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ANTIBACTERIAL ACTIVITY OF MARIGOLD FLOWER (TAGETES ERECTA L.) ETHANOL EXTRACT CREAM AGAINST STAPHYLOCOCCUS AUREUS

AKTIVITAS ANTIBAKTERI KRIM EKSTRAK ETANOL BUNGA MARIGOLD (TAGETES ERECTA L.) TERHADAP STAPHYLOCOCCUS AUREUS

Research Report
Penelitian

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ABSTRACT

Background: *Acne is caused by inflammation and obstruction of the skin pores, which traps excess oil (acne vulgaris). The bacterium Staphylococcus aureus is the causative agent of acne. Treatment and prevention efforts can be made traditionally.* ***Purpose:*** *Testing the antibacterial efficacy of the marigold flower ethanol extract cream (Tagetes erecta L.) against Staphylococcus aureus.* ***Method:*** *This type of research is descriptive exploratory and experimental. Samples of ethanol extract from marigold flowers (Tagetes erecta L.) were formulated into three treatments, namely concentrations of 25%, 50%, and 100% with three replications. Antibacterial efficacy testing was carried out by the agar well diffusion method. Data analysis used One-way ANOVA and Tukey HSD tests.* ***Result:*** *The inhibition zones produced by the ethanolic extract of marigold flowers were 8.59 ± 0.047, 9.23 ± 0.026, and 10.23 ± 0.044 respectively. Thus, a higher concentration of the ethanolic extract of marigold flowers in the cream produced more significant inhibitory activity.* ***Conclusion:*** *The ethanol extract cream of marigold flower (Tagetes erecta L.) showed antibacterial activity against Staphylococcus aureus and is suitable to be applied as a cream for the traditional treatment of acne (acne vulgaris).*

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ABSTRAK

Latar belakang: Peradangan dan penyumbatan pori-pori kulit mengakibatkan terperangkapnya minyak berlebih yang memicu jerawat (*acne vulgaris*). Bakteri *Staphylococcus aureus* adalah agen penyebab jerawat. Upaya pengobatan dan pencegahan dapat dilakukan dengan cara tradisional. **Tujuan:** Pengujian khasiat antibakteri krim ekstrak etanol bunga marigold (*Tagetes erecta L.)* terhadap *Staphylococcus aureus*. **Metode:** Jenis penelitian deskriptif eksploratif dan eksperimental, sampel ekstrak etanol bunga marigold (*Tagetes erecta L.*) diformulasikan menjadi tiga perlakuan yakni konsentrasi 25%, 50 %, dan 100% dengan replikasi sebanyak tiga kali. Pengujian efikasi antibakteri dilakukan dengan metode difusi sumur agar. Analisis data dengan uji *One-way* ANOVA dan Tukey HSD. **Hasil:** Zona hambat yang dihasilkan oleh ekstrak etanol bunga marigold secara berurutan antara lain 8,59 ± 0,047; 9,23 ± 0,026; dan 10,23 ± 0,044. Demikian, semakin tinggi konsentrasi ekstrak etanol bunga marigold dalam krim maka semakin besar daya hambat yang dihasilkan. **Kesimpulan:** Krim ekstrak etanol bunga marigold (*Tagetes erecta L.*) memiliki aktivitas antibakteri terhadap *Staphylococcus aureus* dan layak dimanfaatkan sebagai krim untuk pengobatan jerawat (*acne vulgaris*) secara tradisional.

Kata kunci:
Antibakteri, Jerawat, Ekstrak etanol, Bunga marigold, Obat tradisional

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INTRODUCTION

Inflammation and clogging of the skin pores accumulate oil trapped in the skin and cause acne (acne vulgaris) (Mandias *et al*., 2022). Acne is found in adolescents to adults aged 12-25 and grows independently. Under normal conditions, the bacteria *Staphylococcus aureus*, *Staphylococcus epidermis*, and *Propionibacterium acnes* proliferate very quickly, which causes the skin to experience acne in the follicular tissue (Widjanarko *et al*., 2021; Priani *et al*., 2018). Nearly 56% of the incidence of acne occurs on oily skin, and 44% occurs on normal and dry skin (Zhang *et al*., 2020). The androgen dihydrotestosterone hormone causes excess oil production in the skin. This hormone can increase the size of the sebaceous glands, which has implications for sebum production in the skin (Cahyaningrum *et al*., 2020; Rajkumari *et al*., 2021). This situation makes it easier for acne causing bacteria to grow ideally (Puluh *et al*., 2019). In sebum, there are triglycerides and fatty acids, which are nutrients for acne causing bacteria. Then, through these bacteria, the triglycerides will be broken down into free fatty acids and colonized, causing inflammation in acne (Mekvimol *et al*., 2020; Priani *et al*., 2018). Inflammation must be overcome so that it does not worsen acne conditions and reduces skin health, elasticity, and shape or structure (Pramitha *et al*., 2017) by interrupting the growth of acne causing bacteria.

S. aureus is a common acne causing bacteria that attack the skin. These bacteria can cause necrosis, ulcers, and clogged pores on the skin (Widjanarko *et al*., 2021). If this happens, it will cause dolor on the skin, heat due to infection, tumors in specific areas such as the face and blush, namely redness as a sign of infection and inflammation (Widjanarko *et al*., 2021; Parwanto *et al*., 2020). Acne vulgaris can be managed using modern medicine and folk remedies (Kosmadaki and Katsambas, 2017). In modern medicine, anti acne drugs are generally administered with topical preparations to relieve inflammation, heat, and pain produced in the local area (around which the infection occurs in the skin tissue). However, long term use results in resistance, and excessive use is not able to cope with the infection as a whole, as revealed by Walsh *et al*. (2016), who found the presence of topical antibiotic resistance reaching >50% for bacterial strains *Propioni acnes* tested on topical macro lesions.

Furthermore, Greydanus *et al*. (2020) explained in their findings that with the treatment of acne vulgaris using topical retinoids, topical benzoyl peroxide, antibiotics (topical, oral), oral contraceptive pills, and isotretinoin, there is an increase in antibiotic resistance in the 21st century so that primary and secondary management is needed, especially treatment with natural ingredients. D'Epenoux *et al*. (2022) revealed that it is necessary to consider broad-spectrum quinolone antibacterial agents derived from natural ingredients as an acne treatment in the era of antimicrobial resistance. Efforts can be made to inhibit the development of *S. Aureus* bacteria on the skin by using traditional medicines made from plants, one of which is marigold flowers (*T. erecta L.*). Marigold flowers are known to contain bioactive compounds in the form of phenols (Pramitha *et al*., 2017), flavonoids (Mekvimol *et al*., 2020), and carotenoids in the form of lutein (Zhang *et al*., 2020).

Previous research revealed that marigold flowers have antibacterial effects (Cahyaningrum *et al*., 2020), antimicrobial (Padalia and Chand, 2015), antimalarial (Edy *et al*., 2019), antihyperlipidemic (Kresnapati *et al*., 2021), and anti-inflammatory (Selvam *et al*., 2021). Flavonoid compounds have maximum inhibitory activity of *K. pneumonia* (Rajkumari *et al*., 2021). Marigold flower essential oil has antifungal activity and the maximum apoptotic rate of 90%, as seen from the HepG2 cell line at 82 and 122 g/ml (Safar *et al*., 2020). Furthermore, polyphenolic compounds in marigold flowers contain laricitrin and glycosides, these compounds are helpful and potentially antibacterial (Mandias *et al*., 2022; Verma *et al*., 2020). Flavonoid compounds and tannins work by damaging the cytoplasmic membrane and forming complexes with proteins through hydrophobic interactions, which ultimately disrupt the metabolism of bacterial cells resulting in lysis (Burlec *et al*., 2022; Zanovello *et al*., 2021; Zhang *et al*., 2020). Furthermore, previous research revealed that parts of the marigold flower have pharmacological benefits (Fu *et al*., 2019; Romes *et al*., 2021) and potential cosmetic raw materials (Kumar *et al*., 2021; Mekjaruskul *et al*., 2021).

Research evaluated the benefits of marigold flowers (*T. erecta L.*) as an antibacterial is essential to assess the efficacy of ethanol extract cream from marigold flowers used to inhibit *S. aureus*. This research is necessary to produce an effective, safe, and tested antibacterial and antiacne cream formulation. In the study, variations in concentration were emphasized to obtain adequate and reasonable formulations to inhibit bacteria. The concentration variations tested were 25%, 50%, and 100% cream of marigold flower ethanol extract (*T. erecta L.*) against *S. aureus*.

MATERIAL AND METHOD

Research design

This study used two research designs, exploratory and experimental descriptive (Darwin *et al*., 2021). The exploratory, descriptive research includes making the marigold flower (*T. erecta L.*) ethanol extract cream preparations and experimentally using the antibacterial properties of marigold flower (*T. erecta L.*) extract cream preparations against *S. aureus*. The test bacteria used in this study were *Staphylococcus aureus*. The selection of bacteria is because *S. aureus* is one of the commonly reported acne causing bacteria. In testing cream products, these bacteria are used as a standard test using the excellent diffusion method.

Instruments and materials

Rotary evaporator (Heidolph), Buchner funnel, incubator, Pyrex glassware, spatula, autoclave, micropipette, oven, Brook Fild DV-1 viscometer, and pH meter were all utilized. Meanwhile, the following ingredients were used marigold flowers (*T. erecta L.*), *S. aureus*, ampicillin, 96% technical ethanol, stearic acid, cetyl alcohol, ceteareth-20, cera alba, triethanolamine, methyl propylene glycol, methylparaben, propylparaben, silicone, Natrium Agar (NA) medium consists of oil, glycerin, and sterile distilled water.

Research procedure

Material preparation

As much as 5 kg of fresh marigold flowers (*T. erecta L.*) was collected from Pasar Badung, Bali, located on Sulawesi Street Number 1, Dauh Puri Kangin, West Denpasar District, Denpasar City, Bali 80233. The flower was cleaned from adhering dirt and washed under running water for 10 minutes. Then all the marigold flowers were dried by the wind for five days. Drying is considered sufficient when the marigold flowers are dry and fragile. Dried flowers are further crushed using a blender until they become powdered, and sifting was carried out using a 40 mesh sieve. The powder obtained is called marigold flower simplisia.

Marigold flower (*Tagetes erecta L.*) extract preparation

The extract was prepared using the maceration method. Marigold flower (*T. erecta L.*) extracted as much as 5 kg of wet disortation to give 1.500 g of dried marigold flower powder. Furthermore, dried marigold flowers were macerated with 96% ethanol in a ratio of 1:10 for five days using continuous stirring. The amount of solvent used to produce the extract was 8 liters. All macerate results were collected using a rotary evaporator at 60°C to give a viscous extract. As much as 1.500 g of dried marigold flower powder produced 31.4 g of viscous extract. Extracted marigold flower extract had a dark yellow color, a solid bitter smell, and a thick texture.

Antibacterial activity assay of marigold flower (*Tagetes erecta L.*) ethanol extract

The antibacterial activity of marigold flower extract was tested at concentrations of 25, 50, and 100% (v/v) using the agar well diffusion method. a primary method for determining the antimicrobial activity of a compound and active substance. This method was carried out by making holes in the agar and inoculating test bacteria on petri dishes containing NA. Then, each hole was filled with samples at a predetermined concentration, and the cup was incubated for one day at 37°C. Observations of antimicrobial activity were carried out by reviewing clear zones indicating the inhibition zones (Balouiri *et al*., 2016; Kumar *et al*., 2021). A total of 20 L of tested extract, negative control (aquades), and positive control (chloramphenicol) were put into the well, then incubated at 37°C for one day. The inhibitory diameter was observed after incubation, and the results were recorded.

Preparation of marigold flower (*Tagetes erecta L.*) ethanol extract cream

A cream base was made with a formula according to Table 1. Three variations of marigold flower (*T. erecta L.*) cream treatments were made with a concentration of 25%, 50%, and 100% of the cream base. The process of cream preparation was begun with a cream base preparation, which was prepared in the form of a mixture of A (in a stainless steel container) materials in the form of acetyl alcohol, ceteareth-20, stearic acid, cera alba, silicon oil heated at 70°C until it melts. In container B, they were heated to a temperature of 70°C, namely distilled water, glycerin, and triethanolamine. Subsequently, mixture B was added to mixture A and stirred until homogeneous. The prepared cream base was then added with the ethanolic extract of marigold flower (*T. erecta L.*) and tested physically, chemically, and microbiologically. Physical testing was carried out by reviewing the texture of the cream, its color, and its aroma. Furthermore, chemical and microbiological testing reviewed the efficacy of marigold flower extract (*T. erecta L.*) in inhibiting *S. aureus* with different formulations.

Efficacy test of marigold flower ethanol extract cream against *Staphylococcus aureus*

The inhibitory quarter dimension was utilized as a metric of antibacterial activity in a cream dosage form. The examination was carried out with the help of the agar well diffusion method. The examination was performed by placing the medium in a petri dish with a bacterial solution. The mixture was then added to n-hexane, chloroform, ethyl acetate, and water so that it amounted to 10 µL. The positive control included 30 µg chloramphenicol, whereas the negative control contained 100 mL of distilled water. Using a micropipette, the extract was dripped into each well. The incubation period was then extended to 1 day at 37°C. The zone of inhibition was measured with a caliper and an accuracy of 0.01 mm. The inhibition zone was estimated by measuring the outside diameter of the generated inhibition zone and then subtracting the excellent diameter.

Data analysis

The results obtained in this study were defined as the inhibition zone value of the marigold flower ethanol extract cream against *S. aureus*. Furthermore, the data were analyzed descriptively to measure the inhibition zones of three variations in cream preparation concentrations. Statistical analysis was carried out with a *One-way* ANOVA test to compare differences in each treatment group using the SPSS Inc. version 25.0. The data continued with a post-hock test with Tukey HSD, with a level of evidence of 95% (*p-value*<0.05) (Darwin *et al*., 2021). The actual data were presented in tables, figures, and narrations.

Table 1. Cream formulation of marigold flower ethanol extract (*Tagetes erecta L.*)

Composition	Base	Formulation		
		F1	F2	F3
Marigold flower ethanol extract	-	2	4	8
Cethyl alcohol	4	4	4	4
Ceteareth-20	3	3	3	3
Stearic acid	2	2	2	2
Silicon oil	1	1	1	1
Methyl propyleneglycol	1	1	1	1
Triethanolamine	1	1	1	1
Cera alba	1	1	1	1
Glycerin	1	1	1	1
Methylparaben	0.02	0.02	0.02	0.02
Propylparaben	0.18	0.18	0.18	0.18
Aquades	100 mL	100 mL	100 mL	100 mL

Source: research results, 2021

Table 2. Antibacterial assay of marigold flower ethanol extract against *S. aureus*

Sample	Inhibition zone (mm)	*Shapiro-Wilk*	F	*p-value*
Positive control (Chloramphenicol)	19.3 ± 0.000	0.964	5335.31	0.000
Formulation 1 (25%)	13.8 ± 0.200	0.907		
Formulation 2 (50%)	18.4 ± 0.500	0.893		
Formulation 3 (100%)	28.9 ± 0.608	0.842		

Table 3. Antibacterial activity of marigold flower ethanol extract

Treatment group	Inhibitory assay results (mm)			
	R1	R2	R3	Mean ± SD
Positive Control (K+)	12.62	12.70	12.74	12.69 ± 0.061[a]
Negative Control (K-)	7.78	7.70	7.72	7.73 ± 0.042[b]
Formulation 1 (25%)	8.54	8.61	8.63	8.59 ± 0.047[c]
Formulation 2 (50%)	9.21	9.22	9.26	9.23 ± 0.026[d]
Formulation 3 (100%)	10.30	10.37	10.38	10.23 ± 0.044[e]

Description: K+: Chloramphenicol; K-: Vanishing cream base; F1: Cream formulation of 25% marigold flower ethanol extract; F2: Formulation of 50% marigold flower ethanol extract cream; F3: 100% marigold flower ethanol extract cream formulation; R1: Replication 1; R2: Replication 2; R3: Replication 3; letter notation is different: there is a significant difference (*p-value*<0.05)

RESULT

Antibacterial activity assay of marigold flower ethanol extract (*Tagetes erecta L.*)

Before the assay, the antibacterial activity of the cream dosage form and the ethanol extract of the marigold flower were tested for the antibacterial activity against *S. aureus* bacteria using an NA medium with an agar well diffusion method. The solidified media was cultured with bacteria and then divided into three on one plate and perforated to place the control and concentration of the extract. The negative control was distilled water and the positive control was chloramphenicol. Evaluation of antibacterial activity was observed after two days of bacterial cultivation in the form of the diameter of the inhibition zone in the form of a clear zone on the media.

Based on the test on the clear zone with *S. aureus* bacteria, 25% marigold flower ethanol extract showed an inhibition zone of 13.800 ± 0.200 mm, marigold flower extract of 50% offered an inhibition zone of 18.400 ± 0.500 mm, which was equivalent to the results of positive control of 50 mg chloramphenicol which showed an inhibition zone diameter of 19.300 ± 0.000 mm against *S. aureus* bacteria with a strong category of inhibition against bacteria. In comparison, 100% marigold flower ethanol extract showed a zone of inhibition against *S. aureus* of 28.900 ± 0.608 mm, which showed powerful inhibition against bacteria. The inhibition zone diameter is presented in Table 2 and Figure 1.

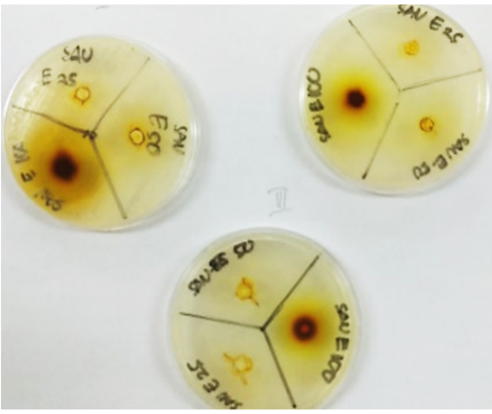

Figure 1. Inhibition zone of marigold flower ethanol extract with concentration of 25%, 50%, and 100%

Antibacterial activity assay of marigold flower ethanol extract (*Tagetes erecta L.*) cream

Based on the cream antibacterial test, it was found that the ethanol extract cream of marigold flowers at concentrations of 25%, 50%, and 100% showed antibacterial activity against *S. aureus*. The resulting inhibition zones were 8.59 ± 0.047; 9.23 ± 0.026; and 10.23 ± 0.044 mm, respectively. The higher the ethanolic extract of marigold flowers added to the vanishing cream base, the larger the inhibition zone diameter against *S. aureus* bacteria. The positive control treatment using chloramphenicol showed an inhibition zone of 12.689 ± 0.061 mm. This happened because chloramphenicol is an antibiotic with bacteriostatic properties. The results of the antibacterial activity test against *S. aureus* are presented in Table 3.

DISCUSSION

The marigold plant (*T. erecta L.*) produces flowers and can be used in traditional medicine (Rajkumari *et al*., 2021; Salehi *et al*., 2018). This plant has compounds that can be used as cosmetics, overcoming skin irritation and treating itching, packaged in cream preparations obtained from the extraction process (Kumar *et al*., 2021; Mekjaruskul *et al*., 2021; Faraz *et al*., 2020). Marigold flowers have many names depending on where they live (Perez-Ortega *et al*., 2017; Tyagi *et al*., 2021). In Javanese, this flower is known as "kenikir," while the Balinese are known as "gumitir" or "gemitir" (Chau *et al*., 2021; Edy and Parwanto, 2019; Singh *et al*., 2020). This flower is generally used by the community for various purposes, especially as a means of ceremony by the Balinese people (Cahyaningrum *et al*., 2020). The development of this flower is very strategic because it has excellent prospects and is used as a means of worship, cosmetics, and traditional medicine. Marigold flower ethanol extract has a tremendous benefit, mainly developed as a traditional medicine to treat acne.

The ability of marigold flower extract to inhibit the growth of *S. aureus* bacteria is caused by the content of the extract, which acts as an antibacterial; these compounds in the form of flavonoids, phenolics, carotenoids, and triterpenoids which have been identified and have antibacterial effects (Burlec *et al*., 2022; Kresnapati *et al*., 2021; Zhang *et al*., 2020). The research of Moline *et al*. (2018) and Pramitha *et al*. (2017) revealed that marigold plants contain flavonoid compounds of the quercetin and phenolic groups, which act as antibacterial. Furthermore, Mekvimol *et al*. (2020) revealed that marigold flowers contain polyphenolic compounds of the flavonol group, namely laricitrin and its glycosides and have inhibitory benefits as an antibacterial against *Streptococcus agalactiae*. Romes *et al*. (2021) found marigold flower extract contains natural photoantioxidants such as quercetin, gallic acid, and polyphenols having medicinal properties (antioxidative antityrosinase, antiviral, and antimicrobial). The extract protects the skin from UVA/UVB rays and improves skin and regenerative treatments (Mertha Adnyana and Sudaryati, 2023).

Furthermore, Cahyaningrum *et al*. (2020) discovered that soap prepared from marigold flowers had antibacterial action against *E. coli* and *S. aureus*. Singh *et al*. (2020) revealed that marigold flowers have phytochemical constituents that have pharmacological effects, anti-nociceptive, anti-inflammatory, antioxidant, insecticide, larvicidal, hepatoprotective, antipyretic, wound healing, antibacterial, antimicrobial, antiepileptic, and antifungal properties. The most investigated and therapeutically beneficial effect is antibacterial and has been clinically tested. Previous research by Burlec *et al*. (2022) revealed silver nanoparticles (AgNPs) from *Tagetes erecta L.* flower having UV-Vis Spectrum at 410 and 420 nm. The extract has antioxidant and antimicrobial activity.

The mechanism that causes the inhibition of bacterial growth is the inhibition of bacterial nucleic acid synthesis. Flavonoids bind to nucleic acids and form a complex that interferes with the function of the template DNA so that the synthesis of DNA, RNA, and bacterial proteins is inhibited (Meurer *et al*., 2019; Padalia and Chand, 2015). Previous research found that flavonoids exhibited antibacterial action against all tested pathogens, with a maximal zone of inhibition of 29.50 mm for *K. pneumonia* (Trinh *et al*., 2020). Carotenoid compounds also have a toxic effect on microorganisms, including *S. aureus* (Zhang *et al*., 2020). Marigold flower phenolics will activate macrophages that stimulate the body's immune system to work as an antibacterial (Selvam *et al*., 2021;

Likasari *et al*., 2021; Ventura-Martinez *et al*., 2018; Verma *et al*., 2020). The normality test yielded a probability value of *p-value*>0.05, while the homogeneity test yielded a significance value of 0.179 > *p-value* > 0.05, indicating that the data were homogenous. The *One-way* ANOVA test yielded a probability value of 0.000 less than *p-value*<0.05, indicating that there was a highly significant difference between the concentrations of cream of marigold flower ethanol extract of 25% (F1), 50% (F2), and 100% (F3) against *S. aureus*. In the follow up test results using Tukey HSD, there were significant differences between the three variations in the concentration of 25%, 50%, and 100% (*p-value*<0.05). The addition of marigold flower ethanol extract in F1 and F3 significantly affected the diameter of the resulting inhibition zone. Hence, based on traditional medicine, it can be inferred that the marigold flower ethanol extract cream has antibacterial qualities and can be created to treat acne.

CONCLUSION

Cream formulations of ethanol extract of marigold flower (*Tagetes erecta L.*) with concentrations of 25%, 50%, and 100% inhibited *S. aureus* in diverse ways. The higher concentration, the more potent the inhibition. The resulting inhibition zones were 8.59 ± 0.047, 9.23 ± 0.026, and 10.23 ± 0.044 mm respectively. As a result, the cream of marigold flower ethanol extract showed antibacterial activity against *S. aureus* and was suitable as a cream dosage form for the traditional treatment of acne vulgaris. In the future, it is expected to carry out acute and chronic toxicity tests of marigold flower ethanol extract cream on other bacteria and carry out integrated clinical trials to produce appropriate, effective, and tested formulations for further use as antiacne.

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