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# Phytochemical Test and Antioxidant Activity of Aqueous Extract of Marigold Flower

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**Abstract**. Natural medicines are increasingly in demand for safety reasons and are believed to be able to cure certain diseases. Literacy reveals that the marigold flower (*Tagetes erecta L.*) has medicinal potential as an antioxidant, anti-bacterial, anti-inflammatory, and anti-carcinogen. This research aimed to identify marigold flower aqueous extract's phytochemical content and antioxidant activity. The method used in this exploratory research was the maceration of marigold flower simplicia using distilled water for 24 hours, followed by evaporation to obtain marigold flower aqueous extract. Then, phytochemical screening and antioxidant bioactivity tests were carried out using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The results of phytochemical screening showed that marigold flower water extract contained flavonoids, phenols, alkaloids, and tannins. Meanwhile, the antioxidant activity test obtained an IC50 value of 50.77 ppm. From these results, it can be concluded that the aqueous extract of marigold flowers has medicinal potential with vigorous antioxidant activity.

***Keywords***: Aqueous extract, marigold flower, phytochemical test, IC50

## I. INTRODUCTION

Nowadays, people need alternative medicines other than chemical medicines because continuous use of chemical medicines will cause ongoing side effects. Traditional medicine is a concoction of natural ingredients such as plants, animal ingredients, minerals, and juices mixed and formulated for consumption and believed to cure certain diseases. Many natural ingredients have been developed for faster and more effective wound healing with minimal irritation, including the marigold flower (*Tagetes erecta L.*). The marigold flower has another name, namely the Gemitir flower. In Indonesia, marigold flowers have long been known, especially by the Balinese, who are familiar with them because they are often found at religious events. Marigold flowers have antioxidants and are antibacterial, anti-inflammatory, and anti-carcinogenic in the health sector.

Marigold flowers contain carotenoids, which can be antioxidants, cure mild fever, mild sore throat, natural moisturizer, and natural mosquito repellent, boils, epilepsy, scabies, infected wounds on the skin, stomach aches, eye aches, deworming, and urinary tract infections [1]. Research conducted by Rajvanshi and Dwivedi (2017) showed that the results of phytochemical screening from plants in India contained terpenoids, flavonoids, alkaloids, quinones, phenols, germinins, carbohydrates, and tannins. One function of active phytochemical compounds is that they can be used as antioxidants. Based on research by Santi (2021), the antioxidant activity of marigold flowers is moderate because marigold flowers contain several compounds that act as antioxidants, including flavonoids, phenolics, and carotenoids [2]. These three compounds have been proven to have good antioxidant activity, so marigold flowers have potential as natural medicine.

Local environmental conditions, including soil, nutrients, and stress, influence the production of secondary metabolites in plants. This allows similar plants from different locations to have other phytochemical and antioxidant contents. One way to obtain phytochemical compounds is by extraction. Extraction aims to dissolve the compounds contained in plant tissue into the solvent used for the extraction process. The appropriate type of solvent is selected to bind more active compounds so that a high yield is obtained. In this preliminary research, the reason for using marigold flowers from Sukawana Village, Bangli Regency, Bali Province, which makes it different from previous studies because we want to know the profile of active chemical compounds that appear when they are in the highlands and why water was used as a solvent because water is a universal, economical, and easily available solvent.

## II. METHODS

*Object of Research*

The object of this research was marigold flower extract (*Tagetes erecta L.*), which was obtained directly from Sukawana Village, Bangli Regency, Bali Province. Marigold flowers were determined at LIPI-UPT Plant Conservation Center "Eka Karya" Bedugul Bali Botanical Garden to determine the type of flower used for research. The variables in this research are divided into independent and dependent variables. In this study, the independent variable was the water extract of marigold flowers (*Tagetes erecta L.*). The dependent variables are alkaloids, flavonoids, saponins, tannins, phenols, triterpenoids, steroids, and IC50.

*Research Design*

The first stage begins with the preparation of marigold flowers until they become dry powder, then continues with the extraction process of marigold flowers with solvents, followed by phytochemical tests including alkaloid tests, flavonoid tests, saponin tests, tannin tests, phenolic tests, steroid tests, and triterpenoid tests as well as antioxidant tests.

*Sample Preparation*

Fresh and undamaged marigold flowers were picked and air-dried at room temperature (25°C) for 14 days. Avoid exposure to direct sunlight to avoid damage or loss of desired chemical content. Drying generally functions to reduce water content, which can inhibit the growth of bacteria and mold so that the material is more durable and more accessible to store, as well as making subsequent treatment easier. The flowers were then blended until smooth. Next, the powders were stored at room temperature in a clean, airtight container such as a jar.

*Aqueous Extraction*

The method used was cold maceration; the extraction process begins by mixing simplicia and solvent. The solvent was water with a mixture of simplicia and solvent, namely 1:3, and stirred every 12 hours for two days. Next, the soaking results were filtered, and the filtrate was evaporated using a rotary evaporator. The liquid extract was then placed into a sample flask and heated in a water bath at a temperature of 40°C. Then, the solvent vapor formed will flow into a cold condenser with the help of a vacuum pump, thereby avoiding overheating. This process will be stopped if the solvent from the extract has run out, which is indicated by the absence of dripping of solvent vapor formed in the condenser.

*Flavonoid Test*

A total of 1 mL of the test solution extract was added to enough magnesium powder and 10 drops of concentrated hydrochloric acid. The formation of a reddish-black, yellow, or orange color indicates the presence of flavonoids [3].

*Saponin Test*

A total of 2 mL of test solution in a test tube was added with 5 mL of hot distilled water, shaken 22 vertically for 10 seconds, then left for 10 minutes. The formation of foam 1-10 cm high, which is stable for no less than 10 minutes, indicates the presence of saponin. When adding one drop of 2 N HCL, the foam does not disappear [4].

*Triterpenoid Test*

A total of 2 mL of test solution was added to 1 mL of vanillin solution and 5% sulfuric acid. The formation of a red color indicates the presence of terpenoid compounds [4].

*Alkaloid Test*

A total of 5 mL of the test solution was put into a test tube, then 2 mL of chloroform and 5 mL of 10% ammonia were added, then ten drops of sulfuric acid were added to clarify the separation of the formation of 2 different phases. The top part of the phase formed was taken, and Mayer's reagent was added. The presence of alkaloids in the sample is indicated by the formation of a red precipitate [3].

*Phenol Test*

Two milliliters of the test extract solution were added with 2% FeCl3 reagent. The formation of a blue-black solution indicates the presence of phenol.

*Tannin test*

A total of 2 mL of the test extract solution was added with 10% Pb acetate reagent. The formation of a blue-black solution indicates the presence of phenol [4].

Steroid test

A steroid examination was carried out using the Liebermann-Burchard reaction. A 2 mL solution was added with 2 mL of anhydrous acetic acid. Next, 2 mL of concentrated sulfuric acid was added through the tube wall. The formation of a blue color indicates the presence of steroids [4].

*Antioxidant Activity Test*

Four milliliters of extract (concentration series 20 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm) were added with 1 ml of 0.5 mM DPPH and then incubated for 30 minutes in dark conditions. Absorption was measured at a wavelength of 517 nm. The ascorbic acid solution was used as a comparison. With the classification criteria indicator, the compound or extract's antioxidant ability is stronger if the antioxidant test using IC50 gets a lower IC50 value, as in Table 1. On the other hand, if the IC50 value is higher, the compound or extract requires a higher concentration. Its antioxidant ability may be weak to achieve an inhibition effect of 50% [5].

*Data Analysis*

The data obtained in this study were analyzed descriptively and qualitatively by describing the identification results with tables, figures, and graphs.

*Location of Research*

This research was conducted at the Veterinary Pharmacy Laboratory, Faculty of Veterinary Medicine, and the Chemistry and Microbiology Laboratory, Faculty of Food Technology, Udayana University.

TABLE I
INHIBITORY CONCENTRATION (IC50) VALUE AND ANTIOXIDANT CATEGORY

| IC50 Value (ppm) | Antioxidant Category |
|---|---|
| IC 50%>200 | Very weak |
| 150 <IC50%<200 | Weak |
| 100 <IC50%<150 | Medium |
| 50 <IC50%<100 | Strong |
| IC50% < 50 | Very strong |

## III. RESULTS AND DISCUSSION

### Results

The water extract of marigold flowers in this study yielded 26.74%. Phytochemical screening of the extract showed that the positive sample contained active compounds, namely flavonoids, phenols, alkaloids, and tannins. Detailed screening results are presented in Table 2. Meanwhile, the antioxidant activity of marigold flower water extract against DPPH radicals obtained an IC50 value of 50.77 ppm, which can be categorized as having vigorous antioxidant activity. The absorbance and inhibition values of the extract are shown in Table 3 and Figure 1.

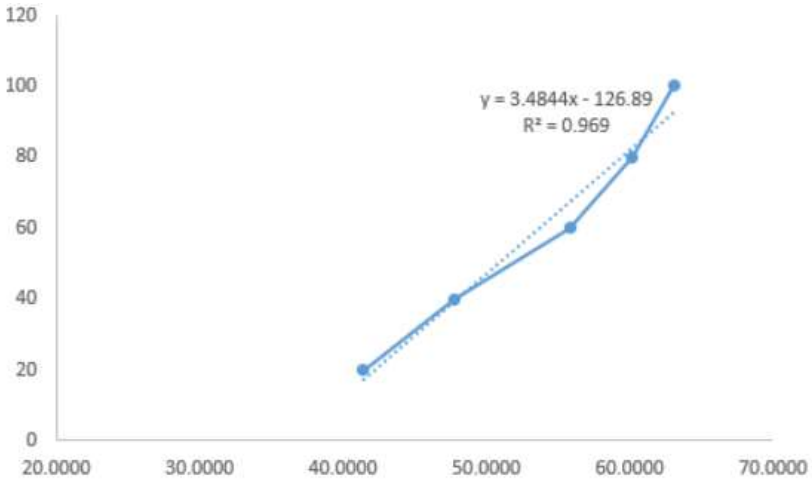


Figure 1. Regression curve of antioxidant of marigold flower water extract

Discussion

Extraction is a method of withdrawing active components/substances from a mixture of solids and liquids using certain solvents. This process is a crucial first step in drug research because preparing crude plant extracts is the starting point for isolating and purifying chemical components found in plants [6]. There are two types of extraction, namely liquid-liquid extraction and solid-liquid extraction. Solid-liquid extraction generally consists of Maceration, Refluxation, Soxhletation, and Percolation. The maceration extraction method was used in this research because it is the easiest/simplest method.

Water is used as a solvent in this research because water is polar. Water is a much better solvent than almost all commonly encountered liquids. Compounds that immediately dissolve in water include neutral organic compounds with polar functional groups such as sugars, alcohols, aldehydes, and ketones [7]. In general, most solvents have polarity due to their internal chemistry. The appropriate type of solvent is selected to bind more active compounds so that a high yield is obtained [8]. Yield compares the resulting sample's dry weight with the raw material's weight [9]. Extract yield is calculated based on the comparison of the final weight (the weight of the extract produced) with the initial weight (the weight of the cell biomass used), then multiplied by 100% [10]. The yield is good if the value exceeds 10% [11]. The yield value is also related to the bioactive content in marigold flowers. In this study, the yield obtained in water solvent was 26.74%.

In Table 2, the results of the phytochemical test of marigold flower water extract are proven to contain flavonoid compounds. The results of the flavonoid test on the water extract of marigold flowers showed positive results, as indicated by a color change that was seen after the test sample was added with magnesium powder and concentrated hydrochloric acid with the formation of a yellow color [3]. Flavonoids are a group of secondary metabolite compounds most commonly found in plant tissues and have potential as essential compounds for research into pharmacology [12]. Flavonoids, as compounds originating from plants, have many positive impacts, including acting as natural antioxidants and affecting many diseases, such as anti-tumor, anti-inflammatory, anti-allergic, and anti-diabetic. This compound can also be used as a medicine for acne, dandruff, and blackheads, preventing baldness and slowing down aging because it contains antioxidants and antibacterials [13].

Examining the phenol of marigold flower water extract, which was added with FeCl3, showed positive results with a blue-black color change in both test solutions. Phenol is an organic compound containing a hydroxyl (-OH) group directly bonded to the carbon atom in a benzene ring [14]. Phenol is an antioxidant that can ward off the dangers of free radicals and contains digestive enzymes that improve the work of the digestive tract, absorb nutrients, reduce digestive stress, maintain pH, maintain intestinal health, and balance the body's natural enzymes [15]. Meanwhile, research from Thoo et al. (2013) stated that the phenol content of noni fruit was 881.57 ± 17.7 mg/100g, with the ability to fight free radicals.

In the examination of steroid water extract and 70% ethanol extract of marigold flowers carried out using the Liebermann-Burchard reaction, in which anhydrous acetic acid and concentrated sulfuric acid were added through the tube wall, it was proven that the test solution did not contain steroid compounds or was negative for steroids. This is because steroids are composed of long-chain hydrocarbon isoprenes, which causes the nature of steroids to be non-polar.

Based on Table 2, related to the test results of marigold flower water extract, it is proven that it does not contain triterpenoid compounds. The results obtained from marigold flower water extract were due to using a polar solvent in the extraction process. Because triterpenoid compounds are non-polar, these compounds cannot be extracted perfectly [17].

In the alkaloid test based on Table 2 related to the results of the phytochemical test of marigold flower water extract, it was proven that marigold flower water extract contains alkaloid compounds, which were indicated by the presence of a red precipitate after the reagent solution was mixed with the test solution. Alkaloids soundly affect health, including triggering the nervous system and reducing pain. Antimicrobial and sedative alkaloid compounds in a plant can also act as bioactive mosquito repellents [18].

The tannin test of marigold flower water extract was proven positive for containing tannin compounds using the Pb acetate reagent. After mixing the test solution, a white precipitate will be produced. Tannin is one of the polyphenols contained in marigold flowers. In plants, tannins function as self-defense from attacks by bacteria, viral fungi, herbivorous insects, and herbivorous vertebrates. In the health sector, tannin has activity as an antibiotic. The working principle of tannin as an antibiotic is to form complexes with extracellular enzymes produced by pathogens or by interfering with the pathogen's metabolic processes [19].

In previous research conducted by Monisa et al. (2016), it was concluded that the tannin compounds in durian leaves have the potential to act as α-glucosidase inhibitors [20]. Tannins are polyphenolic compounds with a huge

molecular weight, namely more than 1000 g/mol, and can form complex compounds with proteins. Therefore, tannins are predicted to act as biological antioxidants [21].

The saponin test based on Table 2 regarding the results of the phytochemical test of marigold flower water extract proved that it does not contain saponin compounds because it does not form stable foam. The appearance of foam in the saponin test indicates the presence of glycosides, which can form foam in water hydrolyzed into glucose and other compounds.

Measurement of the antioxidant activity of water extract and 70% ethanol extract of marigold flowers was carried out using the DPPH method. The DPPH method is a commonly used method to determine the antioxidant activity of a sample because this method is easy to use, fast, accurate, and cheap to measure antioxidant capacity using the free radical 2,2 diphenyl-2 picrylhydrazyl (DPPH). The principle of this method is to react antioxidant compounds with free radical compounds. Antioxidant activity was measured by reacting a 2 mL DPPH solution with 4 ml of extract (concentration series 20 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm) and then incubated for 30 minutes in dark conditions. Absorption was measured at a wavelength of 517 nm. The ascorbic acid solution was used as a comparison. The color change from violet to pale yellow in the DPPH solution is due to the presence of antioxidant compounds in the sample.

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The ability of antioxidant activity is measured by the inhibition ability expressed by the IC50 value. Sample solutions with an IC50 value of less than 200 ppm have potent antioxidant activity [5]. The antioxidant activity test using the DPPH method on marigold flower water extract obtained an IC50 value of 50.77 ppm. From the research results, marigold flower water extract can be categorized in the potent antioxidant category (50 < IC50% < 100 ppm). This potent antioxidant activity is likely due to the content of phytochemical compounds such as flavonoids, phenols, alkaloids, and tannins, which have been reported to have a role as antioxidants that protect against free radicals that damage cells and tissues [2]. Previous research was reported by Siddhu and Saxena (2017), who reported that the antioxidant activity of marigold flower extract was evaluated using the DPPH method using a standard reference compound in ascorbic acid [22]. This research shows that the methanol extract of marigold flowers produces the highest antioxidant activity with an IC50 value of 30.08 ppm, followed by ethyl acetate extract at 96.83 ppm, petroleum ether extract at 108 ppm, and finally, chloroform extracts at 197.22 ppm. Yulia and Ranova (2018) stated that Marigold flower extract produces antioxidant activity, which was tested using the DPPH method [23]. The ethyl acetate extract of marigold flowers (produces higher antioxidant activity than the methanol extract. Marigold flower ethyl acetate extract produced an IC50 value of 53.40 ppm, while methanol extract produced an IC50 value of 181.09 ppm. Several studies above show that the antioxidant activity of marigold flowers is quite intense.

## IV. CONCLUSION

Marigold flower water extract contains phytochemical compounds of flavonoids, phenols, alkaloids, and tannins and has potent antioxidant activity with an IC50 value of 50.77 ppm. For future research, it is hoped that further research will be carried out by examining the phytochemical compound content of marigold flower water extract quantitatively so that we can know in more detail about the content levels and role of marigold flowers in the future and can implement knowledge regarding phytochemical compounds and antioxidant activity of marigold flower water extract as an essential ingredient for herbal medicines.

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TABLE II
PHYTOCHEMICAL COMPARISONS OF AQUEOUS EXTRACT OF MARIGOLD FLOWERS

| Compound | Reaction Change | Results |
|---|---|---|
| Flavonoids | Reddish black, yellow, or orange discoloration occurs | Positive |
| Phenols | Formation of a blue-black solution | Positive |
| Steroid | No blue-green color formed | Negative |
| Triterpenoids | No formation of red color | Negative |
| Alkaloids | Formation of red precipitate | Positive |
| Saponins | No stable foam formed | Negative |
| Tannins | Formed white precipitate | Positive |

TABLE III
ANTIOXIDANT ACTIVITY TEST RESULT OF MARIGOLD FLOWER WATER EXTRACT

| Concentration (ppm) | Absorbents | Inhibition (%) |
|---|---|---|
| 20 | 0,6946 | 41.4223 |
| 40 | 0,6195 | 47.7611 |
| 60 | 0,5239 | 55.8173 |
| 80 | 0,4799 | 60.1434 |
| 100 | 0,4799 | 63,0415 |