Walisongo Journal of Chemistry
Walisongo Journal of Chemistry Vol. 5 Issue 2 (2022), 177-181
ISSN: 2621-5985 (online); 2549-385X (print)
DOI: https://doi.org/10.21580/wjc.v5i2.13292

Potential of Essential Oil *Eucalyptus botryoides* Leaves as an Antibacterial in Hand Sanitizer

Andi Nur Fitriani Abubakar[1*], Syahdam Karneng[1], Firnanelty[2], Rezki Nur Damayanti[1], St. Aisyah[1], Ahmad Bikharuddin[3]

[1]Department of Chemistry, Faculty of Science, Universitas Muhammadiyah Bulukumba, South Sulawesi, Indonesia
[2]Department of Chemistry, Faculty of Science and Technology, Universitas Islam Negeri Alauddin Makassar, South Sulawesi, Indonesia
[3]Graduate School of Medicine, Dentistry and Pharmaceutical Scinces, Okayama University, Japan

*Corresponding author: a.nurfitriani@umbulukumba.ac.id

Received: 17 October 2022; Accepted: 22 December 2022; Published: 25 December 2022

Abstract

Hand sanitizer is an antiseptic product that is widely used to prevent the spread of bacteria and viruses. Hand sanitizer preparations circulating in the market still use alcohol as an antibacterial agent. One of the natural antibacterial Eucalyptus is an antibacterial agent, and essential oil extracted from Eucalyptus can be named as natural ingredient. The purpose of this study was to determine the potential of essential oil E. botryoides leaves as an antibacterial ingredient to make of hand sanitizer. Eucalyptus essential oil is extracted from its leave using steam distillation method and formulated in the making of hand sanitizer gel with various concentrations of 2%, 4%, and 6%. The formulation of hand sanitizer gel was tested for antibacterial, organoleptic, homogeneity, pH, and dispersibility. The results showed that the more concentration of essential oil from Eucalyptus botryoides leaves added to the hand sanitizer gel, the more its antibacterial activity increased. The results of organoleptic observations, homogeneity, pH, and dispersibility of hand sanitizer gel preparations have met the requirements of SNI No.06-2588-1992. Thus, essential oil from E. botryoides leaves has potential as an antibacterial in hand sanitizer gel products.

Keywords: *Essential oil; Eucalyptus botryoides; antibacterial; hand sanitizer*

Introduction

Hand sanitizer is a product that is widely used to prevent the spread of bacteria and viruses. Hand sanitizer can inhibit to kill bacteria and viruses from growing and causing disease. Hand sanitizers on the market are made in gel form and still use alcohol as an antibacterial agent. However, excessive use of alcohol continuously can erode moisture and cause skin irritation (Widyawanti dan Salsabilah, 2020). The most common pathogenic bacteria found on the skin of human hands is *Staphylococcus aureus*. These bacteria in large numbers can cause various kinds of infections such as pneumonia, meningitis, empyema, endocarditis, acne, pyoderma, or impetigo (Tong *et al.,* 2015).

One of the natural antibacterial that can be used to replace alcohol is the *Eucalyptus* species. *Eucalyptus* is one of the essential oil-producing plants, especially in the leaves, and is often reported to have antibacterial properties that can inhibit bacterial growth (Khammassi, 2022; Bhuyan, 2017; Ammer, 2016).

Several studies have shown that essential oil from *Eucalyptus* leaves has a strong inhibition of bacterial growth against *Staphylococcus aureus* and *Escherichia coli* (Sebei *et al.*, 2015; Elaissi *et al*., 2012; Bachir & Benali, 2012). In addition, hand sanitizer gel essential oil from *Eucalyptus camaldulensis* leaves with a concentration of 6% is an effective concentration to inhibit *Staphylococcus aureus* and *Staphylococcus epidermidis* (Matondang, 2019). In another study, it was also reported that the essential oil of *Eucalyptus pellita* has an antibacterial activity which gives *Staphylococcus aureus* a clear zone of 13.1 mm (Astiani, 2014).

One of the *Eucalyptus* species that grows in Indonesia is *Eucalyptus botryoides*. Based on the literature search, *E. botryoides* which grows in Indonesia has never been reported regarding its activity as an antibacterial in hand sanitizers. Therefore, the purpose of this study was to determine the potential of essential oil from *E. botryoides* leaves as an active bacterial ingredient in the manufacture of hand sanitizer gels that are effective and safe to use.

Research Methods

Materials

The tools used are a set of distillation tools, analytical balance, oven, autoclave, laminar air flow, magnetic stirrer HJ-3, pH meter ATC, micropipette, stative, clamps, separating funnel, erlenmeyer, measuring flask, beaker, measuring cup, test tube, petri dish, dropper, tube rack, spatula, bunsen, ossicle needle, and caliper. The materials used were *E. botryoides* leaves, anhydrous sodium sulfate, *Staphylococcus aureus* ATCC 25923, Mueller Hinton Agar (MHA) media, tetracycline, disc paper, triethanolamine (TEA), glycerin, carbopol 940, methylparaben, aluminum foil, and aquadest.

Sample collection and preparation

The leaves of *E. botryoides* were obtained from the Pattapang area, Tinggimoncong, Gowa, South Sulawesi. The leaves were collected in the morning, washed and dried in the room to protect from direct sun light. Then, the dried leaves were chopped to facilitate the distillation process.

Extraction of essential oils from Eucalyptus botryoides leaves

Extraction of essential oil from *E. botryoides* leaves using steam distillation method. A total of 1.7 kg of pieces of *E. botryoides* leaves were put into the distillation apparatus. Furthermore, the distillation was carried out for 4 hours with temperatures ranging from 94-98^{0}C. The distillate obtained was added with anhydrous sodium sulfate to absorb water in the essential oil, then separated between the water and the essential oil.

Making hand sanitizer gel formulation

The hand sanitizer gel formulation was made with three concentration variances of essential oil from *E. botryoides* leaves, namely 2%, 4%, and 6%. The composition of the hand sanitizer gel formulation can be seen in Table 1.

Table 1. Formulation of Hand Sanitizer Gel

Ingredients	F1	F2	F3
Essential oil (%)	2	4	6
Carbopol (g)	0.25	0.25	0.25
Glycerin (ml)	2	2	2
Methylparaben (g)	0,1	0,1	0,1
TEA (ml)	0,1	0,1	0,1
Aquadest (ml)	100	100	100

Antibacterial activity test

The antibacterial activity test of the essential oil hand sanitizer gel formulation from *E. botryoides* leaves was carried out using the Kirby-Bauer disc diffusion method on media MHA. *Staphylococcus aureus*

 | ISSN 2621-5985 (online) | ISSN 2549-385X (print)
Volume 5, Issue 2, 2022

inoculum was streaked into MHA media and incubated for 24 hours at 37°C. Place the paper discs that have been soaked in hand sanitizer gel preparations with variations in essential oil concentrations of 2%, 4%, 6%, negative control (aquadest), and positive control (tetracycline). Then it was incubated for 48 hours at 37°C until an inhibition zone was formed and measured using a caliper.

Physical analysis of hand sanitizer gel

Physical analysis of the hand sanitizer gel preparation includes visual organoleptic observations, homogeneity, pH, and dispersion (Octavia, 2016).

Result and Discussion

Antibacterial activity

An antibacterial activity test was carried out to see the potential of essential oil gel hand sanitizer from *E botryoides* leaves. Antibacterial effectiveness was indicated by the presence of an inhibitory zone around the disc that already contained the sample of the test solution (Handayani *et al.,* 2020). In this method, the diameter of the inhibition zone against *Staphylococcus aureus* was observed. The inhibition zone obtained from the antibacterial test results of essential oil hand sanitizer gel from *E. botryoides* leaves can be seen in Table 2.

Table 2. Antibacterial Activity Test Results Against *Staphylococcus aureus*

Sample	Inhibitory Zone Diameter (mm)
Negative control	0
Positive control	26.76
F1	7.17
F2	7.58
F3	8.14

According to Davis and Stout (1971), the inhibition zone category includes an inhibition zone diameter of 5 mm including a weak category, an inhibition zone of 5-10 mm including a moderate category, an inhibition zone of 10-20 mm including a strong category and an inhibition zone of 20 mm including a very strong category. Based on these criteria, the antibacterial power of essential oil hand sanitizer gel from *E. botryoides* leaves against *Staphylococcus aureus* bacteria with concentrations of 2% (7.17 mm), 4% (7.58 mm) and 6% (8.14 mm) including a moderate category. The higher the concentration of essential oil from *E. botryoides* leaves added to the hand sanitizer gel, the greater the antibacterial activity. Chemical compounds that act as antibacterials in eucalyptus essential oil are 1,8-cineole (Miguel *et al*., 2018). Thus, the essential oil from *E. botryoides* leaves formulated in hand sanitizer gel has the potential to be developed as an antibacterial product.

Physical analysis of hand sanitizer gel

An organoleptic test was carried out by observing visually including shape, color, and smell. The organoleptic results of the three hand sanitizer gel formulas with variations of essential oil from *E. botryoides* leaves at concentrations of 2%, 4%, and 6% can be seen in Table 3.

Table 3. Results of Organoleptic Observations of Hand Sanitizer Gel

Organoleptic	F1	F2	F3
Color	Cloudy white	Cloudy white (+)	Cloudy white (++)
Smell	Typical *Eucalyptus*	Typical *Eucalyptus* (+)	Typical *Eucalyptus* (++)
Shape	Gel	Gel	Gel

The addition of essential oil from *E. botryoides* leaves affects the color, smell, and shape of the hand sanitizer gel. The color intensity of the hand sanitizer gel preparation increases with the increase in the concentration of essential oils. The smell of the hand sanitizer gel preparation became more distinctly *Eucalyptus* with the increase in the concentration of essential oil from the leaves of *E. botryoides*. The shape of hand sanitizer is in the shape of a gel.

Table 4. Physical Properties of Hand Sanitizer Gel

Sample	Test		
	Homogeneity	pH	Spread (cm)
F1	Homogeneous	7.5	5.6
F2	Homogeneous	7.4	5.6
F3	Homogeneous	7.4	5.6

The homogeneity test was carried out by observing the hand sanitizer gel. Tests were carried out to observe the uniform distribution by hand sanitizer gel (Putri *et al*., 2020). All essential oil hand sanitizer gel formulations from *E. botryoides* leaves were physically homogeneous. This shows that the ingredients used in the hand sanitizer gel are dissolved and completely mixed. In addition, the hand sanitizer gel made meets the requirements of SNI No. 06-2588-1992, namely the gel preparation does not have coarse grains or lumps.

The pH test aims to determine the sensitivity of hand sanitizer gel preparations to the skin. The range of requirements for the pH value of hand sanitizer preparations that meet the requirements of SNI No. 06-2588-1992 is 4.5-8.0. The results showed that the pH values of the three hand sanitizer gel formulations with the addition of essential oil from *E. botryoides* leaves met the requirements of SNI No. 06-2588-1992 so that the essential oil gel hand sanitizer from *E. botryoides* leaves is safe to use on the skin.

The spreadability test was carried out to determine the ability to spread hand sanitizer gel on a skin surface (Rohmani and Kuncoro, 2019). The dispersion test value meets the requirements of SNI No. 06-2588-1992 which is 5-7 cm. Based on the results of the spreadability test carried out on the three hand sanitizer gel formulations, the dispersion value was 5.6 cm. This shows that the three formulations of hand sanitizer gel with the addition of essential oil from *E. botryoides* leaves have good dispersion and meet the requirements of SNI No. 06-2588-1992.

Conclusion

Essential oil from *E. botryoides leaves* has antibacterial activity against *Staphylococcus aureus* after being formulated in hand sanitizer gel with various concentrations of 2%, 4%, and 6%. The antibacterial activity increased as the concentration of essential oil from *E. botryoides* leaves was added to the hand sanitizer gel. The results of organoleptic observations, homogeneity, pH, and dispersibility of hand sanitizer gel preparations have met the requirements of SNI No. 06-2588-1992. An essential oil from *E. botryoides* leaves showed antibacterial activity which potentially formulated as the ingredient of hand sanitizer gel.

Acknowledgement

We would like to thank the Directorate General of Higher Education, Research and Technology of the Ministry of Education, Culture, Research and Technology for providing research grants with Decree No. 161/E5/PG.02.00.PT/2022 and contract agreement no. 246/LL9/PK.00/PG/2022. Furthermore, thanks to the chairman and staff of LPPM Muhammadiyah University of Bulukumba for their support and direction.

Reference

Ammer, M.R., Shah, Z., Muhammad, K., Muhammad, B., Shazia, E., Danfeng, H., Shengquan, C., 2016, Optimization of antibacterial activity of *Eucalyptus tereticornis* leaf extracts against *Escherichia coli* through response surface methodology, *Journal of Radiation Research and Applied Sciences*, 376-385.

Astiani, D.P., Jayuska, A., Arreneuz, S., 2014, Uji Aktivitas Antibakteri Minyak Atsiri *Eucalyptus pellita* Terhadap Bakteri *Escherichia coli* Dan *Staphylococcus aureus*, *JKK*, 3(3), 49-53.

Bachir, R.G., Benali M, 2012, Antibacterial activity of the essential oils from the leaves of *Eucalyptus globulus* against *Escherichia coli* and *Staphylococcus*

 | ISSN 2621-5985 (online) | ISSN 2549-385X (print)
Volume 5, Issue 2, 2022

aureus, *Asian Pacific Journal of Tropical Biomedicine*, 2(9), 739-742.

Bhuyan, D.J., Q.V, Vuong., A.C, Chaimers., L.A, Van, A., M.C, Bowyer., C.J, Scarlett., 2017, Phytochemical, antibacterial and antifungal properties of an aqueous extract of Eucalyptus microcorys leaves, *South African Journal of Botany*, 180-185.

Davis, W.W., T.R, Stout, 1971, Disc Plate Method of Microbiological Antibiotic Assay, *American Society for Microbiology*, 2(4), 659-665.

Elaissi, A., Rouis, Z., Salem, N.A.B., Mabrouk, S., Salem, Y.B., Salah, K.B.H., Aounis, M., Farhat, F., Chemli, R., Skhiri, F.H., Khouja, M.L., 2012, Chemical composition of 8 eucalyptus species' essential oils and the evaluation of their antibacterial, antifungal and antiviral activities, *BMC Complementary and Alternative Medicine*, 12(81), 1-15.

Handayani, S.N., Purwanti, A., Windasari, Ardian, M.N., 2020, Uji Fitokimia dan Aktivitas Ekstrak Etanol Daun Kencana Ungu (*Ruellita tuberosa* L.), *Walisongo Journal of Chemistry*, 3(2), 66-70. https://doi.org/10.21580/wjc.v3i2.6119.

Khammassi, M., Flavio, P., Ismail, A., Sana, K., Lamia, H., Filomena, N., Florinda, F., Vincenzo, D.F., 2022, Chemical Composition and Phytotoxic, Antibacterial and Antibiofilm Activity of the Essential Oils of *Eucalyptus occidentalis*, *E. striaticalyx* and *E. stricklandii*, *Molecules*, 27, 5820, https://doi.org/10.3390/molecules27185820.

Matondang, A.F., 2019, Uji Aktivitas Antibakteri Gel *Hand Sanitizer* Minyak Atsiri Daun Eukaliptus (*Eucalyptus camaldulensis* Dehnh.) Terhadap *Staphylococcus aureus* dan *Staphylococcus epidermidis*, *Skripsi*, Universitas Sumatera Utara Medan.

Miguel, M.G., Custódia, G., Maria, D.A., Soraia, L., Maria, L.F., Cristina, M., Isabel, C.G., Javier, V., Ana, C.F., 2018, Antibacterial, Antioxidant, and Antiproliferative Activities of Corymbia citriodora and the Essential Oils of Eight Eucalyptus Species, *Medicines*, 5, 61, doi:10.3390/medicines5030061.

Octavia, N., 2016, Formulasi Sediaan Gel *Hand Sanitizer* Minyak Atsiri Pala (*Myristica Fragrans*houtt.) : Uji Stabilitas Fisik Dan Uji Aktivitas Antibakteri Terhadap Bakteri *Staphylococcus aureus*, *Skripsi*, Universitas Muhammadiyah Surakarta.

Putri, M.A., Saputra, M.E., Amanah, I.N., Musiam, S., Fabiani, V.A., 2020, Hand Sanitizer Ekstrak Daun Pucuk Idat (*Cratoxylum glaucum*) sebagai Antibakteri *Staphycocus aureus* dan *Escherichia coli*, *ALCHEMY Jurnal Penelitian Kimia*, 16(2), 227-231.

Rohmani, S., Kuncoro, M.A.A., 2019, Uji Stabilitas dan Aktivitas Gel Handsanitizer Ekstrak Daun Kemangi, *Journal of Pharmaceutical Science and Clinical Research*, 01, 16-28.

Sebei, K., Sakouhi, F., Herchi, W., Khouja, M.L., Boukhchina, S., 2015, Chemical composition and antibacterial activities of seven Eucalyptus species essential oils leaves, *Biological Research*, 48(7), 1-5.

Standar Nasional Indonesia, 1992, Detergen Sintetik Cair Pembersih Tangan, B*adan Standarisasi Nasional*, No 06-2588.

Tong, S.Y.C., Joshua, S.D., Emily, E., Thomas, L.H., Vance, G.F., Jr., 2015, Staphylococcus aureus Infections: Epidemiology, Pathophysiology, Clinical Manifestations, and Management, *Journal Clinical Microbiology Reviews*, 28(3) doi:10.1128/CMR.00134-14.

Widyasanti, A., Shalsabilla, R.T., 2020, Karakterisasi Sediaan Antiseptik Gel Handmade Dengan Penambahan Bahan Aktif Minyak Atsiri *Eucalytus* Dan *Grapefruit*, *Agroindustrial Technology Journal*, 04(02), 136-144. http://dx.doi.org/10.21111/atj.v4i2.4965

 | ISSN 2621-5985 (online) | ISSN 2549-385X (print)
Volume 5, Issue 2, 2022