Chapter

The Potential of Lutein Extract of *Tagetes erecta* L. Flower as an Antioxidant and Enhancing Phagocytic Activity of Macrophage Cells

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Abstract

Marigold flower (*Tagetes erecta* L.) produces lutein compounds which present biological activities such as antioxidant, antiinflammatory, antimutagenicity, and immunomodulatory effects. The study was to investigate the antioxidant activity of the lutein of *T. erecta* L. and the effect of lutein on the activity and phagocytic capacity of macrophage cells. The antioxidant screening was carried out using diphenyl picrylhydrazyl (DPPH), 2,2′-and-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical scavenging assay with serial concentrations and ferric-reducing antioxidant power (FRAP) method. For the observation of activity and phagocytic capacity of peritoneal macrophages, twenty-eight mice were used and divided into seven groups each comprising four replicates, i.e., Group (I) normal controls, mice were untreated (II) a negative control, mice were induced by *Staphylococcus aureus* (III) positive control, mice were induced by *S. aureus* and treatment of meniran extract (*Phyllanthus niruri*). The treatment group (IV-VII) mice were induced by *S. aureus* and treated crude lutein, respectively: 0.15 mg, 0.30 mg, 0.60 mg, and 0.90 mg. 20 g^{-1} of body weight. The lutein extracted from *T. erecta* shows an antioxidant activity against DPPH radical with an IC50 value of 53.58 $\mu g.ml^{-1}$, while the antioxidant activity against ABTS has an IC50 value of 72.91 $\mu g.ml^{-1}$. The antioxidant activity test results by the FRAP method at each lutein concentrations of 10, 25, 50, and 75 ppm were obtained respectively of 33, 88, 185.5, and 288.5 μmol Fe^{2+}/g extract. The data were analyzed using one-way ANOVA and Duncan's multiple range test (DMRT) after. The phagocytic activity was 45.5%; 54.75%; 57.50% and 67.0%, respectively, while the phagocytic capacity values were 355; 519; 611 and 767 *S. aureus* bacterial cells per 50 macrophage cells. The lutein from marigolds (*T. erecta* L.) is capable of scavenging free radicals and reducing oxidants. Lutein can increase the activity and capacity of phagocytic of peritoneum macrophage cells in mice.

Keywords: *Tagetes erecta* L., lutein, antioxidant, peritoneal macrophages, phagocytic activity

1. Introduction

Marigold flower (*Tagetes erecta* L.) is an annual herbaceous plant commercialized worldwide as an ornamental plant and a natural source of pigment from its yellow/orange flowers. The flower is rich in carotenoids, the extract of which is used as a colorant in a wide variety of food products, including cake mixture, drinks, and ordinary drinks, cereals, chewing gum, dairy analogs, egg products, fats and oils, dairy products, processed fruit and fruit juices, and soups [1, 2]. Marigold flowers are classified as medicinal plants from the Compositae tribe. People empirically use it to treat asthma, bronchitis, fever, ulcers, burns, and swelling [3]. The pigment content in marigold flowers are classified as carotenoids, namely lutein pigments that are yellow to orange [4]. *T. erecta* L. is a major source of lutein for commercial use. In 2010, lutein occupied a $ 233 million share of the world carotenoid market [1, 2, 5]. Lutein is a primary pigment because this pigment is not produced synthetically. It is due to its production, which requires a long process. Lutein from *T. erecta* L. is a pure extract obtained from marigold oleoresin, extracted from the petals of marigold flowers with an organic solvent. After the saponification process, the final product contains the main component lutein and a fraction of zeaxanthin. Lutein (3R, 3'R, 6'R-βε-carotene-3,3′-diol) is a member of the pigment group known as xanthophylls and lacks provitamin A activity. Lutein generally coexists in nature with its stereoisomer zeaxanthin and the double bonds of the isoprene backbone can exist in the all-trans (**Figure 1**). Pure lutein typically appears as a yellow-orange crystalline, lipophilic, solid with the chemical name β,ε-carotene-3,3′-diol (C40H56O2) [3].

Lutein pigments are found in egg yolks, fruits, and vegetables, including tomatoes, carrots, pumpkin, corn, and various green plants [6]. Some research results reported that lutein is efficacious to protect the eyes from macular degeneration and epithelial cancer, and has antioxidant properties [7], and can increase the immune system in the body [8]. Unfortunately, lutein cannot be synthesized in the body; so, it depends on plants [9]. In plants, lutein can be in the form of free lutein such as in spinach, cabbage, and broccoli, or in the form of esters with the fatty acids in the following fruits and vegetables: mango, orange, papaya, red or green peppers, yellow corn etc. [10–12]. Lutein content in natural sources depends on the type, variety, level of maturity, part of the fruit, and also on processing processes such as heating, preservation, or storage [12]. Dried marigold flowers contain 0.1%–0.2% carotenoids with a composition of 80% as lutein ester [4].

Lutein is an antioxidant because it can reduce free radicals and reactive molecules that can inhibit cell damage [13]. Free radicals can be produced from the body's metabolism and also from outside the body, such as the smoke of a cigarette, environmental pollution, radiation, drugs, pesticides, and ultraviolet rays [14]. Free radicals can cause damage because they can react with cell components that are important for maintaining cell life, both structural components (for example, the molecules making up membranes) or functional components (for example, DNA enzymes) [15]. Antioxidants are chemical compounds that can neutralize free

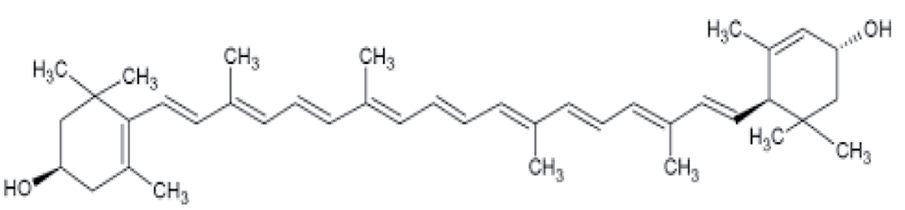

Figure 1.
Structural of all-trans lutein.

radicals by donating one or more electrons to free radicals; so, these free radicals can be suppressed [16]. Lutein is very effective as an antioxidant to protect the eyes because it is able to neutralize free radicals formed by the action of ultraviolet radiation on the retina of the eye and reduce the risk of cataracts due to aging [17]. Humans are unable to synthesize lutein, so they can obtain it by consuming fruits, vegetables, and dietary supplements [12]. Testing the antioxidant potential of lutein compounds from marigold flowers (*T. erecta* L.) can be carried out using DPPH, ABTS, and FRAP methods.

The lutein pigment in marigold flowers (*T. erecta* L.) can act as an immunomodulator, especially to protect the eyes from pathogenic elements such as viruses, bacteria, and infectious diseases intra-ocular inflammation [18]. Humans have resistance to a disease/infection from microorganisms or foreign substances, which is called the immune system. The body's immune system's decreased function can be caused by stress, an unhealthy lifestyle, aging, and chronic disease. The human body's immune system consists of specific and non-specific immune systems. One of the defenses made by the body's non-specific immune system in preventing the entry of antigens is by doing phagocytosis. Phagocytosis is the process of ingesting, digesting, and destroying antigens/microbes. The cells that play their role in carrying out phagocytosis are macrophage cells [19].

Previous research conducted *in vivo* showed that lutein derived from plants with a yellow pigment had an immunomodulatory activity increasing the regeneration of the immune system [8]. Immunomodulators are substances that can restore and repair the immune system, which function is disturbed or to suppress its excessive function [19]. In previous studies, researcher found that lutein activity was able to stimulate an immune response. In addition, it was found that 10 mg of lutein consumed daily by cats for 12 weeks led to an increase in the percentage of CD4+ and CD21+ lymphocytes, plasma IgG concentrations, and NK cell activation. It showed that lutein could stimulate mediator cells and humoral immunity in cats, which was done *in vivo*; so, stimulating the activity and capacity of macrophage cells in the body as an immune response [8]. Testing of immunomodulatory activity was carried out using observing the activity and capacity of macrophage cells from test animals (mice) induced by *S. aureus* bacteria [20].

This research has two objectives. First, this study examined the potential of lutein compounds from marigold flowers (*T. erecta* L.) as antioxidants using the DPPH, ABTS, and FRAP methods. Second, this study examined the possibility of lutein extract as an immunomodulator through a non-specific immune system, namely the activity and capacity of phagocytosis of mice's peritoneal macrophage cells (*in vivo*) induced by *S. aureus*.

2. Experiment

2.1 Material collection

Marigold flower (*T. erecta* L.) belongs to the Asteraceae family obtained from Taman Bunga Nusantara, Cipanas - West Java. Plant determination was carried out at Herbarium Bogorienses, Research Center for Biology, Indonesian Institute of Sciences (LIPI).

2.2 Lutein extract from marigold flowers (*T. erecta* L.)

Dry powder of Marigold flower crown was weighed as much as 20 g into Erlenmeyer to extract of lutein [21, 22]. The weighing was carried out six times.

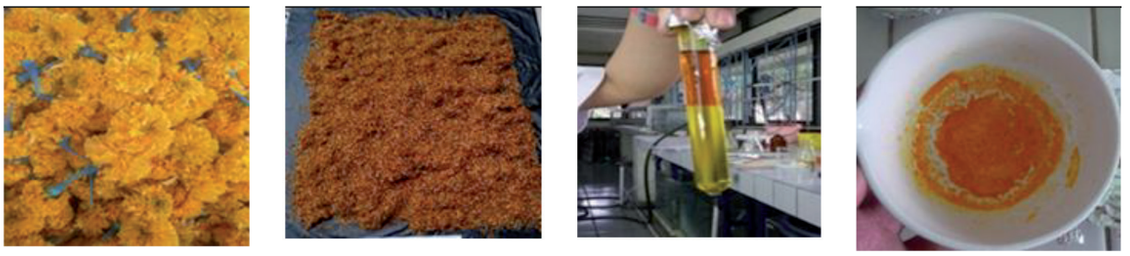

Figure 2.
Extraction of lutein from the crown of marigold flowers (T. erecta L.).

Each Erlenmeyer was added with 300 ml of n-hexane (Merck) and was shaken out for 24 h. The extracted liquid was filtered with filter paper to separate the filtrate and residue. The residue was remastered by adding 200 ml of n-hexane and was shaken out for 24 h, then was filtered, and the filtrate was combined.

The filtrate was concentrated with a rotary evaporator. The extract was obtained, and it was dried at 40°C until hexane extract was obtained. The n-hexane extract was digested by adding isopropanol solution, then was stirred for one hour at 50°C. At the same temperature, it was saponified with 50% NaOH solution, stirred homogeneously for one hour until two layers were formed, i.e., semisolid and liquid. Next semisolid and liquid were cooled, the semisolid part is separated from the liquid. The semisolid solution was digested with distilled water repeatedly until the other carotenoids were completely separated. The solution was centrifuged for ten minutes at a speed of 3000 rpm; yellow deposits were formed, then the filtrate was removed. The precipitate was dried in a water bath at 40°C; so, the lutein extract was obtained (**Figure 2**).

2.3 Antioxidant activity of lutein extract from *T. erecta* L.

2.3.1 DPPH free radical-scavenging activity

The ability of Lutein extract of *T. erecta* L. to scavenge the DPPH radical was estimated using the method described by Kushwaha and Verma [23]. An aliquot of 50 μL of various sample concentrations was added to a volume of 2 mL from the DPPH methanolic solution (60 μM). The reaction mixture was well shaken and incubated for 20 min at room temperature in the dark and the absorbance was recorded at 517 nm. The blank was constituted by methanol instead of the extract. The percentage inhibition of the DPPH radical by the samples was calculated using the following equation:

$$\%\, inhibition = \frac{Ao - As}{Ao} x100. \tag{1}$$

where A_0 is the absorbance of blank and As is the absorbance of the lutein sample. The sample concentration providing 50% of inhibition (IC50) was determined from the plotted curve of inhibition using several concentrations. Vitamin E were used as a comparative antioxidant compound.

2.3.2 ABTS [2,2′-azinobis (3-ethylbenzothiazoline-6-sulphonic acid)] free radical scavenging activity assay

The ABTS● + stock solution was prepared by reacting ABTS (Sigma Aldrich aqueous solution 7 mM) with 2.45 mM aqueous solution of potassium persulfate (Merck) in equal quantities; the mixture was allowed to stand in the dark at room temperature for 12–16 hours before use [24–25]. The working solution of

ABTS● + was obtained by diluting the stock solution in methanol to give an absorbance of 0.70 ± 0.02 at 734 nm. Then, 1.0 mL of ABTS● + solution was mixed with 0.5 mL of the Lutein extracts at different concentrations (25–100 ppm). The mixture was then incubated at room temperature for exactly 10 min in the dark. The control was prepared by mixing 1.0 mL of ABTS● + solution with 0.5 mL of distilled water. The absorbance was measured against a blank at 734 nm using spectrophotometer (Shimadzu UV-160). The percentage of scavenging activity of each extract on ABTS● + was calculated as % inhibition (I%) using the following equation:

$$\%\ Inhibition = \left[\frac{(Ao - As)}{Ao}\right] x\ 100 \quad (2)$$

Where Ao is the absorption of blank and As is the absorption of the lutein extract solution.

2.3.3 Ferric ion reducing antioxidant power (FRAP) assay

The ferric reducing capacity of extracts was investigated by using the potassium ferricyanide-ferric chloride method [26]. Briefly, Reagents for FRAP assay a) Acetate buffer 300 mM pH 3.6: b) TPTZ (2, 4, 6-tripyridyl-s- triazine): (M.W. 312.34), 10 mM in 40 mM HCl (M.W. 36.46). c) FeCl3. 6 H2O: (M.W. 270.30), 20 mM. The working FRAP reagent was prepared by mixing a, b and c in the ratio of 10:1:1 just before testing. All the reagents were prepared from Merck (Germany) company. FRAP solution (3.6 mL) add to distilled water (0.4 mL) and incubated at 37° C for 5 min. Then this solution was mixed with certain concentration of the plant extract (80 mL) and incubated at 37° C for 10 min. The absorbance of the reaction mixture was measured at 593 nm. For construction of the calibration curve, five concentrations of FeSO4, 7H2O (25, 50, 75, 100, 150, 200, 300 μmol) were used and the absorbance values were measured as for sample solutions. The sample concentration providing 0.5 of absorbance (IC50) was calculated by plotting absorbance against the corresponding sample concentration [27, 28]. Vitamin E were used as a comparative antioxidant compound.

2.4 Measurement of *in vivo* phagocytosis activities and capacity of macrophage

Activity and capacity were observed under a 10x100 magnificent light microscope [29]. Animal experimental was performed in the Animal laboratory of the Faculty of Veterinary Medicine, IPB University- Dramaga Bogor. The experimental animal used was male mice (*Mus musculus*) strain DDY. A total of 28 mice were used in 6–8 weeks old, with 18–21 g of body weight. The mice were acclimated for seven days ahead. The mice were randomly divided into seven experimental groups of four mice in each group: (I) Normal control (mice were given vegetable oil), (II) Negative control (mice were given vegetable oil + *S aureus*, (III) Positive control (mice were given immunomodulator stimulator 0.078 mg + *S. aureus*), (IV) Lutein extract dose 0.15 mg/20 g BW + *S. aureus*, (V) Lutein extract dose of 0.30 mg/20 g BW + *S. aureus*, (VI) Lutein extract dose of 0.60 mg/20 g BW + *S. aureus*, (VII) Lutein extract dose 0.90 mg/20 g BW + *S. aureus*. Lutein extract was administered orally for 14 days. On the 15th day, all experimental animals were injected intraperitoneally with 0.5 ml of *S. aureus* bacterial suspension (1 x 10^6 CFU/ml), except the mice in normal control group. Those experimental animals were euthanatized one hour after infection, and then their peritoneal liquid was collected. Swab preparation was made for all samples, and then fixated using methanol for 5 minutes, stained with Giemsa and left for 20 minutes, and then washed using distilled water.

2.4.1 Phagocytosis activity

Phagocytosis activity value is the number of macrophage cells that actively phagocyte *S. aureus* in 100 macrophage cells. Phagocytosis activity was presented in percent, with the formulation [30] as the following:

$$\%\ Activity = \frac{The\ number\ of\ active\ macrophage}{The\ number\ of\ whole\ macrophage}\ x\ 100 \quad (3)$$

2.4.2 Phagocytosis capacity

Phagocytosis capacity value is the number of bacteria ingested by 50 active macrophages [30]. This parameter was observed by staining with Giemsa and then the bacteria number was counted under microscope. Phagocytosis is a devouring process on bacteria or strange objects by enfolding those things using macrophage cytoplasm.

2.5 Data analysis

Determining of immunomodulatory activity of crude lutein from *T. erecta* L. was through macrophage activity and capacity induced by *Staphylococcus aureus* bacteria by *in vivo*. Data were analyzed by one way ANOVA with 4 replications using SPSS ver 22.0 with P = 0.05. This analysis was then followed by Duncan Multiple Range Test.

3. Results

3.1 Antioxidant activity of lutein extract from *T. erecta* L.

Antioxidant activity is a complex procedure usually happening through several mechanisms and is influenced by many factors, which cannot be fully described with one method. Therefore, it is essential to perform more than one type of antioxidant capacity measurement to consider the various mechanisms.

3.1.1 DPPH free radical-scavenging activity and ABTS radical cation scavenging activity assay

The potential test of lutein extract as an antioxidant using the DPPH method has some advantages, such as it is easy to conduct, and the process is relatively fast. The test method using the DPPH was based on a decrease in absorbance caused by a change in the DPPH solution's color. DPPH will react with hydrogen atoms from free radical absorbing compounds to form DPPH Hydrazine, which is more stable. DPPH reagent that reacts with antioxidants will change color from purple to yellow; the intensity of the color formed depends on the antioxidants' ability [31]. The data processing technique was carried out by comparing the concentration with the percentage value of antioxidant activity for each sample in a regression graph (**Figure 3**).

The results of the marigold flower lutein extract antioxidant test using the DPPH and ABTS methods can be seen in **Table 1**.

IC50 is a number that shows a concentration (μg/ml) which can inhibit the oxidation process by 50%. The smaller the IC50 value, the higher the antioxidant activity is. Based on the IC50 value, if it is less than 50 ppm, the antioxidant is very strong, 50 ppm–100 ppm is strong, 100 ppm–150 ppm is moderate, and 150 ppm–200 ppm is weak [31].

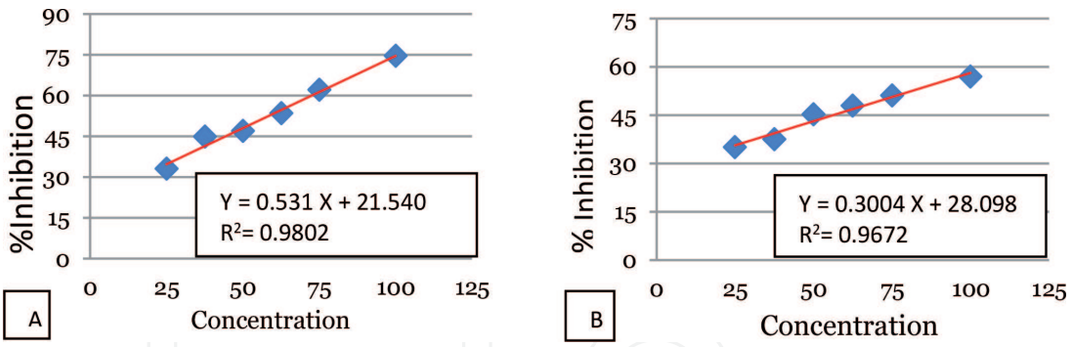

Figure 3.
Relationship between the concentration of lutein extract from Marigold flower (T. erecta L.) (ppm) with % inhibition of (A) DPPH, and (B) ABTS.

Lutein Conc. (ppm)	DPPH		ABTS	
	% Inhibition	IC 50(μg/ml)	% Inhibition	IC 50(μg/ml)
25	33.15	53.58	35.07	72.91
37.5	44.88		37.50	
50	46.96		45.28	
62.5	53.44		47.88	
75	62.07		51.10	
100	74.64		56.92	

Table 1.
Antioxidant activity values of marigold flower (T. erecta L.) using different assays.

Vit E Conc. (ppm)	DPPH		ABTS	
	% Inhibition	IC 50(μg/ml)	% Inhibition	IC 50(μg/ml)
4	34.97	9.60	34.43	12.36
6	40.47		37.81	
8	45.06		42.30	
10	51.14		44.73	
12	56.36		49.76	

Table 2.
Antioxidant activity values of vitamin E using different assays.

The results of testing for antioxidant potential with the DPPH suppression method showed that the lutein extract of flowers (*T. erecta* L.) had strong antioxidant activity with an IC50 value of 53.58 μg/ml (**Table 1**). The IC50 value of Vitamin E, i.e. 9.60, is categorized as a very strong antioxidant (**Table 2**). The antioxidant activity was measured based on the reduction in the purple DPPH intensity which was proportional to the reduction in the concentration of DPPH solution. The color change from purple to reddish purple then to yellow occurred after 30 minutes of giving lutein extract into the DPPH solution. The color change was caused by the reaction between diphenyl picryl hydrazine and the hydrogen atom released by one molecule of the lutein extract component; then diphenyl picryl hydrazine compound was [32].

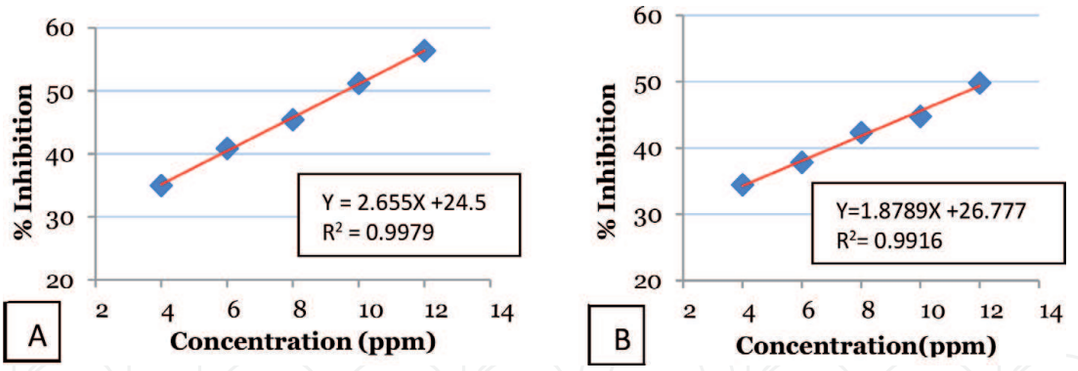

Figure 4.
Relationship between the concentration of vitamin E (ppm) with % inhibition of (A) DPPH and (B) ABTS.

Antioxidant activity testing could be done using the compound 2.2-Azinobic Acid (3-ethylbenzatiazolin-6 sulfonate). ABTS was a water-soluble and chemically-stable compound. This method was used for research on antioxidants that are water soluble, fat soluble, and dissolve in pure compounds. ABTS compounds were converted into radical cations (ABTS ●+) with the addition of potassium per sulfate. Free radical cations were blue green which could absorb light at a wavelength of 734 nm [33].

Maximum absorbance could also occur at other wavelengths. Radical cations were reactive to most antioxidants. ABTS which was blue-green during the reaction turned colorless which was analyzed using a spectrophotometer. From measurements using a UV–VIS spectrophotometer, the absorption value was obtained, and the % resistance was calculated. Furthermore, the relationship between concentration and % inhibition of marigold flower lutein extract (*T. erecta* L.) or vitamin E was plotted and the linear regression was calculated. The results can be seen in **Figures 3** and **4**. Furthermore, from the Eq. 50% activity was plotted, so that the concentration value (IC50) of the marigold flower lutein extract (*T. erecta* L.) was obtained, i.e. 72.91 μg/ml which was included in the strong antioxidant category because it had an IC50 value of around 50–100 μg/ml. The IC50 vitamin E yield of 12.36 μg/ml was in the very strong category. The use of the two DPPH and ABTS methods for testing the antioxidants of lutein and vitamin E extracts showed that there were differences in the IC50 obtained. Thus, the use of the DPPH method was more suitable and showed more sensitive results.

3.1.2 Ferric ion reducing antioxidant power (FRAP) of lutein extracts and vitamin E assay

The FRAP assay is based on the measurement of the ability of the substance to reduce Fe3+ to Fe2+ resulting in the change of color from yellow to blue colored solution of Fe2 + – TPTZ complex (Fe2+ tripyridyltriazine) which has a high absorbance at 593 nm. The FRAP assay provides a reliable method.

FRAP value was obtained by comparing the changes of absorbance values in the sample mixture with those obtained from the increased concentrations of Fe3+. FRAP values were expressed as μmol of Fe2+ equivalents per g sample. **Figure 5** shows the relationship between Fe (II) sulfate concentration and absorption at the wavelength 593 nm. **Table 3** FRAP values of lutein extract of marigold flower and Vitamin E in several concentration. The FRAP value is directly proportional to the concentration of sample. The higher the sample concentration exhibited the higher the FRAP value.

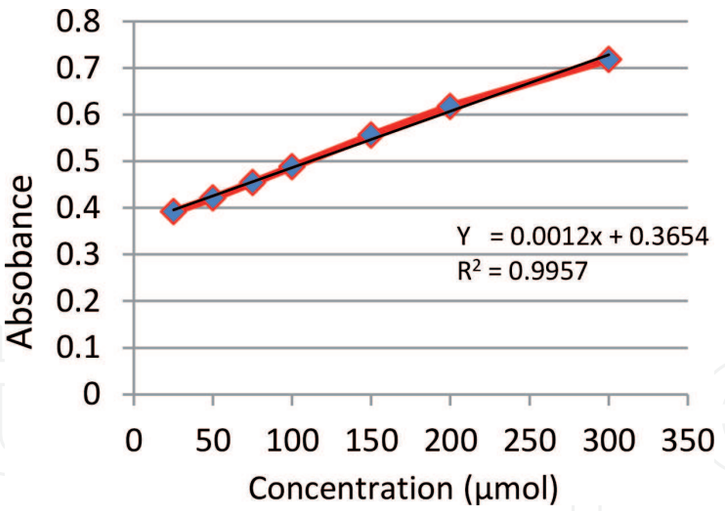

Figure 5.
Standard curve of FeSO4.7H2O.

No	Sample	Concentration of Sample (ppm)	Conc. of Fe(II) (µmol)
1	Lutein Extract of *T. erecta* L.	10	33.0
		25	88.0
		50	185.5
		75	288.5
2	Vitamin E	4	12.16
		6	18.83
		8	25.50
		10	33.83

Table 3.
Antioxidant activity of lutein extracts from marigold flower (T. erecta L.), using FRAP assay.

Generally, the reducing properties are associated with the presence of compounds, which exert their action by breaking the free radical chain through donating a hydrogen atom [34, 35].

3.2 Activity and capacity of phagocytosis of macrophage cells in mice peritoneum liquids

The results of the immunomodulatory activity test upon lutein extract of kenikir flower (*T. erecta* L.) based on the value of phagocytic activity and the phagocytic capacity of mice peritoneal fluid macrophage cells can be seen in **Figure 6** and **Table 4**.

Remarks:

I. Group normal control, mice were given vegetable oil.

II. Group negative control, mice were given vegetable oil+0.5 ml *S. aureus.*

III. Group positive control, mice were given stimuno®0.078 mg/20 g bw + 0.5 ml *S. aureus*.

IV. Group mice were given the extract lutein 0.15 mg/20 g bw/day +0.5 mL *S. aureus.*

V. Group mice were given the extract lutein 0.30 mg/20 g bw/day +0.5 mL *S. aureus.*

VI. Group mice were given the extract lutein 0.60 mg/20 g bw/day +0.5 mL *S. aureus.*

VII. Group mice were given the extract lutein 0.90 mg/20 g bw/day+0.5 mL *S. aureus.*

The results of the Duncan Multiple Range Test on the value of phagocytosis activity for each test group showed that there was no significant difference between the negative control group and the 0.15 mg/20gBW lutein extract treatment group. Treatment of lutein extract 0.30 mg/20gBW, was not significantly different from treatment of lutein extract 0.60 mg/20gBW, while lutein treatment 0.90 mg/20gBW showed a significant difference. The normal control group and the positive control group showed significantly-different phagocytic activity. The lowest phagocytosis activity was found in the normal control group because in the normal control group mice were not given intra-peritoneal *S. aureus* suspension; so, macrophages were not triggered to perform phagocytosis. Macrophages are activated by various stimuli originating from exogenous antigens and microorganisms.

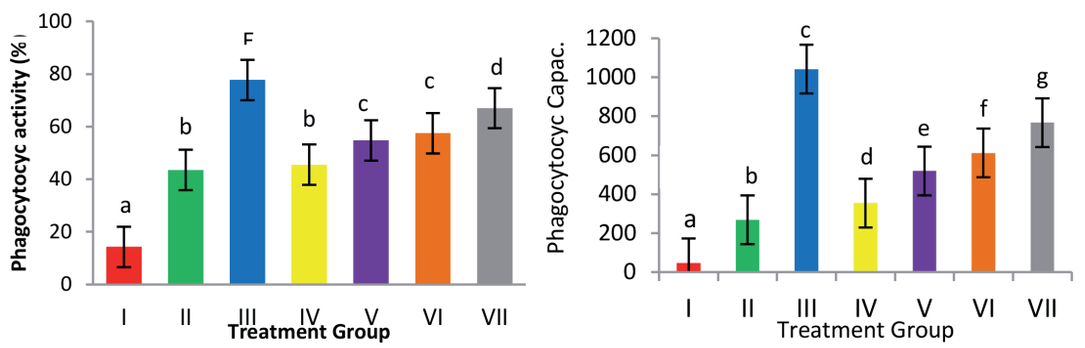

Figure 6.
Histogram of activity and capacity of phagocytosis macrophage cells in mice peritoneum liquids on each test group.

No.	Test Group	Phagocytosis Activity (%)	Phagocytosis Capacity *S. aureus* cells/ 50 macrophage cells
I	Normal Control	$14.25^{a} \pm 2.22$	$46.50^{a} \pm 6.61$
II	Negative Control	$43.50^{b} \pm 2.65$	$268.00^{b} \pm 5.09$
III	Positive Control	$77.75^{e} \pm 4.11$	$1041.50^{c} \pm 15.06$
IV	Lutein Extract 0.15 mg/20 g bw	$45.50^{b} \pm 1.29$	$354.75^{d} \pm 3.59$
V	Lutein Extract 0.30 mg/20 g bw	$54.75^{c} \pm 4.35$	$519.25^{e} \pm 5.12$
VI	Lutein Extract 0.60 mg/20 g bw	$57.50^{c} \pm 2.65$	$611.25^{f} \pm 4.57$
VII	Lutein Extract 0.90 mg/20 g bw	$67.00^{d} \pm 2.58$	$766.75^{g} \pm 6.50$
Note: The numbers followed by the same letter are not significantly different ($P < 0.05$).			

Table 4.
Phagocytosis activity of macrophage cell (per 100 cells) and phagocytosis capacity (the number of bacteria devoured per 50 active macrophage cells).

Both will be recognized, then captured, eaten, and digested (phagocytes), and finally degraded and disappeared from the body. Peritoneal macrophages are freely available in the peritoneal fluid, so that pathogens entering the body [19] are possible to be captured. The highest value of phagocytosis activity was found in the immunosuppressive positive control group and in the treatment group lutein extract 0.90 mg/20gBW. Oral administration of lutein with different doses, namely 0.15 mg, 0.30, 0.60, and 0.90 mg per 20 g BW, resulted an increase in phagocytic activity, respectively 45.50%, 54.75%, 57.50%, and 67.0%. The increase in phagocytic activity of peritoneal fluid macrophage cells of mice occurred simultaneously with the increase in lutein dose.

The results of Duncan's test on the value of phagocytosis capacity in each of the 50 active macrophage cells showed that there were significant differences between groups. The lowest macrophage cell phagocytosis capacity was found in the normal control group. It was due to the fact that in a healthy body condition, macrophages became inactive; so, they did not show any activity and capacity to carry out phagocytosis. The highest value of macrophage phagocytosis capacity was found in the normal control group. The lutein extract treatment group with the highest phagocytosis capacity value was found in the lutein treatment group 0.90 mg/20gBW. The administration of lutein extract for 14 days to experimental animals, at a dose of 0.15 mg/20gBW, 0.30 mg/20gBW, 0.60 mg/20gBW and 0.90 mg/20gBW, was able to increase the phagocytosis capacity of every 50 peritoneum macrophage cells, respectively 355; 519; 611 and 767 of *S. aureus* cells.

Phagocytosis is an important process for nutrition in unicellular organisms, while in multicellular organisms it is found in specialized cells called phagocytes. Phagocytes can ingest microbial pathogens, but importantly also apoptotic cells. Phagocytosis becomes essential not only for microbial elimination, but also for tissue homeostasis [36]. Macrophages are one of the cells that play an important role in the phagocytosis process or as antigen presenting cells (APC) [19]. The phagocytosis ability of macrophage cells is a form of the body's immune system response to overcome the incoming antigen. Bacteria *S. aureus* will stimulate macrophages to produce IL-6 which will activate NK cells and then will secrete IFN-β which will activate macrophages. Macrophages will also be stimulated to produce TNF-α which will activate other macrophages. Experiments of administering lutein extract for 14 days resulted the increased activity and capacity of mice peritoneal fluid macrophage macrophages. It showed that lutein could boost the body's immune system or could act as an immunomodulator. The lutein mechanism could increase macrophage phagocytosis activity, namely by inducing ROS (Reactive Oxygen Species), intracellular and iNOS (inducible nitric oxide synthase), activating MAPK (mitogen activated protein kinase), and RAR β (retinoic acid receptor beta) in macrophage cells that could increase the phagocytosis ability of macrophages [37].

4. Conclusions

The work confirms that lutein extract from marigold flower (*T. erecta* L.) had strong an antioxidant potential. The three protocols used i.e. DPPH, ABTS and FRAP provide a good selection method to use for antioxidant measurements. Determination of antioxidant lutein extract will use to developed new drugs in pharmaceutical fields. The results of *in vivo* experiments showed that Lutein extract was able to increase the activity and phagocytic capacity of *S. aureus*-induced rat peritoneal macrophages. Phagocytic processes in immune cells contribute in particular to homeostasis and defense against disease.

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Conflict of interest

The authors declare no conflict of interest

Animal rights

The institutional and international ethical guidelines for the Care and Use of Laboratory Animals were followed. See the experimental parts for details.

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