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ANTIOXIDANT CAPACITY TEST, TOTAL PHENOLIC, TOTAL ALKALOID, AND TOXICITY OF MARIGOLD FLOWER (TAGETES ERECTA L.)

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KEYWORDS
alkaloids; BSLT; DPPH; Phenolic; Tagetes erecta L.

ABSTRACT
Antioxidants are a set of compounds that can fight the effects of reactive oxygen species (ROS) so as to eliminate their effects in the form of damage to macromolecules of carbohydrates, fats, proteins, and DNA chains. One source of antioxidants that are easily available and widely available is through herbal ingredients such as marigold flowers (Tagetes erecta L.). Marigold flowers are well known and used as a source of antioxidants, anti-inflammatory, antibacterial, diuretic and accelerate wound healing. This flower is often consumed as an edible flower and is also drunk in the form of tea. This study aims to look for the antioxidant capacity, total phenolic, total alkaloids, and toxicity of marigold flowers. Marigold flower extract is obtained by evaporating the results of methanol maceration from dried marigold flowers. Test antioxidant capacity with DPPH (2,2-diphenyl-1-picrilhydrazyl) using Blois method, total phenolic using Singleton and Rossi method, total alkaloid by method by Trivedi et al, and BSLT toxicity by Meyer method. The antioxidant capacity of marigold flowers is measured in IC50 as much as 74.5 µg/mL which includes active antioxidant levels. The total phenolic content of marigold flowers was obtained as much as 10,350.68 µg/mL. Total alkaloid levels were obtained as much as 13.05 µg/mL. The BSLT toxicity test in LC50 is 162.82 µg/mL which is categorized as moderate toxicity. From the results of this study, marigold flowers can be used as a source of antioxidants and have antimitotic properties.

INTRODUCTION

Marigold flowers, which are usually ornamental plants, have also been sold in dried form to brew and drink as tea. Marigold flowers are already known to contain carotenoids such as lutein, neoxantin, zeaxantin, β-carotene, etc. In addition the main compounds in marigold flowers are quercetagetin and syringic acid (Sing, Gupta, & Kannojia, 2020). These two substances belong to the group of flavonoids and phenolics which are powerful antioxidants. There are also antibacterial, antifungal, anti-inflammatory, antiendotoxic, hepatoprotective and cytotoxic effects. (Priyanka, Shalini, & Navneet, 2013) (Vallisuta et al., 2014) Click or tap here to enter text. The antibacterial properties of marigold flowers have been found due to the content of alkaloids (Motamedi, Seyyednejad, Bakhtiari, & Vafaei, 2015)

Secondary metabolite compounds such as phenolics and alkaloids are part of herbal plants that can be utilized. Marigold flowers themselves have the highest phenolic levels compared to other edible flowers (Socha, Kałwik, & Juszczak, 2021). A wide variety of phenolic compounds have been used as anti-inflammatory agents and recent discoveries have found the presence of beneficial effects in the prevention and treatment of type 2

diabetes (Aravind & Dhanavel, n.d.) (Dias et al., 2022). In medicine, alkaloids are known as a source of analgesic, anti-inflammatory and cardioprotective drugs such as morphine, berberine and atropine (Heinrich, Mah, & Amirkia, 2021). Apart from being an antioxidant, there are also cytotoxic properties and antimitotic effects of marigold flowers. This effect is important in inducing apoptosis so that cell death occurs and prevents the presence of unwanted cell division. Reactive oxygen species or ROS is one of the compounds that plays a role in the use of antioxidants and the mitosis process.

ROS is all reactive compounds that contain oxygen. Oxygen in our bodies has many essential functions such as its role as the last electron receiver on the respiratory chain to make energy or ATP in the mitochondria. This process can also occasionally make much more reactive byproducts such as oxygen radicals, hydrogen peroxide, and hydroxyl radicals (Zhao, Jiang, Zhang, & Yu, 2019). This increase in endogenous ROS can be triggered due to external effects such as stress, UV radiation, strenuous exercise, poor diet, and malnutrition (Li, Jia, & Trush, 2016). ROS has physiological functions needed by the body, namely in cell signaling, apoptosis, vasodilation, inflammation and also immunity. Under normal circumstances, ROS is in small amounts but there can be an increase that will result in disturbances in homeostasis (Jakubczyk et al., 2020). This imbalance in homeostasis is referred to as oxidative stress.

Oxidative stress is a condition where there is an imbalance between proxidan compounds such as ROS and antioxidants. This state has many consequences in the systems of our body as in the cardiovascular system can occur atherosclerosis, in the nervous system can appear dementia and Alzheimer's, in the DNA of cells there can be damage resulting in mutations to cancer. (Pizzino et al., 2017)

Antioxidants can counteract ROS in a way called free radical scavenging where antioxidants have the ability to contribute or reproduce electrons. The resulting product can be inactive ingredients or less reactive ingredients than before (Shastri, Srivastava, Jyoti, & Gupta, 2016). Antioxidants can be divided into several groups based on their origin, namely endogenous and exogenous. Endogenous antioxidants originate in the body such as catalase, glutathione peroxidase and superanion dismutase while exogenous antioxidants must be obtained such as vitamin C, vitamin E, selenium and zinc derived from food (Mohamed, 2015)

With the results of previous studies, this encourages the author to conduct various subsequent tests. This study aims to determine the total antioxidant capacity, total phenolic levels, total alkaloid levels, and toxicity of marigold flowers

RESEARCH METHODS

This research was conducted at the Laboratory of Biochemistry & Molecular Biology, Faculty of Medicine, Tarumagarara University, West Jakarta. All data is processed using the GraphPad Prism 9.0 application.

Extract Creation

A total of 515 grams of fresh marigold flowers are dried in a place with room temperature and dark. 52 grams of dried simplisia of marigold flowers were obtained, which were then macerated using methanol. The maceration process is carried out for 24 hours, after which methanol is accommodated and poured new methanol to be re-macerated. This process is carried out three times. The methanol solvent from maceration was evaporated using a rotary evaporator so that 19.77 grams of marigold flower extract was obtained.

Antioxidant Capacity Test

Test this antioxidant capacity using the Blois method with DPPH. A total of 9.86 grams of DPPH powder was dissolved in 500 ml of aquades to obtain a concentration of 50 µM. To find the optimal gelomang length and absorbance control, 3.5 mL of DPPH solution and 0.5 mL of methanol were taken and mixed on one tube. The tubes are incubated and read with a spectrophotometer at wavelengths of 400-800 after 30 minutes.

Vitamin C was used as a comparison standard in this study. Vitamin C solution is prepared with a concentration of 2 µg/mL, 4 µg/mL, 6 µg/mL, 8 µg/mL and 10 µg/mL. From each concentration was taken as much as 0.5 mL and 3.5 mL of DPPH solution was added. The mixed solution is also left incubated for 30 minutes and fed to a UV-Vis spectrophotometer for optimal wavelength to be determined.

Marigold flower extract was weighed as much as 10 mg and dissolved in 10 mL of methanol to obtain a 1mg/mL solution consetration. The stock solution was dissolved again to obtain a concentration of 25 µg/mL, 50 µg/mL, 75 µg/mL, 100 µg/mL, 125 µg/mL. On each tube is added 0.5 mL of each consetration and 3.5 mL of DPPH solution. The tube was left in a dark room for 30 minutes and checked for absorbance using a spectrophotometer with the optimal wavelength of the DPPH solution. This test was carried out twice. All results are recorded for processing.

The percentage of inhibition is calculated by dividing the value of the control absorbance that has been reduced by the absorbance of the sample by the absorbance of the control. The percentage of inhibition is used as the Y-axis and the concentration as the X-axis on the graph of the linear line equation. To obtain the IC value of 50, enter the number 50 on the Y axis and look for the value of X. This value represents the concentration of marigold flower extract needed to inhibit or neutralize 50% of the free radicals from DPPH.

Total Phenolic Level Test

The method by Singleton and Rossi was used in this test. Tannins in powder form are weighed as much as 0.25 grams for use as a standardpem banding. The weighed tannins were dissolved with 5 mL of 95% ethanol. A total of 50 mL of distilled water was added so that a concentration of 5 mg / mL was obtained. The stock solution was re-dissolved to obtain concentrated solutions of 300 µg/mL, 400 µg/mL, 500 µg/mL, 600 µg/mL, 700 µg/mL. From each concentration, 0.2 mL of solution is taken and added with 15.8 mL of distilled water. A total of 1 mL of Folin-Ciocalteu reagent solution is added to each tube. After homogenization, the tubes are left stationary in a dark room for 8 minutes. A 20% solution of sodium bicarbonate is added as much as 3 mL. The contents of the tube are re-homogenized and left at rest for 2 hours. The absorbance of the solution is read with a spectrophotometer at a wavelength of 765 nm.

A solution of a mixture of methanol and distilled water is prepared in a ratio of 1:1. A total of 10 mL of the solution is used to dissolve 0.3 grams of marigold flower extract. The procedure performed is the same as the procedure for making a standard solution of tannin comparison. This test was carried out twice. The absorbance results on the spectrophotometer are recorded for later processing.

Total Alkaloid Level Test

This test uses a method by Trivedi et al. The berberine chloride stock solution was prepared using 1 gram of its powder dissolved with 10 mL of methanol as the comparison standard. The stock solution was diluted to obtain a concentration of 20 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL, 100 µg/mL. Phosphate pH 4.7 and bromocrescol green (BCG) were added at 5 mL each. The pre-mixed solution is transferred on the separator flask, covered

and homogenized. After homogeneous, the solution is left at rest until it separates into two layers. The undercoat is accommodated on a measuring flask and chloroform is added until its volume reaches 10 mL. The absorbance of each solution is measured with a spectrofometer.

For the marigold flower extract test solution, 100 mg of extract dissolved in 3 mL HCl was used. Testing of alkaloid extract levels was carried out duplo to obtain an average value. All results are recorded for processing.

BSLT Toxicity Test

Toxicity testing using the Meyer method. To obtain the larvae of Artemia salina shrimp, 5 grams of eggs are weighed and added to 500 mL of seawater. The tube is left under the lamp with an aerator for 48 hours until the shrimp larvae can be seen moving.

After that a solution of marigold flower extract is made by dissolving 20 mg of extract with 10 mL of seawater to obtain a stock solution. The stock solution is dissolved until it reaches a concentration of 100 µg/mL, 150 µg/mL, 200 µg/mL, 250 µg/mL with a volume of 1 mL. At each concentration are added 10 larvae of Artemia salina shrimp. Each tube is supplemented with seawater to reach a volume of 2 mL. This test is carried out duplo. All tubes are left stationary under the lamp for 24 hours. After 24 hours, the number of shrimp larvae alive and dead is calculated.

The mortality percentage of shrimp larvae is calculated by dividing the number of dead larvae by the number of larvae on each tube. This mortality percentage value is used as the Y-axis and the concentration of the solution on the X-axis to graph the linear line equation. The LCvalue of 50 can be calculated by entering the number 50 on the Y axis and calculating the value of the X axis. This LC value of 50 means that the required concentration of marigold flower extract kills 50% of the population of Artemia salina shrimp larvae.

RESULTS AND DISCUSSION

Preliminary testing is carried out to determine the phytochemical content of marigold flowers. From the qualitative phytochemical test, it was found that there were alkaloid compounds, betasianin, phenolic, flavonoids, glycosides, cardioglycosides, quinones, coumarins, saponins, steroids, sterols, tannins, and terpenoids. Anthocyanin compounds were found to be negative in phytochemical tests.

Antioxidant Capacity Test

Didapatkan optimal wavelength at 516 nm and control absorbance at 0.838. With vitamin C as the comparison standard, a linear line equation with the formula is obtained

Table 1
Concentration, Percentage of Inhibition, and IC_{50} Vitamin C

Konsentrasi Vitamin C (µg/mL)	Persentase Inhibisi (%)	IC_{50} (µg/mL)
2	26,85	5,40
4	39,11	
6	54,97	
8	67,87	
10	81,81	

Y= 6,934X + 12,52 under R^2= 0,9988. IC value$_{50}$ the calculated vitamin C is 5.40 µg/mL (Table 1).

The results of the antioxidant capacity test of marigold flower extract obtained the linear line equalization formula Y = 0.7112X – 6.539 and R^2 = 0.9986. The IC value of 50 marigold flower extracts is 74.50 µg/mL (Table 2).

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

Konsentrasi (µg/mL)	Persentase Inhibisi (%)	IC_{50} (µg/mL)
25	11,34	
50	28,28	
75	48,33	74,50
100	63,37	
125	82,70	

In one study, an IC_{value} of 50 was obtained of 71.6 µg / mL. While in other studies that also used the same method IC(Saani et al., 2018)$_{50}$ 113.32 7.89 µg / mL. The existence of variations from the results of previous studies can be due to environmental factors for the growth of marigold flowers used. IC±(Youssef et al., 2020)$_{50}$ obtained in this study can be categorized as active antioxidants(Martiningsih et al., 2016).

Total Phenolic Level Test

Tannin solution tested as Standards for obtaining curves by the formula of the linear line equation Y= 0.00073X – 0.1568 with a result of R^2= 0,9727. The absorbance of the tannin solution was obtained using a spectrophotometer at a length of 765 nm (Table 3). A standard curve is created with the concentration of tannins as the X axis and the absorbance result as the Y axis.

Table 3
Tannin Concentration and Absorbance

Konsentrasi Tanin (µg/mL)	Absorbansi
300	0,084
400	0,122
500	0,189
600	0,272
700	0,374

Phenolic levels of marigold flower extract were calculated using a standard curve of tannin solution. 516.16 µg/mL and 518.90 µg/mL were obtained for phenolic levels. These two values were averaged to obtain 517.53 µg/mL (Table 4). Because in the test of 20x dilution, the total phenolic content of marigold flower extract was 10,350.68 µg / mL.

Table 4
Absorbance Values and Phenolic Levels of Marigold Flower Extract

Tabung	Absorbansi	Kadar Fenolik (µg/mL)	Rerata Kadar Fenolik (µg/mL)
A	0,220	516,16	517,53
B	0,222	518,90	

Previous studies using marigold flowers and galat acid as comparison standards obtained total phenolic levels of 57.52 1.42 mgGAE/g ±dry weight (Youssef et al., 2020). Another study found 49.76 mgGAE/g on a phenolic level test (Siddhu & Saxena, 2017).

Total Alkaloid Level Test

This test uses *berberine chloride* as a comparison standard. The absorbance of the solution is measured at a wavelength of 420 nm (Table 5). The standard curve obtained

Table 5
Concentration and Absorbance of *Berberine Chloride*

Konsentrasi *Berberine Chloride* (µg/mL)	Absorbansi
2	0,129
4	0,222
6	0,428
8	0,645
10	0,828

has a formula Y= 0.09105x – 0.0959 with an R value^2= 0,9857.

The absorbance of marigold flower extract solution is 0.502 and 0.495. This absorbance value can be fed into the standard curve *of berberine chloride* to obtain total alkaloid levels. The mean result of alkaloid levels was 6.53 µg/mL (Table 6). Due to the dilution of two times in the test, the total alkaloid level obtained was 13.05 µg/mL.

Table 6
Absorbance Values and Alkaloid Levels of Marigold Flower Extract

Tabung	Absorbansi	Kadar Alkaloid (µg/mL)	Rerata Kadar Alkaloid (µg/mL)
A	0,502	6,57	6,53
B	0,495	6,49	

In research on plants of the *Estericeae* family with the genus *Senecio*, the (Hartmann & Zimmer, 1986) total alkaloid content of the flower was found to be 1.0-1.2 mg / g fresh weight. In addition, marigold flowers have also found the presence of *jafrine*

and *6-ethoxy-2,4 dimethylquinoline* which are alkaloid compounds (XU, Juan, QI, & SHI, 2012)

BSLT Toxicity Test

The test result curve was made with the concentration of marigold flower extract on the X axis and the mortality percentage on the Y axis. The formula of the BSLT toxicity linear line equation is Y= 205X – 403.4 with R^2= 0.9700. From the formula, the calculated $LC_{value\ of\ 50}$ is 162.82 µg/mL (Table 7).

Table 7
Concentrations, Concentration Logs, Mortality Percentages and LC_{50} Marigold Flower Extracts

Konsentrasi (µg/mL)	Log Konsentrasi	Persentase Mortalitas (%)	LC_{50} (µg/mL)
100	2,00	11,36	162,82
150	2,18	35,14	
200	2,30	66,67	
250	2,40	93,18	

The $LC_{value\ of\ 50}$ obtained is 162.82 µg / mL which means that there are cytotoxic and antimitotic effects on marigold flower extract. In a study with marigold flowers, an LC(Meyer et al., 1982)$_{50}$ value of 23.29 ppm was obtained. (Mastura et al., 2021) This difference in results can be due to differences in the solvent used, namely ethanol

CONCLUSION

Marigold flowers contain an antioxidant capacity in IC 50 of 74.50 µg / mL. This antioxidant content can be categorized as active. The total phenolic content was 10,350.68 µg/mL. The total alkaloid level obtained was 13.05 µg/mL. In the BSLT toxicity test, LC50 was obtained with a value of 162.82 µg / mL which means that marigold flowers have stitotoxic and antimitotic effects. The LC value of 50 can be categorized as medium. It can be concluded that marigold flowers have the potential to be used as a source of antioxidants and antimitotics.

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