Marigold_Leaf_Extract_Tagetes_Erecta_L.

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Phytochemical Screening and Total Antioxidant Capacity of Marigold Leaf Extract (Tagetes Erecta L.)

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ABSTRACT

Reactive Oxygen Species (ROS) that are not balanced with antioxidants are also known as oxidative stress. ROS are very easy to get, it can be found in the human body itself, because the human body also produce free radical from oxidation processes, inflammation and many more, external causes such as cigarette smoke, and other smoke. This oxidative stress condition can cause premature aging, cancer or other degenerative diseases. Antioxidants can balance these free radicals, one of the natural antioxidants is the marigold plant (*Tagetes erecta*) that contains lutein which is useful for anti-radicals. The purpose of this study is to know the phytochemical content and antioxidant capacity on methanol extracts of marigold leaf. This research is using methanol as a solvent and the extraction process is by maceration. The in-vitro test consisted of a qualitative phytochemical test (Harborne) than for an antioxidant capacity test with 1,1- Diphenyl-2-Picryhidrazyl (DPPH) test (Blois). The results of qualitative testing showed that marigold leaf extract contains alkaloids, anthocyanins and betacyanin, cardiac glycoside, coumarins, flavonoids, glycosides, phenolics, quinones, saponins, terpenoids and tannins. The antioxidant capacity of marigold leaf extract has (IC_{50} = 145.79 g/mL) shown that marigold leaf extract has moderate antioxidant capacity.

Keywords: *Reactive Oxygen Species, Total Antioxidant Capacity, Tagetes erecta.*

1. INTRODUCTION

The amount of Reactive oxygen species (ROS) that is not balanced with the number of endogenous antioxidants produced by our body is also known as oxidative stress. Conditions like this can cause damage to cells, causing various diseases such as cancer, premature aging, atherosclerosis and other degenerative diseases [1].

Reactive Oxygen Species are present in the body, because ROS can come from oxidation processes such as when breathing, inflammation, excessive physical activity or exercise, and exposure to external pollutants such as cigarette smoke, vehicle fumes, solar radiation and also from food. When compared between free radicals and non-radicals, free radicals are more dangerous [1],[2]. Therefore antioxidants are very important for the body to prevent damage from oxidative stress [3]. Fruits and vegetables contain both nutritional and non-nutritive factors that greatly contribute to antioxidants [4].

One example of an exogenous antioxidant is marigold. Marigold is a natural antioxidant, this plant has yellow and orange flowers containing vitamin A and beta carotene which can function as antioxidants, different lutein content in each variety has different anti-radicals as well. Marigold plants in Indonesia are commonly called kenikir flowers or tahi kotok, and in Bali they are often referred to as local names, namely gemitir. This plant comes from Central America, precisely from Mexico, but now it has spread to all corners of Indonesia because this plant has very adaptive properties, this plant can grow in the lowlands and highlands [5].

This marigold plant grows in the tropics. Marigold plants are ornamental plants with flowers that have yellow or orange colours and many grow wild in places that are directly exposed to sunlight. Indonesia has various types of marigold plants, for example in West Java the flowers of marigolds are yellow while in Central Java the flowers of marigolds are orange. The leaves of this marigold plant also have many benefits, besides being high in antioxidants, marigold leaves can

also be used as antimalarials. Because marigold leaves have a pungent odor that can repel mosquitoes [6].

The aim of this research is that the antioxidant ability of marigold leaves can be used to prevent free radicals from being formed and reduce the risk of several diseases due to oxidative stress such as heart disease, neurodegenerative disease, premature aging and cancer.

2. METHOD

This in-vitro experimental research consisted of phytochemical tests based on the book by Harborne [7] and for total antioxidant capacity with DPPH using Blois [8] method. The research was conducted at the Laboratory of Biochemistry and Molecular Biology, Faculty of Medicine, Tarumanagara University from January to May 2021.

The marigold leaf extract was made by drying at room temperature without direct sunlight, and waiting for a week for the plants to dry. ensure that the leaves are not affected by pests and there is no oxidation. then the sample was cut into fine pieces to form simplicia. then maceration process in using methanol liquid in a maceration tube that has been coated with cotton and the outside is wrapped with aluminum foil and this process is stirred every morning and evening. then evaporated with a rotary evaporator to obtain marigold leaf extract in the form of a paste.

Phytochemical examination used several methods or reagents. As in alkaloids using Mayer and Wagner reagents, NaOH for anthocyanins and betacyanin, Keller - Kiliani method for determining cardio glycosides, Ammonia for coumarins, Alkaline reagents for flavonoids, Borntrager for glycosides, Folin Ciocalteau for phenolics, H_2SO4 for quinones, Foam for saponins, Liebermann Burchard for steroids, Salkowski's for terpenoids and Ferric Chloride for Tannin.

For the total antioxidant capacity, first, make stock of marigold leaf extract as much as 1mg/mL, then dilution was carried out to obtain concentrations of 100 μg/mL, 200 μg/mL, 300 μg/mL, 400 μg/mL, 500 μg/mL. and to compare using vitamin C with concentrations of 2 μg/mL, 4 μg/mL, 6 μg/mL, 8 μg/mL, 10 μg/mL. Each of the concentrations will be taken 0.5 mL mixed with 3.5 mL of DPPH. then wait for 30 minutes in a dark place at room temperature. Finally read by spectrophotometry Genesys 30 visible and read the absorbance at the optimal wavelength. Then after obtaining control absorbance and test absorbance, the percent inhibition could be calculated using the formula:

$$\%\ \text{inhibition} = \frac{\text{Control Abs.} - \text{Test Abs.}}{\text{Control Abs.}} \text{ x } 100\% \quad (1)$$

Data processing was using the GraphPad Prism v.9.0 statistical program software. The results are expressed in the average value and displayed in the form of a graph.

3. RESULT

In this study, the percentage of dry yield was 18.31% with the calculation:

$$\%\text{Yield} = \frac{\text{Exract Weight}}{\text{Simplicia Weight}} \text{ x } 100\% \quad (1)$$

$$= \frac{13\,\text{gram}}{71\,\text{gram}} \text{ x } 100\% \quad (2)$$

3.1. Phytochemical Screening

The results of phytochemical screening on marigold leaf extract are positive for alkaloids, anthocyanins, betacyanin, cardio glycosides, coumarins, flavonoids, glycosides, phenolics, quinones, saponins, steroids, terpenoids and tannins (Table 1).

Table 1. Phytochemical Content

Phytochemical	Result	Method/reagen
Alkaloid	+	Mayer, Wagner
Anthocyanins and betacyanin	+	NaOH
Cardio glycoside	+	Keller - Kiliani
Coumarins	+	Ammonia (NH_3)
Flavonoid	+	Alkaline reagent
Glycosides	+	Borntrager
Phenolic	+	Folin Ciocalteau
Quinone	+	H2SO4
Saponin	+	Foam
Steroid	+	Liebermann Burchard
Terpenoids	+	Salkowski's
Tannin	+	Ferric Chloride

3.2. Total Capacity Antioxidant

The optimal absorption wavelength is 516nm and the test absorbance of marigold leaf extract obtained by UV-Vis spectrophotometry is 0.588.

For each different concentration of marigold leaf extract, the absorbance value and percent inhibition were searched using spectrophotometry where the X-axis was the concentration of the marigold leaf extract and the Y axis was the percent inhibition. Next, the DPPH test linear equation curve was made to find the IC_{50} value (Figure 1). In this study, it was found that Y = 0.07448X + 39.14 and the value of R^2 = 0.9687. The calculation results obtained that the IC_{50} of marigold leaf extract was 145,799μg/mL (Table 2).

Table 2. Concentration, Percent Inhibition and IC_{50} of marigold leaf extract

Concentration	Percent Inhibition (%)	IC_{50} (µg/mL)
100	45.74	145.79
200	54.74	
300	65.47	
400	69.38	
500	75.00	

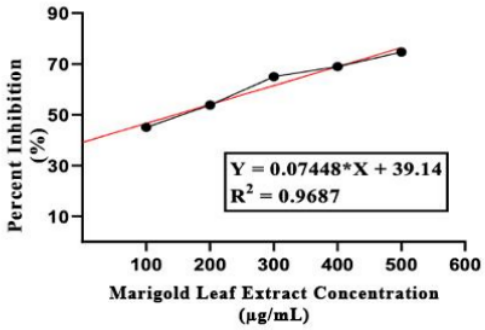

Figure 1. Curve of Total Antioxidant Capacity Test of Extract Marigold Leaf

3.3. Total Antioxidant Test of Vitamin C

Measurement of absorbance of each concentration of vitamin c and percent of inhibition using spectrophotometry Genesys 30 visible. From these measurements, a standard linear equation curve for vitamin c was made (Figure 2). In this study, the linear line equation Y = 6.934X + 12.52 and R^2 = 0.9988 with the X axis is the concentration of vitamin C and the Y-axis is the percent inhibition. Then from these results, the IC_{50} standard of vitamin C was 5.40 g/mL (Table 3).

Table 3. Concentration, Percent Inhibition and IC_{50} of vitamin C

Concentration	Percent Inhibition (%)	IC_{50} (µg/mL)
2	26.85	5.40
4	39.11	
6	54.97	
8	67.87	
10	81.81	

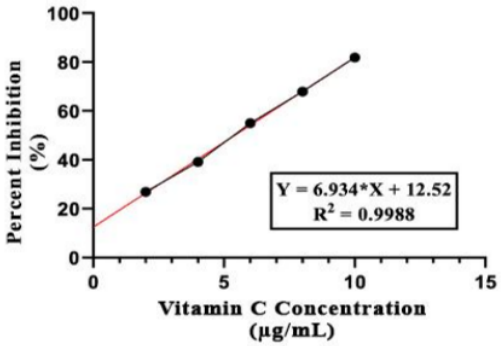

Figure 2. Curve of Total Antioxidant Capacity Test of Vitamin C

4. DISCUSSION

4.1. Phytochemical Test

The results of the quantitative phytochemical screening test on marigold leaf extract showed that marigold leaves contained alkaloids, anthocyanins, betacyanin, cardio glycosides, coumarins, flavonoids, glycosides, phenolics, quinones, saponins, steroids, terpenoids and tannins. From the research conducted by Marini et al [9], marigold leaves contain alkaloids, flavonoids, saponins, and tannins. According to Devika [10] research, it was found that marigold leaves contain cardio glycosides, phenolics and coumarins. Furthermore, marigold leaves contain glycosides and terpenoids [11],[12].

The purpose of the researchers conducting research on anthocyanins is because anthocyanins can function to prevent atherosclerosis by inhibiting the atherogenesis process and also preventing the process of blood clots [13], while for betacyanin because betacyanin contains antiradical effects and high antioxidant activity [14]. The purpose of researchers

doing research on quinones is because quinones can help prevent heart disease and osteoporosis [15]. The purpose of researchers doing research on alkaloid is because alkaloid compounds have long been known, and have also been used as drugs that have a lot of potential, for example, the name of the drug derived from alkaloids is atropine which has an anticholinergic effect, there is also scopolamine which has an antiemetic effect and many others have various effects such as antidepressants, antimalarial, antibacterial, antipyretic, analgesic, anti-inflammatory as for antidiabetic, antihypertensive and anticancer [16]. The purpose of researchers doing research on coumarin is because Coumarin itself can be used as an anti-coagulant, anti-plasmodial and can also be used as an anticancer [17]. The purpose of researchers doing research on flavonoids is because the main antioxidants in food are flavonoids, which are known to prevent cardiovascular disease by reducing the oxidation of low-density lipoproteins. The purpose of researchers doing research on phenolics is because phenolic compounds have fascinated scientists internationally for their peculiar activities, such as anti-inflammatory, antioxidant power, and anti-carcinogenic properties [18]. The purpose of researchers doing research on steroids is because steroids have a similar effect to others such as anti-inflammatory, anticancer but for steroids it has a cardioprotective effect [19]. The purpose of researchers doing research on terpenoids is because terpenoids also have the same effect and have advantages such as anti-mutagenic, anti-cholinesterase, anti-tyrosinase and anti-diabetic properties [20]. The purpose of researchers doing research on cardiac glycosides is because cardiac glycosides are well known and have been used as drugs such as digitoxin, digoxin, ouabain, oleandrin which have long been used for the treatment of heart disease. In addition, cardiac glycosides are also used for heart tonics, diuretics and antiemetics [21]. Then purpose of researchers doing research on saponin is because saponins have long been known and used as natural detergents. However, saponins also have biological activity which can be used for anti-inflammatory and immune-stimulating remedies [22].

Actually the rest such as tannins and others also have the same effect as for example antimicrobial, anticancer and others.

4.2. Total Capacity Antioxidant Test

This study used marigold leaf extract and ascorbic acid as a standard used to measure antioxidant capacity using the DPPH method. In this study, the R^2 for ascorbic acid was 0.9988 and for marigold leaf extract was 0.9687, so from the linear regression equation, the results from the IC_{50} standard of ascorbic acid were 5.4 μg/mL. and for marigold leaf extract was 145,799 μg/mL. Based on the classification, IC_{50} 250-500 μg/mL is classified as weakly active, 101 – 250 μg/mL is moderately active, 50 – 101 μg/mL is strong active and for IC50 less than 50 (<50 μg/mL) is very active. For marigold leaf extract, it was found that 145,799 μg/mL can be classified to the moderately active group, when compared with ascorbic acid, it can be said that marigold leaf extract lost, but we will look back, ascorbic acid only contains antioxidants and if you consume excess ascorbic acid or vitamin C it can cause stomach irritation. and when compared, marigold leaf extract has other advantages besides only containing antioxidants, it can be used as antimalarial, anti-inflammatory, antiviral, antibacterial and can also be used as a cancer treatment.

Based on research conducted by Yulia et al [23] on marigold flower extract, it was found for antioxidant levels to have an IC_{50} of 181.09 μg/mL and IC_{50} for ascorbic acid is 30.30 μg/mL, But here it can be seen that the IC_{50} obtained by Yulia et al is slightly different in number from that obtained in the current study, this can be due to the difference in the location of the plant growth used which is also related to the temperature at which Yulia uses marigolds taken from Bukittinggi with an average temperature The average is 25°C while in this study the plants were taken in the Jakarta area which has an average temperature of 32°C. In the research conducted by Yulia also using a different solvent where Yulia used N-hexane while in this research used methanol, this is also can be one of the factor that can affect the IC_{50} levels that can be obtained. However, although the results obtained are slightly different from those obtained, the results still show that the IC_{50} content of ascorbic acid is higher than that of marigold plants.

But based on research conducted by Kaur et al [24] on marigold plant extracts which are also in accordance with my research which can be used as antiviral, antibacterial, anti-inflammatory and can be used as cancer treatment.

5. CONCLUSION

Based on the discussion and results of the research entitled "Phytochemical Screening, Antioxidant Capacity Test and Toxicity Test of Marigold Leaf Extract, "*Tagetes erecta* L.", the following conclusions can be drawn:

1. There are alkaloids, anthocyanins, betacyanin, cardio glycoside, coumarins, flavonoids, glycosides, phenolics, saponins, steroids, terpenoids, and tannins in marigold leaf extract.
2. The total antioxidant capacity of marigold leaf extract using the DPPH method obtained IC_{50} of 145,799 g/mL, including the category of moderate total antioxidant capacity.

AUTHORS' CONTRIBUTIONS

The author contributed equally to all aspects of the article.

CONFLICT OF INTEREST

The author declare that there is no conflict of interest.

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