IOSR Journal Of Pharmacy
(e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219
Volume 12, Issue 8 Series. I (August 2022), PP. 16-20
www.iosrphr.org

The Antibacterial Effectiveness Test Of*Melaleuca alternifolia* and *Calendula officinalis* Against Some Skin Infecting Bacteria

Annisa Diyan Meitasari[1], Iin Suhesti[2], Hasna Nabila Qotrunada[3], Putri Sekar Kinasih[4]

Pharmacy Study Program, PoliteknikIndonusa Surakarta
Corresponding Author:Annisa Diyan Meitasari
Received 12 August 2022; Accepted 27 August 2022

Abstract: Microorganisms are highly susceptible to skin lesions, especially bacteria. The examples of bacteria that infect the skin are *S.aureus*, *S.epidermidis*, and *Methicillin Resistant Staphylococcus aureus*. Nowdays, many bacteria have resistance to the antibiotics, so an alternative could be effective in inhibit the growth. Calendula are plants with antioxidant, antifungal, and anti-inflammatory activity. Tea tree oil has antifungal, antiviral, antiprotozoal, and antibacterial activity. The aim of the study is to identify the antibacterial activity of the essential oil of calendula, tea tree, and the combination of calendula and tea tree to against*S.aureus*, *S.epidermidis*, and *MRSA*.

Antibacterial test was carried out by well diffusion method. This study used four treatments, that is 25% tea tree oil, 25% calendula oil, 25% calendula and tea tree oil mixture, and 35% calendula and tea tree oil mixture. As a positive control, chloramphenicol was used for *Staphylococcus aureus* and *Staphylococcus epidermidis* and vancomycin for *MRSA* bacteria, 95% ethanol was used as a negative control. Based on the tests carried out, it was found that calendula and tea tree oil inhibited *S.aureus*, *S.epidermidis*, and *MRSA* bacteria in the resistant inhibition category, except for tea tree oil 25% which inhibited *S.epidermidis* bacteria in the intermediate category.

Keywords: *Calendula, MRSA, Staphylococcus aureus, Staphylococcus epidermidis, Tea Tree*

I. INTRODUCTION

The skin is the largest organ in humans which is the body's first line of protection. The skin is susceptible to infections caused by fungi, viruses and bacteria. Several factors for the occurrence of infection are skin surface ecology, topographic location, endogenous factors, and exogenous factors. Studies on the involvement of microorganisms in humans need to be increased so that an antimicrobial therapeutic approach for treatment can be carried out [1].

Microorganisms that are usually found on the skin are *Propionibacterium spp.*, *Staphylococcus spp.*, *Corynebacterium spp.*, *Malassezia ssp.*, and viruses [2]. Examples of skin infections caused by *Staphylococcus aureus* are folliculitis, mastitis, cellulitis, and wound infections. Treatment of infections caused by *Staphylococcus aureus* can be given topically to intravenously depending on the severity. Examples of antibiotics are cephalexin, erythromycin, cefadroxil, and clindamycin.

Tea tree is a plant that is widely found in Australia. Tea tree oil has many properties including antifungal, antiprotozoal, antibacterial, and anti-inflammatory [3].Tea tree oil is extracted from the leaves and twigs of *Melaleuca alternifolia* which is processed through steam distillation and has the main component, namely terpinene-4-ol which functions as an antimicrobial agent [4]. According to Jan et al [5], this plant is generally used as an antiseptic, antimicrobial, antiparasitic, stimulant, diaphoretic, antispasmodic, and antipyretic. In vitro, the extract also has anticancer activity on various tumor cells. *Calendula officinalis* extract is also used in the treatment of gastritis, colitis, and bleeding in duodenal ulcers.

At this time the resistance of bacteria to antibiotics is increasing. The number of antibiotics to control infection is also limited. So it is necessary to research to obtain alternative uses of antibiotics to treat bacterial infections of the skin.This study aims to determine the antibacterial activity of tea tree oil, calendula oil, and their combination at concentrations of 25% and 35% against *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Methicillin-Resistant Staphylococcus aureus* (*MRSA*).

II. MATERIALS AND METHODS

2.1 Plant material Collection and Authentication

The materials used were obtained from the Pharmacy laboratory of PoliteknikIndonusa Surakarta and some of the chemicals were prepared from distributors or chemical stores. Certified essential oil of calendula flower and tea tree is obtained from RumahAtsiriTawangmangu, Karanganyar. *Staphylococcus aureus, Staphylococcus epidermidis,* and *Methicillin Resistant Staphylococcus aureus* bacteria were obtained at the Microbiology Laboratory of the Faculty of Medicine, SebelasMaret University, Surakarta.

2.2 Chemical Materials

The materials use in this study were calendula oil, tea tree oil, positive control disk, Muller Hinton Agar media, 70% alcohol, 95% alcohol, sterile 0.9% NaCl, aquadest..

2.3 Sterilization

Glass utensils used such as erlenmeyer, test tubes, petri dishes, and beaker glass were washed thoroughly, then dried, wrapped in paper, were sterilized using an oven at 180^0C for an hour. Ose sterilized by burning. Tools and materials such as blue tips, yellow tips, and media were sterilized by autoclaving at 121^0C for 15 minutes.

2.4 Media Preparation

The media used for research is MHA (Muller Hinton Agar). MHA media is available in packaged form, so the method of manufacture is only by dissolving the media in distilled water according to the instructions on the packaging. The media was autoclaved at 121^0C for 15 minutes for the sterilization process, then the MHA media was poured into a Petri dish in LAF (Laminar Air Flow) and allowed to stand at room temperature until solidified. Media that is not used immediately is stored in the refrigerator.

2.5 Bacterial Inoculation

Bacteria were obtained from the Faculty of Medicine, SebelasMaret University, Surakarta. Bacterial inoculation is the process of transferring bacteria from one medium to another. The inoculation method used was the streak plate method, namely by scratching 1 loop of bacteria onto a petri dish that previously contained MHA media.

2.6 Preparation of Bacterial Suspension

The bacterial suspension was made by taking 5 bacterial colonies from the streak plate and mixed with sterile 0.9% NaCl. The concentration of the bacterial suspension was equalized using the standard Mc Farland 0.5. If the suspension is still not clear, sterile 0.9% NaCl can be added.

2.7 Preparation of Essential Oil Concentration Series

Each essential oil was treated the same, which was made at a concentration of 25% v/v and 35% v/v. The solution used to dissolve the essential oil is 95% alcohol.

2.8 Antibacterial Activity Test

Antibacterial activity test using well diffusion method. The bacterial suspension whose concentration was equalized with the McFarland 0.5 standard was taken in an amount of 250 µL, dripped on MHA media, and then flattened using a speeder glass and waited for ±10 minutes. Positive control disks, 30 µL of each essential oil solution, and negative control were dropped into wells, then incubated for 24 hours at 37˚C. The next step is to observe the diameter of the inhibition zone formed. The zone of inhibition is characterized by the presence of a clear area around the disc.

2.9Data Collection Technique

Because this research is experimental, the data collection process is carried out when conducting research in the laboratory. The data that will be obtained from this study is the inhibition of essential oil against skin-infecting bacteria.

The diameter of the inhibition zone of essential oil against skin-infecting bacteria can be seen from the area around the disc where there is no bacterial growth, this area is usually clear.

2.10 Data Interpretation

The average inhibition of 3 replications of each bacterium was interpreted using the M100 Performance Standards for Antimicrobial Susceptibility Testing from CLSI according to the positive control used.

III. RESULTS AND DISCUSSION

The samples used were tea tree (*Malaleuca alternifolia*) oil and calendula (*Calendula officinalis*) oil which were obtained from RumahAtsiriTawangmangu, Karanganyar. The pure essential oil was diluted using 95% ethanol as a solvent to obtain concentrations of 25% and 35%. Calendula and tea tree essential oils with the same concentration were then mixed in a 50:50 ratio to obtain 25% and 35% calendula and tea tree oil solutions, respectively. The solvent used is 95% ethanol, this is based on the character of the essential oil which is easily soluble in organic solvents [6].

Antibacterial tests were carried out in the LAF (Laminar Air Flow) to prevent contamination from the environment. The method used in this study is the well diffusion method. According to research conducted by Nurhayati[7], the well diffusion method can produce a better inhibition zone than the disc diffusion method. In the well diffusion method, the agar media is given a hole filled with the test solution which causes the osmolarity of the test solution to be more thorough and more homogeneous[8].

The average inhibition of 3 replications for each bacterium was interpreted using the M100 Performance Standards for Antimicrobial Susceptibility Testing from CLSI (Clinical and Laboratory Standards Institute) according to the positive control used. The positive control used was the antibiotic chloramphenicol for *Staphylococcus aureus* and *Staphylococcus epidermidis*, while vancomycin was used for *MRSA* bacteria. The choice of chloramphenicol as a positive control was because chloramphenicol is a broad-spectrum antibiotic that is capable of killing both gram-positive and gram-negative bacteria [9]. While vancomycin was chosen because vancomycin is the therapy of choice commonly used in the treatment of *MRSA* infections [10]. The category of inhibition of the positive control used according to CLSI [11] can be seen in the following table:

Table 1. Inhibition Zone Category [11]

Antibiotic	Types of Bacteria	Inhibition Zone Diameter (mm)		
		Sensitive	Intermediate	Resistance
Chloramphenicol	All *Staphylococcus*	≥ 18	13-17	≤12
Vancomycin	All *Staphylococcus*	-	-	17-21

In Muller Hinton Agar (MHA) media, 5 wells were made with a diameter of 5 mm. Each well was filled with negative control (95% ethanol), 25% tea tree oil, 25% calendula oil, combination of 25% calendula oil and 25% tea tree oil, and a combination of 35% calendula oil and 35% tea tree oil with each repetition three times. Positive control tests were carried out on different petri dishes. Antibacterial testing carried out on Muller Hinton Agar (MHA) media can be seen in the Fig. 1.

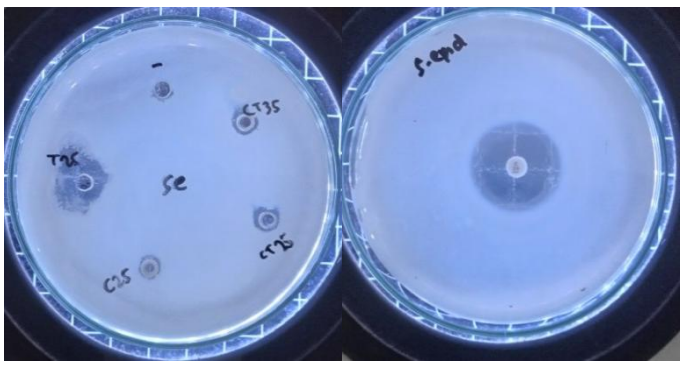

figure 1. calendula, tea tree oil, negative, and positive control tests against *Staphylococcus epidermidis*

The calculation of inhibition zone is measured by calculating the average value of the diameter of the vertical and horizontal inhibition zones and then subtracting the diameter of the well [12]. The results of the calculation of the inhibition zone can be seen in the following Table.

Table 2. Antibacterial Test Results of Tea Tree Oil and Calendula Oil Against *Staphylococcus epidermidis*

Essential Oil	Concentration	Inhibition Zone			Average
		R1	R2	R3	
Tea tree oil	25%	22,5	12	13,5	16
Calendula oil	25%	22	0	1,5	7,8
Calendula and *Tea tree oil*	25%	13	17,5	2,5	11
Calendula and	35%	1,5	15	4	6,8

Tea tree oil					
Chloramphenicol (positive control)	-	-	-	-	27
Ethanol (negative control)	95%	0	0	0	0

At Table 2, it has been seen that the inhibition zones generated from the test solution of 25% tea tree oil, 25% calendula oil, 25% calendula and tea tree oil, and 35% calendula and tea tree oil agains the growth of the *Staphylococcus epidermidis* have a different diameter of the criteria of antibacterial strength. The 25% tea tree oil is showing an average 16 mm hole, with intermediate category. While the inhibition of other test solutions included in the category of resistance, namely ≤12 mm. The positive control used showed an inhibition zone in the sensitive category and in the negative control, there was no inhibition zone at all.

Table 3. Antibacterial Test Results of Tea Tree Oil and Calendula Oil Against *Staphylococcus aureus*

Essential Oil	**Concentration**	**Inhibition Zone**			**Average**
		R1	**R2**	**R3**	
Tea tree oil	25%	9,5	7,5	8,5	8,5
Calendula oil	25%	2,5	3	2	2,5
Calendula and *Tea tree oil*	25%	3,5	6,5	1	3,7
Calendula and *Tea tree oil*	35%	5,5	5	4	4,8
Chloramphenicol (positive control)	-	-	-	-	25
Ethanol (negative control)	95%	0	0	0	0

At Table 3, it has been seen that the inhibition zones generated from the test solution of tea tree oil 25%, calendula oil 25%, calendula and tea tree oil 25%, and calendula and tea tree oil 35% against the growth of *Staphylococcus epidermidis* bacteria have a diameter value of the inhibition zone that is different with different criteria of antibacterial strength. Tea tree oil 25% showed an average inhibition of 16 mm, with intermediate criteria. While the inhibition of other test solutions included in the category of resistance, namely 12 mm. The positive control used showed an inhibition zone which was included in the sensitive category and in the negative control there was no inhibition zone at all.

Table 4. Antibacterial Test Results of Tea Tree Oil and Calendula Oil Against *Methicillin Resistant Staphylococcus aureus*

Essential Oil	**Concentration**	**Inhibition Zone**			**Average**
		R1	**R2**	**R3**	
Tea tree oil	25%	14	4	9,5	9,2
Calendula oil	25%	3	1,5	2	2,2
Calendula and *Tea tree oil*	25%	2,5	2,5	4,5	3,2
Calendula and *Tea tree oil*	35%	2,5	2,5	4,5	3,2
Chloramphenicol (positive control)	-	-	-	-	24,5
Ethanol (negative control)	95%	0	0	0	0

In table 5 it has been seen that the inhibition zones generated from the test solution of tea tree oil 25%, calendula oil 25%, calendula and tea tree oil 25%, and calendula and tea tree oil 35% against the growth of bacteria *Methicillin Resistant Staphylococcus aureus* (*MRSA*) have different inhibition zone diameter values with the same antibacterial strength criteria, which are included in the resistant category (≤12 mm). The positive control used showed an inhibition zone which was included in the sensitive category and in the negative control there was no inhibition zone at all.

The antibacterial test results obtained are different from previous studies conducted by Shi et al [13]which stated that tea tree oil has the ability to inhibit *S.aureus* bacteria with a MIC range of 1-2 mg/ml. The mechanism of bacterial inhibition by tea tree oil is influenced by the presence of a hydrocarbon structure and lipophilicity. Hydrocarbon partitions can enter biological membranes and interfere with the vital functions of bacteria so that bacteria will lyse [3]. According to research conducted by Efstratiou et al [14], methanol and ethanol extracts from calendula flowers have bacterial inhibitory activity with inhibitory powers reaching 16 mm and 13 mm.

IV. CONCLUSION

Tea tree oil 25% has antibacterial activity with intermediate category against *Staphylococcus epidermidis* bacteria and resistant category against *Staphylococcus aureus* and *MRSA* bacteria. Calendula oil 25% has antibacterial activity in the category of resistance to *Staphylococcus epidermidis, Staphylococcus aureus,* and *MRSA* bacteria. The combination of calendula and tea tree oil at concentrations of 25% and 35% did not increase the antibacterial activity to the sensitive category.

Abbreviations

MHA: Muller Hinton Agar; *MRSA*: *Methicillin-Resistant Staphylococcus aureus*; LAF: Laminar Air Flow; CLSI: Clinical & Laboratory Standards Institute.

REFERENCES

[1] E. A. Grice and J. A. Segre, "The Skin Microbiome," *Nat. Rev. Microbiol.*, vol. 9, no. 4, pp. 244–253, 2011, doi: 10.1038/nrmicro2537.The.

[2] C. Schmidt, "Out of Your Skin," *Nat. Biotechnol.*, vol. 38, no. 4, pp. 392–397, 2022, doi: https://doi.org/10.1038/s41587-020-0473-8.

[3] C. F. Carson, K. A. Hammer, and T. V. Riley, "Melaleuca alternifolia (tea tree) oil: A review of antimicrobial and other medicinal properties," *Clin. Microbiol. Rev.*, vol. 19, no. 1, pp. 50–62, 2006, doi: 10.1128/CMR.19.1.50-62.2006.

[4] T. P. Sari, "Efektivitas Obat Kumur Tea Tree Oil 0,2% Dalam Menurunkan Jumlah Koloni Bakteri Rongga Mulut Pada Mahasiswa Kepaniteraan Klinik Departemen Bedah Mulut Dan Maksilofasial Fakultas Kedokteran Gigi Usu," Universitas Sumatera Utara, 2018.

[5] N. Jan, K. I. Andrabi, and R. John, "Calendula officinalis - An Important Medicinal Plant with Potential Biological Properties," *Proc Indian Natn Sci Acad.*, vol. 83, no. 4, pp. 769–787, 2017, doi: 10.16943/ptinsa/2017/49126.

[6] E. Hanani, *Analisisi Fitokimia*. Jakarta: EGC, 2015.

[7] L. S. Nurhayati, N. Yahdiyani, and A. Hidayatulloh, "Perbandingan Pengujian Aktivitas Antibakteri Starter Yogurt dengan Metode Difusi Sumuran dan Metode Difusi Cakram," *J. Teknol. Has. Peternak.*, vol. 1, no. 2, pp. 41–46, 2020, doi: 10.24198/jthp.v1i2.27537.

[8] E. Prayoga, "Perbandingan Efek Ekstrak Daun Sirih Hijau (Piper betle L .) dengan Metode Difusi Disk dan Sumuran Terhadap Pertumbuhan Bakteri Staphylococcus aureus," Universitas Islam Negeri Syarif Hifayatullah, 2013.

[9] S. B. Utomo, M. Fujiyanti, W. P. Lestari, and S. Mulyani, "Uji Aktivitas Antibakteri Senyawa C-4-Metoksifenilkaliks[4]Resorsinarena Termodifikasi Hexadecyltrimethylammonium-Bromide Terhadap Bakteri Staphylococcus Aureus Dan Escherichia Coli," *J. Kim. dan Pendidik. Kim.*, vol. 3, no. 3, pp. 201–209, 2018.

[10] H. Yuwono and M. Biomed, "Pandemi Resistensi Antimikroba : Belajar dari *MRSA*," *J. Kedokt. dan Kesehat.*, vol. 42, no. 1, pp. 2837–2850, 2010.

[11] CLSI, *M100 Performance Standards for Antimicrobial Susceptibility Testing*, 30th Edit. Clinical And Laboratory Standards Institute, 2020.

[12] N. L. A. P. Winastri, H. Muliasari, and E. Hidayati, "Aktivitas Antibakteri Air Perasan dan Rebusan Daun Calincing (Oxalis corniculata L.) terhadap Streptococcus mutans," *Ber. Biol.*, vol. 19, no. 2, pp. 223–230, 2020.

[13] C. Shi *et al.*, "Effect of tea tree oil on Staphylococcus aureus growth and enterotoxin production," *Food Control*, vol. 62, no. November, pp. 257–263, 2016, doi: 10.1016/j.foodcont.2015.10.049.

[14] E. Efstratiou, A. I. Hussain, P. S. Nigam, J. E. Moore, M. A. Ayub, and J. R. Rao, "Antimicrobial activity of Calendula officinalis petal extracts against fungi, as well as Gram-negative and Gram-positive clinical pathogens," *Complement. Ther. Clin. Pract.*, vol. 18, no. 3, pp. 173–176, 2012, doi: 10.1016/j.ctcp.2012.02.003.