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Phytochemical Profile and Hair Growth Activity of Marigold Flower (*Tagetes erecta* L.) Extract

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Abstract: The Marygold flower (*Tagetes erecta* L.) exhibits numerous pharmacological properties. The marigold flower promotes hair development. Antioxidant substances that contribute to hair growth include alkaloids, flavonoids, tannins, and saponins. This research was conducted to determine the efficacy of ethanol extracts from Marygold flowers (*Tagetes Erecta* L.) as hair growers in male rabbits (Oryctolagus cuniculus). In a post-test-only control-group design, this study employed an experimental methodology. Thirty male rabbits served as test subjects in this study, which used one-way ANOVA. The KTL method was used in this study to determine the chemical constituent content of the ethanol extract of Marygold flowers at 5%, 10%, and 20% concentrations. Then, the length and density of the rabbit hair were measured to test the activity of hair growth. On days 7, 14, and 21, hair growth length was measured. On day 21, however, hair weight (density) was measured. Compounds from the flavonoid group (Rf 0.50 & 0.76), tannins (Rf 0.33 & 0.64), saponins (Rf 0.36 & 0.80), and alkaloids (Rf 0.33 & 0.62) were all positively present in the Marygold flower ethanol extract. The 20% concentration of Marygold flower ethanol extract exhibited the highest activity ($p<0.05$) in tests of hair density and length growth. The ethanol extract from Marygold flowers promotes hair growth in male rabbits, making their hair longer and denser.

Keywords: Hair Density; Hair Length; *Tagetes erecta* L.; TLC.

Introduction

Every human being wants to have beautiful hair. Hair is present in nearly all regions of the human body and serves multiple functions, including cosmetic purposes. Hair is often referred to as a crown for women, while for men, hair condition affects self-confidence [1]. Healthy and strong hair is everyone's dream. Hair health is greatly influenced by antioxidant compounds. Some antioxidants play a role in hair growth. This compound acts as an electron donor in a certain amount to inhibit and slow the damage caused by oxidation. Natural antioxidants are usually found in plants, which contain phytochemicals such as flavonoids, flavones, isoflavones, and anthocyanins, as well as vitamin C.

Synthetic antioxidants are in the form of phenolic compounds such as butylated hydroxy anisole (BHA), butylated hydroxytoluene (BHT), tertiary butyl hydroquinone (TBHQ), and esters of gallic acid such as propyl gallate (PG) [2]. Synthetic antioxidants have been widely studied, and their long-term use will influence the body. Therefore, the use of natural antioxidants is more recommended [2]. One plant thought to contain natural antioxidant compounds is the Marygold flower.

The Marygold, also known as the marigold, has various pharmacological activities, including antibacterial, antioxidant, hepatoprotective, and carminative [3]. The color of the Marygold flower is produced by two main pigments, namely a small portion of the flavonoid and carotenoid groups [4]. The class of antioxidant compounds found in Marygold flowers includes flavonoids, saponins, phenols, and carotenoids [5] [6].

Marygold flowers are useful for treating fever, epilepsy, carminative effects, stomach medicine, scabies, and liver complaints. They are also used in the treatment of eye diseases and to purify the blood. Marygold flower petals are often used to make tea or Marygold flower juice as a remedy for blood clots and to treat rheumatism, colds, and bronchitis [7] [8]. Phytochemical tests indicate that the Marygold plant contains flavonoids, carotenoids, saponins, and triterpenoids [9].

In a previous study, a phytochemical screening test of the ethanol extract of Marygold flowers at the same sampling site indicated that the 96% ethanol extract contained flavonoids, saponins, steroids, tannins, and polyphenols [10]. In this study, the compounds contained in Marygold flower samples, such as flavonoids, saponins, alkaloids, and tannins, which are closely related to hair growth, will be tested based on their chromatography profiles.

Flavonoids and saponins have an important role in hair growth. Flavonoids are able to stimulate and control the growth of hair follicles, while saponins help circulate blood to the hair. Reduced blood flow to the hair can cause hair loss [11] [12]. Alkaloid compounds have the effect of triggering hair growth as an irritant that can enlarge the stalks and add weight to the hair [13]. Tannin compounds in the human body serve as hair nutrients, supporting various biological activities. Tannins have various effects in biological systems because they are potential metal-ion chelators, protein precipitants, and biological antioxidants [14]. However,

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there is no research evidence of hair growth activity from Marygold flowers. This research aims to investigate the activity of Marygold flowers (Tagetes erecta L) on hair growth in male rabbits, with hair length and weight measurements.

Research Methods

Main The materials of study were marigold flower, ethanol 96% (Merck), CMC-Na (Merck), aquadest (Bratachem), Dragendroff's reagent (Merck), Silica Gel GF254 aluminium plate(Merck), AlCl3, $FeCl_3$ (Unilab), chloroform (Merck), methanol (Merck), Minoxidil (Regrow)

Marygold Flower Sample Extraction (*Tagetes erecta* L.)

Marygold flower simplicia, weighing 500 grams, was macerated using 1.5 L of 96% ethanol for 5 days. The filtrate was then evaporated using a rotary evaporator (Biobase RE-201D) at 50ºC.

Marygold Flower TLC Test

The extracts were tested by thin-layer chromatography to determine whether they contained flavonoids, tannins, saponins, steroids, and alkaloids. This TLC test used Silica G_{60} F_{254} stationary phase. The TLC plate was 8 cm long and 2 cm wide. As much as 10 mg of the extract was dissolved in 1 ml of ethanol, then spotted on the stationary phase.

Identification of flavonoid compounds

To identify the flavonoid compounds, Marygold flower extract was dissolved in ethanol, then spotted on the TLC plate using a capillary tube, placed 1 cm from the bottom line. Elution was carried out using the mobile phase in the form of a mixture of n-hexane/ethyl acetate/methanol with a ratio of 4:5:1. Then, the plates were observed for spotting under a UV lamp at a wavelength of 254 nm and 366 nm, and with a spot viewer using aluminium chloride (5% AlCl) reagent [15].

Identification of tannin compounds

For tannin identification, a methanol-water mobile phase (6:4) was used. Stain detection was performed with a 5% $FeCl_3$ reagent [16].

Identification of saponin compounds

Merck's Silica Gel GF_{254} aluminium plate was prepared with a length of 8 cm and a width of 2 cm. The extract was dabbed on the bottom edge of the plate and allowed to air for a while. The plate was placed into a measuring cup containing the eluent, a homogeneous mixture of the bottom layer of the solvent chloroform/methanol/distilled water (13:7:2).

The plate was allowed to elute until the eluent propagated and reached the upper edge of the plate. It was then removed and dried in the air. After that, the plate was sprayed with LB reagent and heated at 110 ºC for 10 minutes to clarify the colour of the forming stain. Stain observation was carried out using a UV lamp at 254 nm and 366 nm [17].

Identification of alkaloid compounds

To identify alkaloids, the mobile phase was a mixture of ethyl acetate/methanol/water with a ratio of (100:13.5:10). The TLC plate was sprayed with Dragendorff reagent, which produced blue or yellow spots upon identification [18].

Hair Growth Activity Test

The hair growth activity test began with the adaptation of 30 test animals for 7 days in cages measuring 40 x 30 x 38 cm, with pellets and ad libitum water provided. The test animals were divided into 5 groups, each group containing 6 rabbits.

The rabbit's back was shaved with scissors and a razor, measuring 2 x 2 cm, rinsed with water until clean, and then rubbed with 70% alcohol as an antiseptic. The treatment consisted of applying 1 mL of the extract twice daily for 21 days. Hair was measured on days 7, 14, and 21 by removing the 10 longest strands from each group of test animals and placing them on black paper for measurement using a calliper [19].

Hair weight measurements were also taken to estimate hair density. This measurement was carried out on the 21st day by shaving all the hair in the test area on each rabbit. The hair was then weighed using an analytical balance [20].

The treatment given to the test animals is as follows:

a) Positive control (K1) was given 2% minoxidil.
b) Negative control (K2) was given aquadest.
c) Treatment group (K3) was given 5% concentration of Marygold Flowers (*Tagetes erecta L.*) Ethanol Extract.
d) Treatment group (K4) was given 10% concentration of Marygold Flowers (*Tagetes erecta L.*) Ethanol Extract.
e) Treatment group (K5) was given 20% concentration of Marygold Flowers (*Tagetes erecta L.*) Ethanol Extract.

Data analysis on male rabbit hair length and hair density was carried out using one-way ANOVA on the SPSS 26 application for Windows.

Results and Discussion

The Marygold flower used in this study came from Plaga Village, Badung, Bali. The selection of the Marygold flower was carried out at the Bedugul "Eka Karya" Botanical Garden Characterization Laboratory, Bali-BRIN. The identification results confirmed that the sample used was really a Marygold plant (*Tagetes erecta* L.).

Sampling of Marygold flowers was done by choosing relatively the same diameter and color. The Marygold flower used in this study was taken from Plaga Village, Badung, Bali, because its utilization in that area is not maximized; it is only used as a means of ceremony that serves the interests of tourists and aesthetic objects.

Extract preparation began with washing the Marygold flower samples to remove dirt and other impurities, drying them in the sun, and baking them at 40 °C for 3 days. The

dried Marygold flowers were then blended and sifted to obtain fine simplicia powder.

The extraction method used was maceration. The advantage of this method is that it is simple and easy to use. The risk of damage to the active substance in the extract will be lower because this method does not use heat, which can degrade the active substance in the Marygold flower extract.

Maceration was carried out in stages in a glass jar, the outside of which was covered with aluminium foil. This aims to avoid sunlight and oxidation, namely the decomposition of the active substance's structure, especially in less stable compounds. The glass jar was filled with 300 grams of Marygold flower simplicia, soaked in 96% ethanol at a 1:10 ratio.

The viscous methanol extract obtained was 25 grams with an extract yield of 0.083%. The extract yield is the weight percentage of the total weight of the resulting extract divided by the initial weight of the simplicia powder used, multiplied by 100%. The yield value is related to the amount of bioactive compounds in a plant; the higher the yield value of an extract, the higher the content of substances attracted to the raw material [21]. After obtaining a thick extract, the Marygold flower extract was prepared at concentrations of 5%, 10%, and 20%.

The viscous extract obtained was then subjected to thin-layer chromatography tests for flavonoids, tannins, saponins, and alkaloids. In a previous study, the phytochemical screening of the ethanol extract of Marygold flowers at the same sampling location indicated that the 96% ethanol extract contained flavonoids, saponins, steroids, tannins, and polyphenols [10].

Table 2. Phytochemical Screening Results of Marigold Flower Extract

Phytochemical Screening Test	Result
	Ethanol Extract of Marygold Flowers
Alkaloid	(-)
Flavonoids	(+)
Saponin	(+)
Tannin	(+)
Polyphenol	(+)
Steroids	(+)
Triterpenoid	(-)

Thin-layer chromatography test was carried out in this study to ascertain the content of compounds such as flavonoids, saponins, alkaloids, and tannins in the ethanol extract of Marygold flowers, which are closely related to hair growth. This test was carried out because it was relatively inexpensive, the process was easy, and the results were more accurate [15].

In the TLC test, Silica G60 F254 was used, with a length of 8 cm and a width of 2 cm. 10 mg of Marygold flower extract was dissolved in 1 ml of ethanol and bottled on the stationary phase. Thin-layer chromatography measurements of the ethanol extract from Marygold flowers were performed by calculating the retardation factor. The Rf value is a characteristic parameter of thin-layer chromatography. This value is a measure of the migration speed of a compound on the chromatogram [15].

The ratio of the compound's travel distance to the developer solvent's travel distance is known as the Rf value. One piece of evidence for identifying chemicals is the Rf value. The compound is said to have the same or comparable properties if the identification of the Rf value is the same. Meanwhile, if the Rf value differs, the compound is considered a different compound. Therefore, the Rf number is always less than 1.0 [15].

In the identification of flavonoid compounds in the ethanol extract of Marygold flowers, a mobile phase of n-hexane, ethyl acetate, and methanol (4:5:1) was used. The results showed that the extract contained flavonoid compounds. This was indicated by greenish-yellow stains on the plate after spraying with the AlCl3 spot viewer. In Figure 5.1, after elution and spraying of 5% AlCl3 spotters, there are spots from Marygold flower extract with Rf values of 0.50 and 0.76.

Spots with an Rf value of 0.50 contain 5-OH flavones or flavanols with a substituted 3-O. While the spot with an Rf value of 0.76 may be a flavonoid compound in the form of another glycoside with lower polarity than the first flavonoid compound. In this case, the mobile phase used was n-hexane/ethyl acetate/methanol (4:5:1). This mobile phase is an alcoholic mobile phase. This results in relatively less polar compounds eluting faster than more polar compounds.

Quercetin, as a comparison, produces Rf values of 0.23 and 0.40. The Rf values of the spots correspond to the range of flavonoid-containing compounds, namely 0.2–0.8 [22]. After spraying with 5% AlCl3, the spots obtained were greenish-yellow. This is in line with the theory, which states that a positive sample contains flavonoid compounds if, after being sprayed with the 5% AlCl3 spot remover, the spots change colour to yellow-green in visible light and blue under UV light [18] [15].

In the identification of tannin compounds by the TLC test using the mobile phase methanol/water (6:4), Rf values of 0.33 and 0.64 were obtained. The Rf value of the compound identified as tannin lies in the range of 0.07-0.77, and the Rf value of the tannin standard solution, namely tannic acid, is 0.71 [18]. Marygold flower extract positively contained tannins based on the Rf value, and this was indicated by the presence of black stains on the TLC plate after being sprayed with 5% FeCl3, as shown in Figure 5.2. This is in accordance with the theory, which states that a positive reaction is indicated by the formation of black spots on the TLC plate [16].

For saponin identification, a mobile phase of chloroform/methanol/aquadest (13:7:2) was used. The results showed that the Marygold flower extract contained saponin compounds with Rf values of 0.36 and 0.80. Saponin Rf value of 0.36 indicates the type of saponin. The Rf value of pure saponin as a standard of comparison is 0.80, so the Rf value obtained in this study is in accordance with the Rf value. Saponin-positive extracts were characterised by yellowish-green fluorescence under UV light at 256 and 366 nm. This result is consistent with the theory, which states that after spraying the LB reagent and heating for 10 minutes, a yellowish-green stain will appear on the TLC plate [17].

In the identification of alkaloids, the mobile phase of ethyl acetate/methanol/water was used with the ratio of 100:13.5:10. The test results showed that the ethanol extract of Marygold flowers positively contained alkaloids with Rf values of 0.33 and 0.62. The Rf value of the most common alkaloids is 0.07-0.62 [18]. The standard Rf value for alkaloid comparison is 0.26 [23]. A positive result is also

indicated by the presence of a yellowish-blue stain when observed under UV light. This is in accordance with the theory, which states that, after the plate is sprayed with Dragendorff reagent, it will show spots with a yellowish-blue color [18].

Hair Growth Activity Test

The rabbit hair growth activity test begins with a 7-day adaptation period to ensure the test animals can adapt to the trial environment and avoid stress. During the adaptation period, rabbits are given rabbit food and water ad libitum. The male rabbit's hair was shaved on the back of the rabbit with a size of 2 x 2 cm using scissors and a razor (day 1). The 1-ml extract was applied twice a day, in the morning and evening. Group I (K1) was given aquadest (negative control), group II (K2) minoxidil 2% (Regrou) (positive control), group III (K3) 5% Marygold flower extract, group IV (K4) 10% Marygold flower extract, and group V (K5) 20% Marygold flower extract. Observations were made for 21 days by taking the 10 longest hairs from each test area. Hair length was measured on days 7, 14, and 21 using a calliper. Observations were also made on the density of rabbit hair. Measurements were made on the 21st day by shaving all the rabbit hair in each test area and then weighing it using an analytical balance [20].

Observation of Hair Length

Based on the results of the average hair length measurement on day 7 (Figure 1), the positive control group (K2) had the best hair length of 4.4 mm, which was significantly different from the 3.2 mm in the negative control group (K1) with a value $p<0.05$ (Table 1).

Table 1. Hair growth activity test for hair length for various treatments

Group	Length of Hair (mm) ± SD		
	7th Days	14th Days	21st Days
K1	3.25 ± 0.15	6.05 ± 0.28	12.1 ± 0.20
K2	4.45 ± 0.21	10.6 ± 0.20	17.9 ± 0.20
K3	3.58 ± 0.18	8.86 ± 0.26	14.3 ± 0.20
K4	3.61 ± 0.11	9.2 ± 0.18	15.05 ±0.20
K5	4.13 ± 0.14	9.81 ± 0.21	16.7 ± 0.20

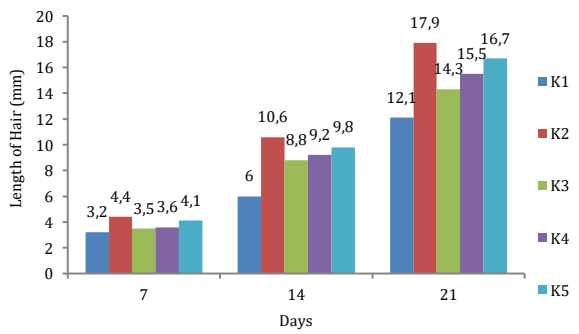

Figure 1. Diagram of length Hair Measurement

This shows that there is a difference in hair length growth among the test animals given 2% minoxidil. The same results were also obtained in the 20% concentration group (K5), the 10% concentration group (K4), and the 5% concentration group (K3), with $p<0.05$ compared to the negative control group (K1). The average hair length in the 5% and 10% concentration test groups was almost the same ($p>0.05$; Table I), indicating no significant difference.

The results of hair length measurements on days 14 and 21 showed that the positive control group (K2) had the best hair length growth with an average of 10.6 mm on day 14 and 17.9 mm on day 21. This had a significant difference ($p<0.05$) from the negative control group (K1), which had hair length growth of 6.0 mm on day 14 and 12.1 mm on day 21. The groups with concentrations of 20% (K5), 10% (K4), and 5% (K3) also had a higher average hair length than the negative control group (K2) ($p<0.05$).

The average hair length growth on days 7, 14, and 21 in the extract groups with concentrations of 5%, 10%, & 20% showed significant differences compared to the positive control ($p<0.05$). Marygold flower ethanol extract shows activity in hair growth, but it is less effective than the positive control.

Hair Weight Observation

Based on the measurement results of the average hair weight on day 21 (Figure 2), the positive control group (K2) had the best hair weight of 39.08 mg, which was significantly different from the negative control group (K1), which had the hair weight of 35.94 mg, with a p-value <0.05 (Table 2).

Table 2. Hair growth activity test for hair weight for various treatments

Group	Hair Weight 21th Day (mg) ± SD
K1	35.94 ± 0.47
K2	39.08 ± 0.95
K3	37.59 ± 0.33
K4	38.6 ± 0.10
K5	38.8 ± 0.05

This shows that administering 2% minoxidil resulted in greater hair weight in the test animals.

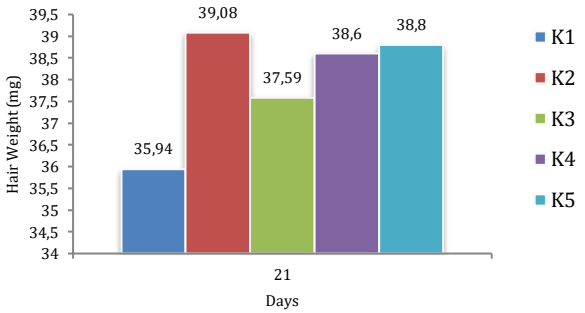

Figure 2. Diagram of Hair Weight Measurement

The 20% concentration group (K5), the 10% concentration group (K4), and the 5% concentration group (K3) also had higher weight values than the negative control group (K2) and showed significant differences ($p< 0.05$; Table II). The average hair weight on day 21 of the extract group with concentrations of 5%, 10%, & 20% had a significant difference compared to the positive control ($p<0.05$). Marygold flower ethanol extract shows activity in hair growth, but it is less effective than the positive control.

Hair follicle cells will continue to renew themselves following the hair growth cycle. The hair growth cycle alternates between the anagen, catagen, and telogen phases. On the 7th day, new hair growth entered the anagen phase. Marygold flower ethanol extract stimulated hair growth in male rabbits, as shown in Figure 6.1, which compares the average hair length between the negative control group and the extract treatment group. In the anagen phase, matrix cells undergo mitosis to form new cells, which are pushed to the skin's surface. This phase occurs in the first week when new hair grows [24].

Hair growth on days 14 and 21 began to enter the catagen phase, the transition from the anagen to the telogen phase. Figures 6.1 and 6.2 show a significant increase in hair growth between groups. The catagen phase is characterized by stopped mitotic activity of matrix cells and well-coordinated apoptosis. Migration of the dermal papillae from the subcutaneous fat to the dermis during this phase is necessary for the continuation of the hair follicle cycle. The duration of this phase is between 2-3 weeks [24], [25].

Factors that affect hair growth are extrinsic factors and intrinsic factors. Extrinsic factors are environmental conditions that can affect the scalp. These environmental factors include extreme weather changes, exposure to ultraviolet, x-rays, radioactivity, chemical irritation, and stress. Stress doesn't directly cause hair loss, but behavioral and mental changes do. Stress causes neurogenic inflammation in neurons, preventing nerve growth factor (NGF), which is involved in hair follicle development, from exerting its effects [26].

An intrinsic factor of hair growth is the blood circulation to the follicle. Hair will not grow without an adequate blood supply to fill the hair follicles with the necessary nutrients. Sex hormones play an important role in the growth, distribution, and pigmentation of human hair, especially during puberty, when hormones trigger secondary hair growth [27].

The hormone estrogen slows hair growth during the anagen phase but prolongs its duration. Whereas tyrosine accelerates anagen activity, cortisone actually slows it down. Androgen hormones can increase the speed of hair growth and the size of the diameter of the hair. However, in scalp biopsies from patients with androgenetic alopecia, androgens decrease the diameter of the hair shaft, the rate of hair growth, and the duration of the anagen phase [27].

Based on the TLC test results, Marygold flowers contain flavonoids, saponins, alkaloids, and tannins. These compounds have a role in stimulating hair growth. Flavonoids regulate vascular-mediated hair follicle growth. Flavonoids can increase the phosphorylation of vascular endothelial growth factor (Vascular Endothelial Growth Factor) [11]. Flavonoids can improve blood circulation, thereby increasing hair growth and preventing hair loss. To promote optimal hair development, flavonoids also reduce the activity of type II 5α-reductase enzymes [28].

Saponin compounds in the human body help regulate blood pressure in the hair. Reduced blood flow to the hair follicles can affect hair follicles and cause hair loss [12]. Saponins are components of the ethanol extract of Marygold flowers and can stimulate hair growth in cases of alopecia caused by hormonal or hereditary factors. Saponins can create foam, remove impurities from the skin, and act as counterirritants, increasing peripheral blood circulation and promoting hair development. Alkaloid compounds have the effect of triggering hair growth as an irritant that can enlarge the stalks and add weight to the hair. The mechanism of action of alkaloids is as vasodilators of blood vessels, which increase the delivery of nutrients and oxygen to hair roots, resulting in optimal hair growth [13]. The tannin compounds in the human body serve as nutrients for hair, supporting various biological activities. Due to their capacity as biological antioxidants, protein precipitators, and metal-ion chelators, tannins have a variety of effects on biological systems [14].

Conclusion

Based on the results of the research conducted, it can be concluded that the ethanol extract of Marygold flowers (*Tagetes erecta L.*) positively contains flavonoid (Rf 0.50 & 0.76), tannins (Rf 0.33 & 0.64), saponins (Rf 0.36 & 0.80), and alkaloids (Rf 0.33 & 0.62). Ethanol extract of Marygold flowers (*Tagetes erecta L.*) with concentrations of 5%, 10%, and 20% has hair growth activity in male rabbits (*Oryctolagus cuniculus*). The 20% concentration of ethanol extract of Marygold flowers (*Tagetes erecta L.*) showed the best activity for hair regrowth by increasing the length and density of hair growth in male rabbits (*Oryctolagus cuniculus*).

Author's Contribution

L. Puspitasari: Drafting the research design and writing the paper; Putu Indrayoni: Determining the type of analysis and data analysis techniques; P. A Septiarini: conducting research using experimental animals, preparing the extract, and performing phytochemical tests.

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