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Review: Potential Effect of Black Garlic *(Allium sativum* L.*)* as Antioxidant

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ABSTRACT
Black garlic is a fermented garlic product that is heated at a specific temperature and humidity are a certain time without additional treatment. Numerous studies have mentioned that black garlic extract exhibits several capabilities, inclusive of antioxidant, antiallergic, antidiabetic, antiinflammatory, and anticarcinogenic effects. The antioxidant activity of black garlic can be stimulated by the processing method and the condition of the garlic by controlling the temperature and water content. The purpose of this study was to explain the potential of black garlic as an antioxidant based on the IC_{50} value obtained through the antioxidant activity tests of DPPH, ABTS, and FRAP studied from various research sources that have been carried out. This research method was carried out by searching the literature and obtaining 31 journals from several databases of *Researchgate*, *National Center for Biotechnology Information* (NCBI), *Multidisciplinary Digital Publishing Institute* (MDPI*)*, *International Journal of Research and Review* (IJRR), *Science Direct*, and *Google*. The potential of black garlic as an antioxidant is determined by the IC_{50} value. The smaller the IC_{50} value in black garlic products, the higher the antioxidant activity. This review concluded that the antioxidant activity test using the DPPH, ABTS, and FRAP methods showed an increase in the antioxidant activity of black garlic. The results of the antioxidant activity test of black garlic with any method showed a fairly good IC_{50} value.

Keywords*: Allium sativum, Black Garlic, Antioxidant*

1. INTRODUCTION

Black garlic is a fermented garlic product which is heated at a temperature of 70-90°C for 12-40 days without any additional treatment [1]. Several studies detail that black garlic extract shows several benefits, such as antioxidant, anti-allergic, anti-diabetic, anti-inflammatory, and anti-carcinogenic effects [2];[3]. The antioxidant activity of black garlic can be influenced by the processing method and condition of the garlic by controlling the temperature and water content. Black garlic products contain high levels of polysaccharides, proteins, phenolic compound, and sulfur compound. The content of water-soluble sugars, polyphenols, amino acids, and flavonoids increased or decreased during the heating interaction. This can be proven based on the research of Lee *et al*. (2009), there was a more significant decrease in free radicals in large quantities in black garlic compared to new garlic with increased polyphenols and the amount of flavonoids in black garlic increased significantly during the heating interaction [4].

Flavonoids act as antioxidant by reacting with free radicals, including superoxide anions, peroxyl radicals and hydroxyl radicals. Flavonoids with hydroxyl groups will function as free radical scavengers and the more hydroxyl groups will increase activity as antioxidant [5]. Flavonoids act as antioxidants and protect the body against reactive oxygen species (ROS). *S-allylcysteine* (SAC) is a water-soluble bioactive compound known for its high antioxidant properties [6]. SAC is formed by *γ-glutamylcysteine* catabolism and suppresses oxidative damage associated with maturation and other diseases. *Tetrahydro-β-carbolin* derivatives, which have been found in black garlic, also exhibit antioxidant effects.

Antioxidant are compound that are able to delay, slow down, or inhibit oxidation reactions caused by free radicals [7]. The mechanism of inhibition of antioxidant occurs during the initiation or propagation of reactions inside the oxidation of fats or other molecules in the body by absorbing and neutralizing free radicals or decomposing peroxides [8]. The antioxidant pastime check method using the DPPH method (2,2-diphenyl-1-picrylhydrazyl) is a quantitative measurement of antioxidant activity by plotting the concentration and percent antioxidant activity. According to [9] the 2,2'-azino-bis method (3-ethyl-benzo-thiazoline-6-sulphonic acid) (ABTS) as an antioxidant activity test method is a method of determining antioxidant activity obtained from the oxidation of potassium persulfate by ABTS diammonium salt. The FRAP method is a method to verify antioxidant activity through the ability to donate electrons. In FRAP, the ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex was reduced to ferric tripyridyltriazine (Fe^{2+}-TPTZ) by the sample.

Based on the above, it is necessary to conduct a literature study because there is still a lack of research that discusses the potential of black garlic as an antioxidant so that the authors are interested in discussing it in a review article. In this study, we will further examine the potential of black garlic as an antioxidant based on the IC_{50} value through the antioxidant activity tests of DPPH, ABTS, and FRAP from research journal obtained previously.

2. MATERIALS AND METHODS

2.1 Article Collection and Selection Strategy

In this study, literature searches were conducted from several databases of *Researchgate*, *National Center for Biotechnology Information* (NCBI), *Multidisciplinary Digital Publishing Institute* (MDPI), *International Journal of Research and Review* (IJRR), *Science Direct*, and *Google*. Literature search with keywords "Black Garlic", "DPPH method", "ABTS method", "FRAP method", "antioxidant", and "IC_{50} value". After searching for journal, a journal screening process is carried out, then the filtered journal are selected according to the keywords and research topics. After that, a feasibility test was carried out by reading all journal according to the inclusion and exclusion criteria.

2.2 Data Collection Criteria

The inclusion criteria in this review journal are (i) English and Indonesian language journal; (ii) is a research journal of research article and original article available in full text; (iii) discusses the antioxidant activity of black garlic which is expressed by the IC_{50} value and tested by the DPPH, ABTS and FRAP methods; (iv) the journal published are research from the last 10 years from 2010 to 2020.

The exclusion criteria in this review journal are (i) journal published before 2010; (ii) journal reviews; (iii) the journal does not state the IC_{50} value with a clear nominal.

3. RESULTS AND DISCUSSION

3.1 Article Selection

The literature selection process was obtained with a total number of relevant journal discussing the topic, there were 18 journal that explained the potential of black garlic as an antioxidant based on the IC_{50} value obtained through the DPPH, ABTS and FRAP tests which were reviewed from various research sources that have been conducted. This study will also explain the advantages and disadvantages of the DPPH, ABTS, and FRAP antioxidant activity test methods.

3.2 Article Characteristics

Article characteristics about the potential of black garlic as an antioxidant based on the IC_{50} value obtained through the antioxidant activity test of DPPH, ABTS, and FRAP which were reviewed from various research sources that have been carried out with a literature search process summarized in **Table 1**. In the original article there are 5 article, and the research article there are 4 articles that make observations using the DPPH, ABTS and FRAP methods, the review article found there are 4 articles that compare the DPPH, ABTS, and FRAP methods, while there are 18 other articles that discuss the method with a value IC_{50} and without IC_{50} value.

Year publication of obtained article can be seen in **Figure 1.**

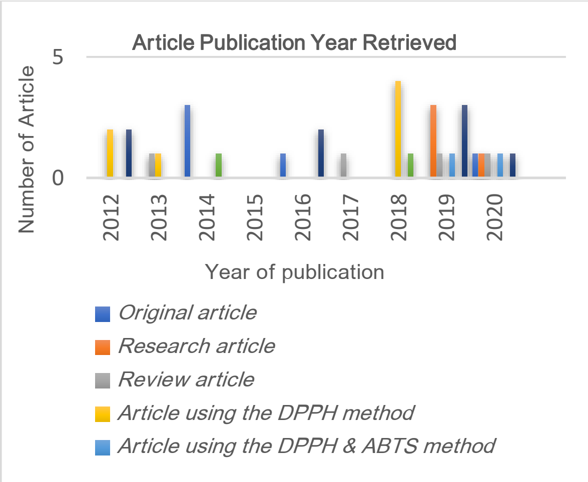

Figure 1 Year of publication

Table 1. Black Garlic Antioxidant Activity Review Results

Black Garlic Component	Method			Source	IC_{50} Value (ppm)	Antioxidant Group	Research
	DPPH	ABTS	FRAP				
Polysaccharides, glucose, proteins, phenolic compound, sulfur organic compound, and melanoidins.	√			Kastamonu, Turkey	180	Weak	[10]
S-allylcysteine (SAC), polyphenols, flavonoids, and ascorbic acid.	√			Bali, Indonesia	1.529	Very strong	[11]
S-allylcysteine (SAC), *tetrahydro-β-carbolines*, alkaloids, and flavonoids.	√			Lamongan, Indonesia	3475	Weak	[12]
S-allyl cysteine (SAC), flavonoids, polyphenols and amino acids.	√			Bogor, Indonesia	361.07	Weak	[13]
Polysaccharides, glucose, proteins, phenolic compound, and sulfur compound.	√			Surabaya, Indonesia	2.41; 2.93; 2.27	Very strong	[14]
Flavonoids, saponins and tannins.	√			Bogor, Indonesia	14.957	Very strong	[15]
Flavonoids, phenols, saponins, alkaloids and tannins.	√			West Sumatra, Indonesia	637795.5	Weak	[16]
5-hydroxymethylfurfural (5-HMF), sulfur compound, phenols, and polyphenols.	√			Kastamonu, Turkey	290; 190	Weak	[17]
Polyphenols, polysaccharides, *5-hydroxymethyl-2-furaldehyde* (5-HMF).	√			Luzhiye, China	514	Weak	[18]
S-allylcysteine (SAC), *tetrahydro-β-carbolines*, alkaloids, flavonoids and polyphenols.	√			Magelang, Indonesia	2400	Weak	[19]
Polyphenols and *S-allylcysteine*.	√			Córdoba, Spain	30	Very strong	[20]
Flavonoids and phenols.	√			Kastamonu, Turkey	1.3654	Very strong	[21]
Polyphenols dan flavonoids.	√			Namhae-gun, Korea	11520	Weak	[22]
Polyphenols, flavonoids, *5-hydroxymethylfurfural* (5-HMF).	√			NTB, Indonesia	16.45	Very strong	[23]
S-allylmercaptocysteine (SAMC) dan *s-allylcysteine* (SAC).	√			Tianjin, China	46600	Weak	[24]
Polysaccharides, Glucose, Proteins, Phenolic compound, Sulfur organic compound, and Melanoidins.		√		Kastamonu, Turkey	280	Weak	[10]
Flavonoids and phenols.		√		Songkhla, Thailand	1.5819	Very strong	[21]
N-(1- deoxy-D-fructos-1-yl)-L-arginine (Fru - Arg), phenols, polysaccharides, glucose, proteins, sulfur compound, and melanoidins.		√		Nanjing, China	1240	Weak	[25]
Polyphenols and flavonoids.		√		Namhae-gun, Korea	6500	Weak	[22]
S-allylmercaptocysteine (SAMC) dan *s-allylcysteine* (SAC)		√		Tianjin, China	0.1669	Very strong	[24]
Polysaccharides, Glucose, Proteins, Phenolic compound, Sulfur organic compound, and Melanoidins.			√	Kastamonu, Turkey	4.8539	Very strong	[10]
Flavonoids and phenols.			√	Songkhla, Thailand	0.5653	Very strong	[21]
N-(1- deoxy-D-fructos-1-yl)-L-arginine (Fru - Arg), phenols, polysaccharides, glucose, proteins, sulfur compound, and melanoidins.			√	Nanjing, China	650	Weak	[25]

3.3 Discussion

From the results of the literature review that has been obtained, the majority of article explain that black garlic is a product of garlic development. Making black garlic is to store garlic for a certain period of time under high temperature and humidity [6]; [1]; [26]. Another method is by enzymatic treatment and curing [27]. Compared with garlic, the advantages of black garlic include an increase in *S-allylcysteine* content by 5-6 times [6], longer shelf life, 5-8 times higher content of phenolic compound [28], and reduced off flavor [26]. According to previous studies, black garlic contains more phenols, flavonoids and various sulfur compounds, such as (SAC, hydrophilic) and disulfides (hydrophobic) than fresh garlic. In addition, SAC has high free radical scavenging activity [29]; [30]. This is supported by the literature search results in Table 1, which indicate that the content of black garlic is composed of SAC, flavonoids, polyphenols, and sulfur compounds. Flavonoids have the function of scavenging free radicals, such as superoxide anions, peroxy radicals, hydroxyl radicals, and effective alkoxy radicals. Flavonoids also have the ability to bind to metal ions, such as iron and copper, which can catalyze the generation of free radicals and catalyze lipid peroxidation [32]. The antioxidant mechanism of flavonoids is to suppress the formation of reactive oxygen species by activating enzymes or by binding to elements involved in the production of free radicals, reducing reactive oxygen species, protecting the body's antioxidants [31].

The black garlic extraction process causes the bioactive components to be dissolved in the solvent used. The extract preparation only contains bioactive components whose polarity is the same as the solvent used [33]. The antioxidant content of black garlic can decrease during storage. Factors causing damage to antioxidant compound include oxygen, light, water vapor and high temperature. This damage will shorten the shelf life of the product. The high antioxidant and overall phenol content are the functional value of black garlic. This value will not be maximized if the product has been damaged (degradation/degradation of quality) during storage [33].

The black garlic extraction method usually uses the maceration method using polar solvents such as methanol and ethanol, as well as distilled water as a solvent. According to research by Wahyuningtyas *et al*., (2017) stated that flavonoids are polyphenolic compound that are polar and soluble in polar solvents such as water, ethanol, methanol, acetone, butanol, dimethyl sulfoxide, dimethyl formamide, [34]. Because based on research results from several related article, the extraction method by maceration is the most frequently used. Although this maceration method requires a lot of time and solvent, it can avoid the destruction of compound that are sensitive to high temperatures [35]. All groups of flavonoids, both in the form of glycosides and flavonoids in free form, can be dissolved in methanol as solvent. In addition, the boiling point of methanol has a fairly low value (64.5°C), so it is easier to separate it [36].

The purpose of extracting black garlic is to obtain the active compound content. Before being extracted, the black garlic was mashed first to reduce the contact surface area with the solvent [14]. The yield of black garlic obtained was 68.62%. The water content obtained is 8.87%. The water content needs to be determined to meet the requirements in making simplicia. The water content in making simplicia must be less than 10%. The smaller the water content will facilitate the diffusion of the solvent which will attract the flavonoid group compound contained in black garlic. The high or low water content of black garlic depends on the high and low aging temperature used [37]; [12].

Based on research by Agustina *et al* (2019), methanol solvent is able to extract polar and semi-polar compound because the dielectric constant is 80. The dielectric constant indicates the degree of polarity, the greater the dielectric constant, the greater the polarity of the solvent, so it can be said that the polarity of the methanol solvent is lower. Than aqueous solvents [14]. This shows the appropriate result that black garlic extraction using distilled water is more widely used as a solvent than other solvents. The use of water as a solvent can maintain the stability and overall charge of the molecule so that it can help maintain the quality of its bioactivity. Aquades/water solvent is the most polar solvent compared to other solvents, so that polar components such as carbohydrates are also extracted and cause the total flavonoids per sample weight to be low [38]. According to Liu (2011), the solubility of flavonoids is determined by their chemical structure. Flavonoid compound are expected to be attracted to more polar solvents. The more polar the solvent used for extraction, the darker the color of the resulting extract.

The antioxidant activity of black garlic is related to the content of phenolic and flavonoid compound in black garlic. Diverse research have proven that the antioxidant compounds of black garlic, including polyphenols and total flavonoids, increased significantly before 21 days of aging ($p < 0.05$), correspondingly, the antioxidant activity of black garlic via DPPH, ABTS, FRAP measurements and power reduction test, the highest at the 21st day of aging [1]. Antioxidant activity test using the DPPH method to determine how much activity is required for a sample to resist DPPH stable radicals by donating hydrogen atoms. Antioxidant activity test using the DPPH technique depends on the loss of purple color due to the decrease in DPPH by antioxidants. Use an ultraviolet-visible spectrophotometer to estimate the intensity of the missing purple at a frequency of 515 nm [39]. The DPPH method has advantages, namely the analysis method is simple, fast, easy and sensitive to samples with small concentrations, but testing using DPPH is limited because DPPH must be dissolved in an organic solvent so it is quite difficult to break down hydrophilic compound [40]. This proves that many antioxidant studies use the DPPH method compared to other methods. This is also supported by the research of Nassur *et al*., (2017) the DPPH method is better than the ABTS method in determining antioxidant activity [41]. Research by Maesaroh *et al* (2018) stated that the antioxidant activity test method against DPPH radicals was found to be the most effective and efficient among the three test methods used, namely the FRAP and FIC methods.

Antioxidant test using the ABTS method is based totally at the loss of blue shade because of the reduction of ABTS through antioxidant. The intensity of this blue coloration became measured at a wavelength of 750 nm [39]. The ABTS method has the advantage of providing a specific absorbance at visible wavelengths and a faster reaction time. In addition, ABTS can be dissolved in organic solvents and water so that it can detect lipophilic and hydrophilic compound, but testing the use of ABTS does not describe the body's protection device in opposition to loose radicals so ABTS can only be used as a comparison method because it does not represent the body's biological system [40]. In particular, the ABTS technique is considered to be more effective than the DPPH technique because ABTS can degree hydrophilic and hydrophobic materials [42].

The FRAP test method relies on the reduction of the iron tripyridyl triazine (Fe (III)-TPTZ) complicated to iron tripyridyltriazine (Fe (II)-TPTZ) through a reducing agent at a low pH of 3.6. Fe (II) - TPTZ forms a blue color which may be measured at a wavelength of 593 nm. This makes it possible to measure all the antioxidant components in the sample individually [43]. The FRAP test method is greater tedious and time ingesting in phrases of getting ready chemical solutions for the job. The FRAP method is a simple and less expensive method and doesn't need the utilization of specific synthetic compound. The results obtained by the FRAP method can be produced for all concentrations. In this way, FRAP is a suitable technique to ensure antioxidant activity [44]. Another factor that affects the high antioxidant activity is the maximum sample maceration time during the extraction process which is for 5 days so that compounds that are efficacious as antioxidants are extracted properly.

4. CONCLUSION

The potential of black garlic as an antioxidant is determined by the IC_{50} value. The smaller the IC_{50} value in black garlic products, the higher the antioxidant activity. This review concluded that the antioxidant pastime check the usage of the DPPH, ABTS, and FRAP methods showed an increase in antioxidant pastime in black garlic. Testing the antioxidant pastime of black garlic with any method, showed a fairly good IC50 value. The three methods have their respective advantages in determining antioxidant activity, namely the DPPH analysis method which is simple, fast, easy and sensitive to samples with small concentrations, ABTS has advantages in providing specific absorbance at visible wavelengths and faster reaction times, and FRAP is straightforward and cheaper and does no longer require the usage of proprietary chemicals.

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