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Phytochemical Screening, Total Antioxidant Capacity and Toxicity Test of White Jasmine Flower Extract (*Jasminum sambac*)

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ABSTRACT

Oxidative stress is a state of imbalance of antioxidants and reactive oxygen species levels in the body. Prolonged oxidative stress can cause degenerative diseases, malignancies, or premature aging. This situation can be prevented by antioxidants, compounds that can inhibit the oxidation process. One of the Indonesian plants, namely white jasmine flower, is known to contain a lot of secondary metabolites and have the potential to be antioxidants. Therefore, this research was conducted to determine the content of secondary metabolites, antioxidant potential, and toxicity of white jasmine flowers. Jasmine flower extract was made by maceration method using methanol solvent. Phytochemical screening was carried out in a semi-qualitative manner based on Harborne's book. The total antioxidant capacity test was carried out by the Blois method using DPPH (1,1-diphenyl-2-picrylhydrazyl) solution. Toxicity test was carried out using the BSLT (Brine Shrimp Lethality Test) method. In the phytochemical screening, jasmine flower extract was found alkaloids, flavonoids, cardio glycosides, glycosides, saponins, coumarins, phenolics, quinones, betacyanins, steroids, terpenoids, and tannins. It was found that jasmine flower extract had a total antioxidant capacity (IC_{50} = 460.24 μg/mL) which was categorized as weak antioxidant (200 ppm ≤ IC_{50} ≤ 500 ppm), and the level of toxicity (LC_{50} = 111.43 μg/mL) which has cytotoxic properties (LC_{50} ≤ 1000 ppm) and belongs to the moderate toxic category (100 ppm ≤ LC_{50} ≤ 1000 ppm). In summary, jasmine flower extract has the potential as an antioxidant and has the potential to be an antimitotic agent.

Keywords: *Jasminum sambac, Phytochemical Screening, DPPH, Brine Shrimp Lethality Test.*

1. INTRODUCTION

Indonesia is the largest archipelagic country in the world, has more than 17,000 islands. Indonesia is located between two continents (Asia and Australia) and two oceans (Indian Ocean and Pacific Ocean), which makes Indonesia has high biodiversity of flora and fauna. Indonesia has 120.6 million hectares of forest area, which is 63% of its total land area. In the 2018 Indonesian Forestry Status, Indonesia has 91.251 species of spore-bearing plants, 120 types of gymnosperms, and 19,112 species of flowering plants (angiosperms) [1]. Many Indonesian plants are used as medicinal plants, an estimated 9,600 plant species are known for their medicinal properties and about 200 of them have been used as raw materials for the traditional medicine industry [2].

The medicinal properties in plants come from non-nutrient chemical compounds, namely phytochemical compounds, which have a role as a self-defence mechanism against the surrounding environment [3]. One of the important roles of phytochemicals is as an antioxidant. Antioxidants are molecules that can neutralize reactive radicals that become less active by accepting or donating electrons to prevent oxidative stress in the body [3,4]. Antioxidants can be categorized based on their activity into enzymatic or non-enzymatic antioxidants, based on their solubility, based on size, or based on source. Secondary metabolite compounds can be grouped into 6 based on their structure and

biosynthetic pathways, namely terpenoids, steroids, phenylpropanoids, polyketides, flavonoids, and alkaloids [4-6].

Oxidative stress is a state of imbalance between free radicals and antioxidants in the body due to a lack of antioxidants or an increase in free radicals such as reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive sulphur species (RSS) [4-6]. Free radicals have unpaired electrons so they can easily react with other molecules in the body and have the main function for apoptosis, cell signalling, ion transport, and gene expression, but can cause cell damage when excessive and bind to macromolecules such as carbohydrates, fats, proteins, and nucleic acid, which leads to accelerated aging, malignancy, and degenerative diseases [4-6].

White jasmine (*Jasminum sambac*) is one of Indonesia's national flowers. White jasmine is often used in Indonesian traditions and is known as a symbol of purity. In addition, white jasmine flowers are also used as traditional medicines to treat diarrhoea, fever, stomach pain, asthma, toothache, and infertility. White jasmine flowers are also believed to have antioxidant, anticancer, antimicrobial, antiviral, vasodilator, antidepressant, analgesic, anti-inflammatory, gastroprotective, and wound healing abilities [7]. In Kunhachan's research (2016), which was conducted in Thailand, found coumarin, cardiac glycosides, essential oils, flavonoids, phenolics, saponins, and steroids compounds, as well as antioxidant potential in white jasmine flower extract [8]. However, there is very little information about the level of toxicity and antioxidant potential in white jasmine flowers in Indonesia, thus encouraging researchers to find out more about the antioxidant abilities and toxicity of white jasmine flowers in Indonesia. The aims of this research are to investigate, antioxidant activity, and toxicity level of white jasmine flower (*Jasminum sambac* (L.) Aiton). In hope, the white jasmine flower can be a candidate for anti-cancer and prevention of oxidative stress diseases such as early aging, cancer, and degenerative diseases.

2. METHODS

The flowers of white jasmine were picked and sent from Tegal Central Java in a fresh state. The collected sample was identified at Herbarium Bogoriense, Research Center for Biology, Indonesian Institute of Sciences with the results of *Jasminum sambac* (L.) Aiton species and *Oleaceae* family.

Collected white jasmine flowers were washed and dried indoors without sunlight exposure for 14 days. Dried flowers then were made into simplicia and macerated with methanol. Then, the extract was concentrated through evaporation process using a rotary evaporator.

This research is an experimental research consisting *in-vitro* and bioassay tests. The *in-vitro* tests consist of phytochemical screening and total antioxidant capacity test. With a bioassay test in the form of a toxicity test.

Phytochemical screening in this research consisted of 13 types of compounds tested. The methods and reagents used in this phytochemical screening are based on the book written by Harborne [9]. The compounds tested in this phytochemical screening were alkaloids, flavonoids, cardio glycosides, glycosides, saponins, coumarins, phenolics, quinones, anthocyanins, betacyanins. steroids, terpenoids, and tannins.

Total antioxidant capacity test was carried out using Blois method [10]. The reagent DPPH (1,1-diphenyl-2-picrylhydrazyl) solution was used and prepared with a concentration of 50 μM. The absorbance and optimal wavelength of DPPH solution was measured using Genesys 30-Vis Spectrophotometer. Then, standard solution was made by mixing 10 mg of white jasmine flower extract and 10 mL of methanol. The standard solution was then diluted into concentrations of 100 μg/mL, 200 μg/mL, 300 μg/mL, 400 μg/mL, and 500 μg/mL. Each of the concentrations was then mixed with DPPH solution and the absorbance of the solution was measured at the optimal wavelength. The same action was also carried out on vitamin C as a standard of comparison. From the absorbance obtained, inhibition percentage, and regression curves were made, and the linear equation was applied to calculate the IC_{50}.

Toxicity test was carried out using the Brine Shrimp Lethality Test (BSLT) method conducted by Meyer [11]. The shrimp larvae used were *Artemia salina* shrimp. Shrimp eggs were incubated in Erlenmeyer tubes filled with seawater. Shrimp larvae that had lived for two days were used for the test. Then, standard solution was made by mixing 20 mg of white jasmine flower extract and 10 mL of seawater. The standard solution was then diluted into concentrations of 25 μg/mL, 50 μg/mL, 100 μg/mL, and 200 μg/mL. A total of 10 shrimps were added into each concentration and observed for 24 hours. Then, the number of dead larvae was calculated to get the mortality percentage and a standard curve is made out of it.

Statistical tests or data analysis was performed using the application program GraphPad prism v.9.0 La Jolla, California, USA. Data is displayed in the form of tables and graphs.

3. RESULTS

In this research, the wet weight of the sample was 2 kilograms and became 204.57 grams after being dried. The amount of simplicia used to make the extract was 90 grams with 900 mL of methanol and 650 mL of extract was obtained. After the evaporation process, the paste was obtained weighing 12.49 grams. So that the yield is 13.88%.

$$\text{Yield}(\%) = \frac{12{,}49}{90} \times 100\% = 13{,}88\% \quad (1)$$

Phytochemical Screening

Phytochemical examination to determine the content in jasmine flowers includes alkaloids, flavonoids, cardio glycosides, glycosides, saponins, coumarins, phenolics, quinones, anthocyanins and betacyanins, steroids, terpenoids, and tannins. (Table 1).

Table 1. Phytochemical Content

Phytochemicals	Method/Reagent	Extract
Alkaloids	Mayer/Wagner	+/+ +
Flavonoids	NaOH	+ + +
Cardio Glycosides	Keller Kiliani	+ +
Glycosides	Modified Borntrager	+
Saponins	Foam	+ +
Coumarins	NaOH+Chloroform	+
Phenolics	Folin Ciocalteau	+ + + +
Quinones	H_2SO_4	+ + +
Anthocyanins	NaOH	-
Betacyanins	NaOH	+ +
Steroids	Liebermann Burchard	+
Terpenoids	Salkowski	+ +
Tannins	Ferric-Chloride	+ + + +

Total Antioxidant Capacity Test

Jasmine Flower Extract

The absorbance and optimal absorption wavelength obtained using the Genesys 30-Vis Spectrophotometer were 0.62 and 516 nm.

The absorbance value of each concentration of jasmine flower extract that was tested by DPPH was obtained using the Genesys 30-Vis spectrophotometer and used to calculate the inhibition percentage (Table 2). Then a linear equation curve was made from the DPPH test results to obtain a linear equation with the X-axis as the concentration of the extract and the Y-axis being inhibition percentage (Figure 1). The linear equation obtained is Y = 0.0669X + 19.21 with R^2 = 0.9826. By using the linear equation that has been obtained, the IC_{50} value of jasmine flower extract was obtained at 460.24 μg/mL.

Table 2. Concentration, Inhibition Percentage, and IC_{50} of Jasmine Flower Extract

Concentration (µg/mL)	Inhibition Percentage	IC_{50}(µg/mL)
100	25, 32	460, 24
200	34, 52	
300	38, 23	
400	44, 68	
500	53, 71	

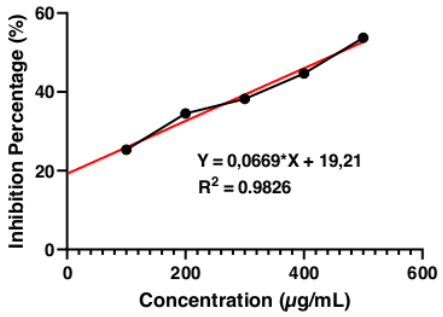

Figure 1 Jasmine Flower Extract DPPH Test Results Curve

Standard Vitamin C Comparison

Each concentration of vitamin C that has been tested with DPPH is read the absorbance value using a Genesys 30-Vis spectrophotometer and the absorbance value is used to obtain inhibition percentage (Table 3). The standard linear equation curve for vitamin C is drawn with the X-axis as vitamin C concentration and the Y-axis being inhibition percentage (Figure 2). The equation of the linear line is Y = 6.934X + 12.52 with R^2 = 0.9988. From the equation of the line obtained, the calculated IC_{50} result of vitamin C standard is 5.40 μg/mL.

Table 3. Concentration, Inhibition Percentage, and IC_{50} of Standard Vitamin C

Concentration (µg/mL)	Inhibition Percentage	IC_{50} (µg/mL)
2	26, 85	5, 40
4	39, 11	
6	54, 97	
8	67, 87	
10	81, 81	

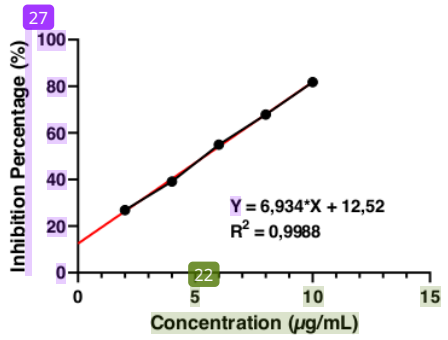

Figure 2 Vitamin C Standard Curve

Toxicity Test

Jasmine flower extract was given to Artemia salina larvae with different concentrations. The log concentration and percentage of mortality of Artemia salina larvae were calculated at each concentration of jasmine flower extract (Table 4). From a curve was made with the concentration log as the X-axis and the percentage of deaths as the Y-axis (Figure 3). The equation of the linear line Y = 77.977X – 109.62 is obtained with R^2 = 0.945. LC_{50} was calculated using the linear equation Y = 77.977X – 109.62, and the LC_{50} result was 111.43 μg/mL.

Table 4. Concentration, Mortality Percentage and LC_{50} of Jasmine Flower Extract

Concentration (µg/mL)	Log Concentration	Mortality Percentage	LC_{50} (µg/mL)
25	1, 4	5, 26	111, 43
50	1, 7	17, 78	
100	2	38, 89	
200	2, 3	76, 47	

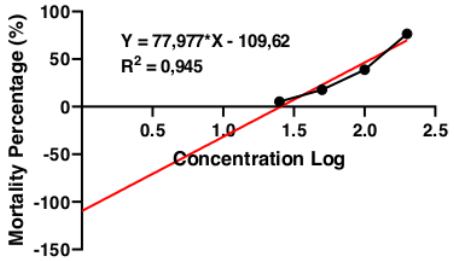

Figure 3 Jasmine Flower Extract Toxicity Test Results Curve

4. DISCUSSION

Phytochemical Screening

Phytochemical screening in this research examined 12 phytochemical content contained in jasmine flower extract. According to Asaduzzaman et al [3], plants that contain phytochemicals have potential as antioxidants, anticancer, antiaging, anti-inflammatory, antidiabetic, antimicrobial, antiparasitic, antidepressant, and wound healing. The phytochemical test results obtained from jasmine flower extract in this research containing alkaloids, flavonoids, cardio glycosides, glycosides, saponins, coumarins, phenolics, quinones, betacyanins, steroids, terpenoids, and tannins (Table 1). The phytochemical screening carried out is a semi-qualitative test, where a positive result is obtained from a colour change that matches the positive criteria for each content. The results of this research are in line with the results obtained by Al-Snafi [12], that jasmine

flower extract contains flavonoids, glycosides, saponins, coumarins, phenolics, steroids, and tannins. In the research of Hossain et al [13], it was found that jasmine flower extract contains alkaloids and terpenoids. In the research of Adnyana et al [14], it was stated that jasmine flower extract contains quinones. In their research, Kunhachan et al [8] also found the content of cardio glycoside in jasmine flower extract.

Total Antioxidant Capacity Test (DPPH)

Jasmine flower extract and comparison standard of vitamin C or ascorbic acid were used to obtain antioxidant capacity by calculating IC_{50}. According to research by Rivero-Cruz et al [15], IC_{50} is the concentration required for a substance to inhibit 50% of biological activity, which in this research was used to measure antioxidant activity in jasmine flower extract. The lower the IC_{50} value, the higher the level of antioxidant capacity in the jasmine flower extract.

In the jasmine flower extract curve and the standard curve of ascorbic acid, the values of $R^2 = 0.9826$ and $R^2 = 0.9988$ are obtained, so that the linear equation made is interpreted as having a reliable level of accuracy. The IC_{50} values for jasmine flower extract and ascorbic acid were 460.24 μg/mL and 5.4 μg/mL (Table 2 and Table 3). According to the research of Widowati et al [16], the IC_{50} value of jasmine flower extract was 94.13 ± 10.54 μg/mL. There is a considerable difference because, in their research, Widowati et al used ethanol as a solvent. According to research by Mustarichie et al [17], the IC_{50} value of 250-500 ppm belongs to the category of weak antioxidant and the IC_{50} value of ascorbic acid obtained is 4.41 ± 0.01 μg/mL. This shows that the level of antioxidant capacity of jasmine flower extract is lower than ascorbic acid and belongs to the category of weak antioxidants. The low IC_{50} value of jasmine flower extract may be caused by factors of storage time and shipping method from plant stores. However, according to research by Rambabu et al [18], jasmine flower extract has advantages over vitamin C, namely, it has gastroprotective and antidiabetic effects. In addition, according to Lim [7] in his book it is stated that jasmine flowers have antiviral, antidepressant, vasodilating, antimicrobial, analgesic, anti-inflammatory, and wound-healing effects.

Toxicity Test (BSLT)

Toxicity testing is a bioassay examination, in this research Artemia salina larvae were used to test the toxicity of jasmine flower extract. According to research by Rachmawati et al [19], the toxicity test using the BSLT method is useful for screening antimitotic abilities in plants, with cytotoxicity activity and antimitotic potential correlated with the content of terpenoids and flavonoids.

In this research, the value of $R^2 = 0.945$ was obtained so that the linear line equation made had a reliable level of accuracy. The higher the concentration of jasmine flower extract, the higher the mortality rate of Artemia salina larvae. Based on the equation of the linear line on the curve, the sample concentration that can kill 50% of Artemia salina larvae within 24 hours of observation is LC_{50}. The LC_{50} value obtained from the jasmine flower extract was 111.43 μg/mL. The results of the toxicity test of jasmine flower extract in this research were similar to research conducted by Rahman et al [20] on jasmine leaf extract, namely the LC_{50} value of jasmine leaf extract was 50 μg/ml. According to Meyer et al [11], the LC_{50} value < 1000 ppm indicates that the compound tested by the BSLT method has cytotoxic properties against proliferating cells. According to Swan et al quoted by Ismail et al [21], the LC_{50} value of 100-1000 ppm is categorized as moderate toxic compound. It can be concluded that white jasmine flower extract has cytotoxic properties with moderate toxicity category, so it has potential as antimitosis.

5. CONCLUSIONS

Based on the results and discussion of this research entitled Phytochemical Test, Total Antioxidant Capacity and Toxicity of White Jasmine Flower Extract (Jasminum sambac), it can be concluded:

1. The white jasmine flowers contains alkaloids, flavonoids, cardio glycosides, glycosides, saponins, coumarins, phenolics, quinones, betacyanins, steroids, terpenoids, and tannins.
2. The total antioxidant capacity of white jasmine flowers in IC_{50} is 460.24 μg/mL, which is included in the category of weak antioxidants.
3. The level of toxicity of white jasmine flowers in LC_{50} is 111.43 μg/mL, which is included in the category of moderate toxicity so that it has the potential as antimitosis.

6. SUGGESTIONS

It is recommended to do further research by using experimental animals to find out more about the antioxidant potential of jasmine flower extract.

It is recommended to test the phytochemical content, antioxidant capacity, phenolic content, alkaloid content, and toxicity level using other parts of the white jasmine plant.

AUTHOR'S CONTRIBUTIONS

The authors contributed equally to all aspects of the article.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest

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