioxidant qualities. Temanggung is located in a highland area with mountains, these conditions affect the climate and soil. Temanggung has a cool climate, with volcanic soil and andosol. This type of soil is very suitable for growing garlic.

The garlic has also become a national superior garlic commodity with a productivity of 14 tons/hectare in 2023.

Isolating the garlic's antioxidant components is best carried out by extraction, the process of which requires the separation of the desired compound(s) from any interfering or undesired chemical mixture. We consider the step vital to obtain pure bioactive natural compounds for medical, scientific, and commercial applications. Meanwhile, while extracting, there are several factors which need to be emphasized in order to boost the efficiency, among them measuring the weight, measuring the volume, undertaking mixing, diluting, heating, cooling, fractionating, purifying, and preserving [10]. In addition, we also highlight another factor, namely the solvent used, when designing an extraction procedure. An effective solvent will contribute positively to the extracted compounds' selectivity, quantity, and quality.

[DOI: 10.22034/FSCT.22.160.35]

Solvent extraction is the most common technique for the extract antioxidant compounds from plant [11]. Different polarities characterize different solvents and play a critical role in determining bioactive compounds' solubility and, in turn, the overall extraction efficiency [12]. In selecting an appropriate extraction solvent, we consider the chemical characteristics of the natural products extracted and the end product desired. There are two types of solvents typically used by people to extract natural products, which are polar solvents like acetone, ethanol, methanol, and water, and non-polar ones like ethyl acetate, chloroform, and hexane. They come with unique properties supportive of the extraction of compounds with a particular polarity [13].

The solvent mixture treatment was carried out to obtaining the best ethanol concentration ratio for extracting single-bulb garlic. The polarity of the solvent affects the type of antioxidant extracted. Water and ethanol both have polar properties, but ethanol has amphipathic properties (has polar and non-polar parts in its molecular structure). Water solvents are more relatively effective in extracting allicin than ethanol [14], but distilled water cannot extract antioxidant compounds which dissolve in polar solvents, e.g., DADS. Bajac et al. [15] convey that [16]ethanol provides better DADS extraction than distilled water. Accordingly, based on the background elaborated above, this research aims to describe antioxidant characterization in single-bulb garlic (var. Temanggung) under the conditions of different solvent mixtures (ethanol and water) .

The results of this research will provide information regarding the use of local Temanggung garlic either as a functional food, or as a natural antioxidant ingredient that can inhibit oxidative damage to food [17],[16], [18] . Garlic is widely used as an inhibitor of oxidative damage. The findings in this research will be important information for local farmers because they can provide the selling value of the single-bulb garlic var Temanggung.

2.Material and Method

2.1*Materials*

Tools employed was a blender (philips), sonicator (BRANSON 2510) , filter paper, UV-Vis spectrophotometry (Genesys 10S), GC-MS (Thermo Scientific Trace OQ301), and digital scale. Meanwhile, the materials included methanol, single-bulb garlic (var. Temanggung), distilled water, ethanol 96% , 1,1-diphenyl-2-picrylhydrazyl (DPPH), quercetin, Folin Ciocalteu 50%, Na_2CO_3 20%, Gallic Acid, FeSO4.7H2O, 5% phenyl methylpolysiloxane, and Helium.

1.1 Research Population

Single-bulb garlic (var. Temanggung) was obtained directly from local farmers in Temanggung, Central Java, Indonesia. Samples were uniform in size and age, but we only used garlic intact, uniform in size, not affected by diseases, smooth outer skin, not dry, not spotted, and not green in color. Samples were then tested for determination to decide the species of the samples used.

1.2 Experimental Design

A completely randomized design (CRD) with a mixture of distilled water and ethanol was applied, as demonstrated in **Table 1.** The results will be analyzed

[DOI: 10.22034/FSCT.22.160.35]

using analysis of variance (ANOVA), if there are significant differences, Duncan's Multiple Range Test (DMRT) will be tested at a 95% confidence level.

1.3 Single-Bulb Garlic Extraction *[19]*

The garlic washed, crushed, and combined garlic cloves with the solvent in a 1:2 weight ratio according to the treatment. The garlic mixture into a sonicator for 15 minutes at room temperature and continued with the filtering process. The resulting supernatant was collected, decanted for 24 hours (refrigerator temperature), and kept in a sterile glass container as storage at 4°C for testing.

Table 1. Experimental Design Of Single-Bulb Garlic Extraction

Treatment	Mixed Solvent (%)	
	Water	Ethanol
F1	100	0
F2	20	80
F3	40	60
F4	60	40
F5	80	20
F6	0	100

1.4 Antioxidant Activity through DPPH and IC-50 Methods *[20]*

Blank antioxidant analysis was conducted by taking 4 mL of DPPH solution, vortexing it, and storing it in a place with minimum light at 37°C as the set temperature. Furthermore, absorbance was recorded at a wavelength of 517 nm. Meanwhile, 3.5 mL of DPPH was combined with 0.5 mL of the sample solution from each concentration to assess antioxidant activity. The combined solution was then vortexed and stored in a dark place at 37°C before measuring the absorbance at a wavelength of 517 nm.

$$\%SA = 1 - \frac{\text{sample absorbance}}{\text{DPPH absorbance}} \times 100\%$$

The percent inhibition was plotted on the y-axis. Meanwhile, the concentration of extract samples or the antioxidant quercetin was plotted on the x-axis to acquire the linear regression equation. This equation, represented as y = a + bx, was invoked to determine lC_{50} (50% inhibitor concentration) values for the respective samples by setting the y value to 50 as well as including the x value obtained from lC_{50}. lC_{50} values stated the concentrations of sample solutions which could reduce DPPH free radicals by 50%.

1.5 Total Phenolic Content (TPC) *[21]*

The Folin-Ciocalteu method was used to identify the total phenolic content (TPC) [21]. To begin with, a gallic acid solution was prepared to serve as a standard. The stock solution was prepared to create 100 ppm of a standard gallic acid solution, then diluted to 10, 20, 30, 40, and 50 ppm concentrations. 2 mL of each standard solution was combined with 5 mL of distilled water as well as 0.5 mL of 50% Folin Ciocalteu reagent and then vortexed. Next, they were incubated at 20-25°C for five minutes, followed by the addition of 1 mL of 20% Na2CO3 and incubation at room temperature for approximately 60 minutes. Absorbance was assessed at a

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maximum wavelength of 784 nm. 0.5 mL of 50% Folin Ciocalteu reagent and 5 mL of distilled water were mixed with about 2 mL of solution, vortexed, and then incubated at 20-25°C for five minutes. The mixture was added 1 mL of 20% Na2CO3 and incubated for 60 minutes at room temperature. The extraction was performed three times. Absorbance was measured at 784 nm as the maximum wavelength. Total phenolic content (TPC) was reported as milligrams of Gallic Acid Equivalents (GAE)/g. The TPC data collected from the absorption values of the respective samples were plotted against a standard gallic acid curve and calculated with the following formula.

$$TPC = \frac{c \times V}{M}$$

TPC = total phenolic content

c = concentration from the calibration curve

V = volume of the extract

1.6 FRAP Analysis *[20]*

$FeSO_4.7H_2O$ stock solution of 10.000 µmol/L was made by dissolving 2.78 grams of $FeSO_4.7H_2O$ using 1.000 mL of distilled water and diluted to 1, 2, 3, 4, and 5 µmol/L concentrations. As much as 1 mL of the standard solutions was taken, combined with 3 mL of FRAP reagent, and measured at wavelengths between 588 and 598 nm with a UV-Vis spectrophotometer. 0.1 mL of the respective extract solutions was added 3 mL of FRAP reagent and placed into test tubes for absorbance measurement. Meanwhile, the solution absorption was recorded with a spectrophotometer at 596 nm.

1.7 Identifying Compounds in Optimum Garlic Extracts with Gas Chromatography-Mass Spectrometry (GC-MS) *[14]*

In identifying compounds in optimum garlic extracts, we used chromatography criteria (retention time) as well as spectrometry criteria (standard compounds, mass spectral interpretation, and comparison with library information). A gas chromatograph (Thermo Scientific Trace OQ301) was coupled with a DSQ mass spectrometer (electron impact ionization, eV 70; Thermo Scientific) to gather chromatographic and spectroscopic data. The DB-5MS (J & W Scientific) column was 30 meters in length and had a static phase of 5% phenyl methylpolysiloxane, a thickness of 0.25 µm, and an internal diameter of 0.52 mm. The GC-MS was programmed in the following modes: 50°C as the initial temperature for 1 minute, which was then increased by 10°C/minute until achieving a temperature of 280°C, with a hold at the latter for 15 minutes. The temperature of the injection port was regulated to 250°C, whereas transmission line's temperature was 280°C. The carrier gas was helium (99.99%), operating at 27 cm/min (constant current 1 ml/min) and a pressure of 155 KPa. Chromatograms and mass spectrometry were calibrated using mass scanning within 45-500 m/z at a rate of 5.1 scans per second.

3.Result and Discussion

1.8 Total Phenolic Content (TPC)

The organic molecules consisting of hydroxyl groups (-OH), phenolic compounds were attached to carbon atoms in aromatic rings. As antioxidants, phenolic compounds donated hydrogen atoms, reducing free radicals into a more stable form [22]. Determination of total phenolic content (TPC) was carried out with the Folin-Ciocalteu reagent using gallic acid as a standard. The principle of

[DOI: 10.22034/FSCT.22.160.35]

the Folin-Ciocalteu method is that phenolic compounds are oxidized by the Folin-Ciocalteu reagent so that the test solution is blue which can be measured with a visible spectrophotometer at a wavelength of 750 nm. The total phenolic analysis method using the Folin-Ciocalteu reagent is a relatively reliable method and is often used in research and industrial applications, especially for the determination of total phenol in various types of food samples, but is less suitable when applied to pure samples [23] .

The single-bulb garlic extracts' total phenolic content (TPC) exhibited in **Table 2** was significantly influenced by different extraction treatments ($p > 0.05$). The highest TPC of 65.29 ± 0.4 GAE/g was obtained in the F5 treatment, while the lowest of 63.28 ± 0.2 (GAE/g) was acquired in the F1 treatment. Results indicated that TPC fluctuated in each treatment because the polarity of each different solvent produced different types of phenolic compounds extracted. Garlic had 20, and even above, phenolic compounds, and the number was exceeding that found in other vegetables [24]. It was defined that garlic from Tawangmangu had the highest TPC of 92.2 mg GAE/g. The primary phenolic compound was β-resorcylic acid, with other significant compounds including quercetin, protocatechuic acid, routine, gallic acid, and pyrogallol. Single-bulb garlic (var. Temanggung) had a relatively higher TPC level than that garlic grown in five regions in Korea had the highest TPC of 49.89 mg GAE/g [25].

The total phenol content in garlic extracts varied according to the cultivar, which could differ from one to another. Plants experiencing stress, also covering those which were exposed to various signal molecules or elicitors, would undergo the accumulation of secondary metabolites. Secondary metabolites were crucial for plant adaptation to their environment and coping with stressful conditions. Environmental conditions influenced antioxidant activity, flavonoids, and phenolics levels. A high-temperature stress in the environment would likely increase antioxidant activity, flavonoids, and phenolics levels generated [26].

A positive and highly significant relationship between total phenolics and antioxidant activity in plant products has been previously demonstrated. Miean & Mohamed [27]found relatively high concentrations of the myricetin, quercetin and apigenin flavonoids in garlic. The growing location and cultivation method influence the quality of garlic TPC. Garlic taken from 4 locations in Spain had a total phenolic content varying from 3.4 mg gallic acid equivalent (GAE)/g dry matter (dm) to 10.8 mg GAE/g dm [28] Meanwhile, local garlic from purbolinggo (Indonesia) has a total phenolic content of 28,756 mg GAE/g [29], This study had a higher TPC of 63.28 in the F2 treatment, higher than garlic from Spain, or from Purbalingga (Indonesia).

Local people consume single-bulb garlic var Temanggung to increase body endurance and reduce cholesterol levels. Functional food based on single-bulb garlic, apple vinegar, red ginger, honey and lemon can reduce triglyceride levels in hypercholesterolemic white mice ($p < 0.05$) [30]. However, the research did not explain in detail the variety of single-bulb garlic. Single-bulb garlic var Temangung has high antioxidants, so it is possible that the ability to reduce cholesterol levels in the body will also be higher.

[DOI: 10.22034/FSCT.22.160.35]

3.2 **Antioxidant Activity (DPPH Method)**

The DPPH method could be applied for either liquid or solid samples but was not working specifically when deployed for certain antioxidant components. It measured the samples' total antioxidant capacity by identifying the hydrogen capture reaction by DPPH from antioxidant substances [31] [18], [32]. During an interaction between the free-radical DPPH and an odd electron, peak absorption was notable at 517 nm and indicated a purple color. A free-radical scavenger antioxidant reacted with DPPH to form DPPHH, which came with reduced absorbance compared to the first due to its less hydrogen content. If we compared it to the DPPH-H state, this radical form caused decolorization, engendering a yellow hue, when the electrons collected increased in number [33].

However, the DPPH method had some limitations. For instance, it could only dissolve in organic solvents, challenging us when we desired to analyze hydrophilic compounds. Metal ions, hydrogen, and water contributed to the mechanisms of the free radical process. The presence of these ions in samples with antioxidant potential was hence a crucial parameter of research. For instance, flavonoids had the capability of forming complexes with Cu (II) and Fe (III), which often demonstrated enhanced activity against free radicals and, therefore, had a reaction with DPPH stronger relative to compounds lacking metal ions [34] .

Table 2. Antioxidant And Total Phenol Levels Of Single-Bulb Garlic Extracts With Different Solvents

Treatment	TPC (GAE/g)	IC_{50}(ppm)	DPPH (%RSA)	FRAP (µMeq Fe(II)/g)
F1	63.28 ± 0.20 ^{b}	28.93 ± 7.54 ^{b}	91.39 ± 0.08 a	96.51 ± 2.49 c
F2	61.01 ± 0.17 ^{a}	10.3 ± 0.85 ^{a}	93.27 ± 0.01 b	94.14 ± 10.18 c
F3	63.98 ± 0.18 ^{c}	32.78 ± 0.01 ^{c}	93.96 ± 0.20 b	102.47 ± 2.99 c
F4	64.36 ± 0.26 ^{c}	107.78 ± 12.61 ^{c}	94.01 ± 1.21 b	79.40 ± 8.98 b
F5	65.29 ± 0.40 ^{d}	82.19 ±0.01 ^{d}	95.47 ± 0.47 c	44.14 ± 6.42 a
F6	62.88 ± 1.01 ^{b}	11.93 ± 2.12 ^{b}	96.21 ± 0.33 c	52.74 ± 1.39 a

*Different small letters in each row represent a significant difference at a 5% level.

$^{a, b, c, d}$Means with different superscripts within a row are significantly different ($p < 0.05$)

The highest antioxidant value namely 96.21 ± 0.33%RSA was obtained with the F6 treatment, while the lowest was 91.39 ± 0.08%RSA. The increase in TPC was directly proportional to increased antioxidant activity. Ethanol had groups of hydroxyls which were polar and groups of alkyls which were non-polar. It could dissolve all secondary metabolite compounds, in contrast to water, which had more polar properties. Extraction using water and extraction using ethanol resulted in different antioxidant results. Since DPPH was also dissolved in organic compounds, an ethanol solvent was considered more suitable. Various types of garlic and specifically addressing their antioxidant activity, the garlic extract with the highest antioxidant results was single-bulb garlic instead of imported [35]. The stability of antioxidants was not examined in this research, but in general antioxidants have sensitive properties and are easily damaged due to several things, including

[DOI: 10.22034/FSCT.22.160.35]

oxygen, light, temperature and storage methods. Because of this, to increase antioxidant stability, a multilayer encapsulation will be made. Encapsulation will protect antioxidants from damage caused by environmental conditions.

3.3 ***IC_{50}***

IC_{50} values and antioxidant activity were correlated inversely, where lower IC_{50} values indicated a higher antioxidant activity [36]. A low IC_{50} value indicated a better antioxidant quality. The classification of a compound based on its IC_{50} value was as follows: weak (an IC_{50} value of above 150 ppm), moderate (an IC_{50} value of 101-150 ppm), strong (an IC_{50} value of 50-100 ppm), and very strong (an IC_{50}value of less than 50 ppm). The F2 treatment was the best as the IC_{50} value was the lowest, which was 10.3 ± 0.85 ppm. (**Table 2**). The treatment also had a strong antioxidant quality because its IC_{50} value was less than 50 ppm that was higher than the control quercetin, i.e., 3.9 ppm. Single-bulb garlic had an IC_{50} value of 10.61 mg/ml, while the local variety Ciwidey was 13.61 mg/ml [37].

Table 3 shows that the IC_{50} value in this study is better than the findings from garlic extracts from Porbolinggo (Indonesia) and West Sumatra (Indonesia), and close to the results obtained from garlic from Spain and Bangladesh. The IC50 value of garlic from Uganda and Portugal is smaller than in this study, namely less than 5 ppm, but it is still classified as a strong antioxidant.

Figure 1 shows that TPC was directly proportional to antioxidant levels tested using the DPPH method. It was because DPPH reacted in two ways, namely by hydrogen atom donor and electron donor mechanisms, where antioxidant compounds would provide hydrogen atoms or electron pairs to DPPH which was a radical. It would reduce the presence of free radicals in the sample [38].

The inhibitory potential of extracts was influenced by the position and number of the groups of hydroxyls, as well as the phenolic compounds' molecular weight. Phenolic compounds were identified as showing more efficient actions when being hydrogen donors. Accordingly, they functioned as antioxidants with effective characteristics. Besides, their inhibitory effect on DPPH increased when the concentration or degree of hydroxylation enhanced. Phenolic compounds as antioxidants could stabilize free radicals by releasing hydrogen atoms through electron transfer mechanisms, transforming phenol into phenoxyl radicals. Through resonance effects, phenoxyl radicals could undergo stabilization. This property made derivatives of phenol effective hydrogen donors in inhibiting reactions caused by radical compounds. The scavenging activity of phenolic compounds against free radicals was affected by the quantity of phenolic hydrogen positioned within their molecular structure. The higher the hydroxyl groups in phenolic compounds, the greater the antioxidant activity produced [39]. Using alcohols, including ethanol or methanol, contributed to the extracts' acquisition of strong antioxidant activity on account of the phenolic compounds' presence [40].

Table 3 IC_{50} values for various types of garlic in previous studies

Varieties	IC_{50}	Solvent	
Local Variety Garlic Of Bangladesh	7.8±0.8 µg/mL	ethanol	[41]
Local Garlic From Paimban, (Indonesia)	671.7395 µg/mL	ethanol	[42]

[DOI: 10.22034/FSCT.22.160.35]

Local Variety Garlic Of Uganda	4.01 mg/mL	ethanol	[43]
Local Variety Garlic Of Uganda	5.64 mg/mL	water	[43]
Local Garlic From Probolinggo (Indonesia)	257,75 µg/mL	water	[29]
Local Variety Garlic Of Portugal	4.88 µg/mL	water	[44]
Garlic The Polish 'Harnaś' Cultivars (Spain)	6.52 µg/mL	water	[44]
Garlic 'Castano' (Spain)	7.59 µg/mL	water	[44]

3.4 Antioxidant Activity (FRAP Method)

A simple, fast, and efficient method for testing antioxidants, Ferric Reducing Antioxidant Power (FRAP) analysis required no special measurement equipment. However, the reagent in the FRAP test method was less stable, therefore it had to be used immediately. Additionally, the Fe^{3+}/Fe^{2+} reduction potential could be detected using the FRAP method despite no antioxidants contained [45] [31]. The FRAP method operated on the principle of reducing ferroin analogs, which were the Fe3+ complex from tripyridyl triazine $Fe(TPTZ)^{3+}$ to an intense blue $Fe(TPTZ)^{2+}$ complex by antioxidant compounds in an acidic environment. Another disadvantage of this method was the tendency for the reagent to settle, forming a suspension and contaminating the measurement equipment. Differences in antioxidant content between using the FRAP method and using the DPPH method were because of different testing principles. The highest antioxidant content of 102.47 ± 2.99 µMeq Fe(II)/g) was presented by the F3 treatment, while the lowest 44.14 ± 6.42 µMeq Fe(II)/g) was shown by the F5 treatment. The higher concentration of ethanol solvent produced lower antioxidants in the FRAP method.

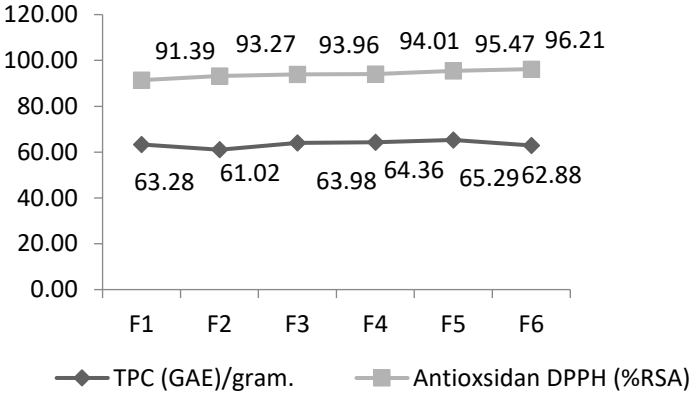

Figure 1. Comparison of TPC and Antioxidant (FRAP Methods)

Different solvent mixtures produced antioxidant content that was significantly different using the FRAP method since the mixture of solvents would provide different solubility properties. Flavonoids, alkaloids, carbohydrates, glycosides, and tannins were found exclusively in methanol extracts and water [46]. Meanwhile, water extracts included tannins, saponins, total protein, and carbohydrates yet demonstrated neither steroids nor alkaloids. Water has high polarity so it becomes one of the most suitable solvents for plant extraction [47],

[DOI: 10.22034/FSCT.22.160.35]

[48]. Therefore, ethanol extracts had the most abundant secondary plant substances (steroids, alkaloids, saponins, tannins, total protein, and carbohydrates) if compared to garlic bulb extracts obtained with other solvents.

3.5 Chemical Compound Identification

Table 4 exhibits the chemical components in each extract. Different solvent mixtures had different effects on the extract content. In treatments F6, F5, and F4, the presence of the compound 5-(Hydroxymethyl) Furfural (5-HMF) was identified. 5-HMF could be developed as a novel natural antioxidant [49], Meanwhile, in treatments F3 and F2, the presence of oxalic acid was identified. Oxalic acid was an organic acid which functioned as an antioxidant in plants and vegetables. The synthesis of oxalate was documented from ascorbate or isocitrate. Several enzymes, e.g., oxalyl-CoA synthetase oxalate oxidase, and oxalate decarboxylase, were engaged in breaking down oxalate, varying with the tissue type, cell, or species of the plant concerned [50]. Plants from the same variety could be different in terms of the quantitative and qualitative compositions of their antioxidant compounds and the composition of their constituents, whose impact on the antioxidant characteristic assessment was often ignored or rarely considered. Some examples of the constituents were metal ions, natural acid, covering succinic acid, malic, ascorbic, oxalic, citric, and others, and water. As a result, some wonders arose concerning how varying concentrations of metal ions, hydrogen, and water affected the evaluation of these compounds' antioxidant characteristics [51]. Xanthosine was expressed in treatments F6 and F6. The lower the ethanol content, the lower the Xanthosine until it was no longer detected. Likewise, Guanosine was detected in treatments F6, F5, and F4 and disappeared in treatments F3 and F2.

Lestari et al. [37] also found Xanthosine and Guanosine in black garlic extract. Guanosine and Xanthosine were nucleosides, which were substrates for adenine deaminase and purine nucleoside phosphorylase. Purine nucleoside phosphorylase was an enzyme contributing to the pathways of purine catabolism. The enzyme catalyzed the reversible phosphorylation of N-riboside nucleotide bonds, hence resulting in the production of ribose 1-phosphate and purine bases. The purine bases created were hypoxanthine, which were substrates for xanthine oxidase and guanine deaminase, xanthine, and guanine. Subsequently, the guanine deaminase enzyme converted guanine into xanthine, while the xanthine oxidase enzyme catalyzed the conversion of hypoxanthine to xanthine, then ultimately to uric acid [52].

[DOI: 10.22034/FSCT.22.160.35]

Table 4. Chemical Compound Identification By Gc-Ms

F6	F5	F4	F3	F2
5-(Hydroxymethyl) Furfural(HMF)	5-(Hydroxymethyl) Furfural(HMF)	5-(Hydroxymethyl) Furfural(HMF)	Ethanedioic Acid/Oxalic Acid	Ethanedioic Acid/Oxalic Acid
Xanthosine	Xanthosine	Xanthosine	1-Chloroisopropyl Alcohol	1-Chloroisopropyl Alcohol
Cytidine	Cytidine	Cytidine	3-Chloro-4-Methyl-2-Pentanol	Hexanol
Guanosine	Guanosine	Guanosine	4-(1'-Azepanyl)-2,6-Diphenylpyridine	5,5-Dimethylimidazolidin-2,4-Diimine
4,5-Dimethylhex-4-En-3-One	4,5-Dimethyl-4-Hexen-3-One	2-Amino-9-(3,4-Dihydroxy-5-Hydroxymethyl-Tetrahydro-Furan-2	1-Methoxy-2-Propanone	
Cis-5-Methyl-4-Hepten-3-One	Cis-5-Methyl-4-Hepten-3-One	Tris(Hydroxymethyl)Nitromethane		
4-Methyl-4-Hepten-3-One	4-Methyl-4-Hepten-3-One			
Tris(Hydroxymethyl) Nitromethane	Isobutyl Glycerol			
Guanosine Hydrate				
Triethanolamine Borate				
2,3,5,6-Tetrabromopyridine				

Aqueous and alcoholic garlic extract (GE) contain S-allyl-mercapto cysteine (SAMC), S-methyl-l-cysteine, Spropenyl-l-cysteine, and S-allyl-cysteine, all of which are derived from g-glutamyl-S-allyl-L-cysteines [53]. Table 4 indicates the absence of allicin or DADS as the test used GC-MS on a dilute sample with a ratio of garlic solvent (1:2 w/w). Garlic contains a number of antioxidants which play a role in fighting oxidative stress and protecting the body from damage caused by free radicals. Guanosine plays a role in biological mechanisms related to redox balance and protection against oxidative stress.

In the Future, the extraction results will then be encapsulated in order to maintain the quality of single-bubl garlic extract (var Temanggung). Encapsulated single-bulb garlic extract will protect the bioactive components of the garlic, besides that storage and transportation will also be easier. For this reason, it is very possible for encapsulated single-bulb garlic extract (var Temanggung) to be marketed not only locally but also internationally.

4. Conclusion

Single-bulb garlic var Temanggung contains a variety of antioxidants with strong antioxidant activity. The solvent mixture treatment gave significantly different results to the quality and quantity

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of single-bulb garlic antioxidants. The best treatment was F2 (ethanol: water = 20%:80% v/v) that produced the highest antioxidant activity values (IC_{50} DPPH of 10.35 ppm and a FRAP value of 9.41 μM equivalent Fe(II)/g) and a TPC value of 61.02 GAE/g).

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