

Antifungal Ability as an Antimicrobial of Eucalyptus Oil (*Melaleuca leucadendra*) in Inhibiting the Growth of *Candida albicans*

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ABSTRACT

The Indonesian community recognizes eucalyptus oil as one of the traditional medicines. Eucalyptus oil is known for its antimicrobial properties, which are antibacterial, antiviral, and antifungal. This study aimed to determine the antifungal effect as an antimicrobial of eucalyptus oil (*Melaleuca leucadendra*) produced by MSME Sendang Arum Lamongan in inhibiting the growth of *Candida albicans*. The study used a true experimental post-test-only control group design with the antifungal diffusion test method. Sampling was done by simple random sampling with eucalyptus oil concentrations of 20%, 40%, 60%, 80%, and 90%. The data obtained was inhibition zone diameter, which was analyzed using IBM SPSS statistics 26 software. In this study, the diameter of the inhibition zone of positive control, negative control, 40% concentration, 60% concentration, 80% concentration, and 90% concentration was 19.63 mm, 0.00 mm, 26.36 mm, 41.15 mm, 49.69 mm, 56.09 mm, 61.36 mm, respectively. Non-parametric statistical analysis using the Kruskal-Wallis test obtained $p < 0.05$, indicating an effect between eucalyptus oil (*M. leucadendra*) on the ability to inhibit the growth of *Candida albicans*. The post-hoc test with Dunnett T3 found that the concentration of eucalyptus oil 40%, 60%, 80%, and 90% had significant differences from the positive control (Nystatin 100 IU). Eucalyptus oil (*M. leucadendra*) can inhibit the growth of *Candida albicans*. Therefore, eucalyptus oil has great potential as an alternative or adjunctive therapy for treating candidiasis caused by *Candida albicans*.

Keywords: Antimicrobial, Eucalyptus oil, *Melaleuca leucadendra*, *Candida albicans*, public health

INTRODUCTION

People have recognized eucalyptus oil (*Melaleuca leucadendra*) as a traditional medicine for addressing various health complaints.¹ *Melaleuca leucadendra*, is a tropical plant, can grow well in Indonesia, including Lamongan.² Eucalyptus oil has a potent antimicrobial ability, proven by being able to inhibit the growth of *Pseudomonas aeruginosa*, *Candida albicans*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi*.³⁻⁶ Eucalyptus oil contains 45 active compounds, with the most significant proportion being the compound 1,8-cineole, as much as 72.30%.^{7,8} The compound can destroy the cell walls of bacteria and fungi.⁹ Compared to drugs, herbal compounds have the advantages of low toxicity, diverse mechanisms of action, and rarely cause side effects.¹⁰ Therefore, eucalyptus oil is widely researched as an alternative treatment, one of which is to overcome *Candida albicans* infection.

Candida albicans can cause mild skin infections to life-threatening systemic infections, especially in immuno compromised patients, and is the most common organism causing oral candidiasis (51%) and vulvovaginal candidiasis (42%).¹¹⁻¹⁴ The increasing incidence of opportunistic infections needs attention to reduce the risk of morbidity and mortality in immunocompromised patients.¹⁵ In recent decades, the incidence of resistance of *Candida sp.* to several antifungal therapies has become a concern for health workers.¹⁶ Antifungal treatment generally uses four main classes of drugs: polyenes, pyrimidine analogs, echinocandins, and triazoles.¹⁷ Azole group drugs, such as Fluconazole, have a high % resistance to *Candida albicans* of 35.3%, while Nystatin has a resistance of 5.33%.¹⁸

In the research of Keereedach *et al.*, eucalyptus oil can potentially inhibit the growth of *Candida albicans* through the mechanism of damaging membrane integrity and inhibiting biofilm formation. The study also found the ability of eucalyptus oil to reduce

Candida albicans resistance to Fluconazole expression of the MDR1 gene, which plays a role in *Candida albicans* resistance to Fluconazole.¹⁹⁻²¹

Research on the antifungal ability as an antimicrobial of eucalyptus oil needs to be developed to explore the potential of eucalyptus oil as an alternative medicine or additional compound in overcoming *Candida albicans* infection. In this study, the eucalyptus oil used came from MSME Sendang Arum Lamongan, Indonesia. Sendang Arum MSME's is one of the eucalyptus oil producers in Lamongan, managed by the local village community. Eucalyptus oil from MSME Sendang Arum was tested for antibacterial properties and found to be effective in inhibiting the growth of *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella typhi*.³⁻⁶ This research is expected to drive the local community's economy and improve the Indonesian people's health level in accordance with SDG's points 3 and 8.

METHODS

This study was an experimental study that tested the antifungal activity as an antimicrobial of eucalyptus oil against the growth of *Candida albicans* fungus at several concentration variations: 20%, 40%, 60%, 80%, and 90%. This study employs variations in concentration, utilizing five distinct levels ranging from lowest to highest, to identify the particular concentration of eucalyptus oil that exhibits antifungal properties. The selection of concentration was informed by prior research conducted by Targanski *et al.*, which employed various concentrations. This research was conducted at the Microbiology Laboratory, Department of Medical Microbiology and Parasitology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.

In this study, samples were used in the form of *Candida albicans* clinical isolates obtained from the Microbiology Laboratory, Department of Medical Microbiology and Parasitology, Faculty of Medicine,

Universitas Airlangga. Clinical isolates were taken as much as 1 Ose to be mixed in Phosphate-Buffered Saline (PBS) solution, then homogenizing this solution using a vortex at 120 rpm. The fungal suspension that has been made equalized turbidity with 0.5 Mc Farland standard solution (1.5×10^8 CFU/mL) (Biosan DEN-1B Mc Farland Densitometer, No cat. BS-050104-AAF, Latvia).

The eucalyptus oil used in this study is produced by Sendang Arum MSMEs in Sambeng District, Lamongan Regency, East Java, Indonesia. The process of making eucalyptus oil is done through a distillation process. The Herbal Materia Medica Laboratory, Batu, Indonesia, identified the eucalyptus species used in this study. PERHUTANI Laboratory has tested the cineole content (73%) in eucalyptus oil. The eucalyptus oil will be dissolved in ethyl acetate and divided into five different dilution concentrations: 20%, 40%, 60%, 80%, and 90%.

The well diffusion method was used in this study to determine the inhibitory ability of eucalyptus oil against *Candida albicans* growth. Beginning with the preparation of *Candida albicans* suspension and preparation of eucalyptus oil. *Candida albicans* suspension was streaked in solid Sabouraud Dextrose Agar (SDA) media (Oxoid CM0041) using a sterile Ose. It made wells with a diameter of 6 mm on SDA media using a corkborer produced by the Microbiology Laboratory. One hundred μ L of each eucalyptus oil concentration was taken, put in the wells, and labeled. This study was repeated three times.

The positive control used was Nystatin antifungal disc (Liofilchem 9078) 100 IU, and the negative control was ethylacetate. The results were read by measuring the inhibition zone formed with a caliper after incubation for 2x24 hours at 37°C. Then, the diameter of the inhibition zone was measured.

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Table 1. Diameter of zone of inhibition of eucalyptus oil against *Candida albicans*

Intervention	R1 (mm)	R2 (mm)	R3 (mm)	Average Zone of Inhibition (mm)
Control+	19.63	19.63	19.63	19.63
Control-	0	0	0	0
20%	28.08	26.23	24.77	26.36
40%	39.65	43.77	40.02	41.15
60%	50.04	47.33	51.71	49.69
80%	56.05	57.24	54.98	56.09
90%	62.04	58.33	63.71	61.36

Table 2. Diameter of inhibitory of Nystatin based on the CLSI

Antifungal	Species	Category Interpretation and Zone Diameter Limits		
		Sensitive	Intermediate	Resistant
Nystatin 100 IU	<i>Candida albicans</i>	≥15	10-14	≥10

Table 3. Dunnet T3 test of the zone of inhibition produced by several concentrations of eucalyptus oil against the growth of *Candida albicans*

Intervention	Control +	Control -	20%	40%	60%	80%	90%
Control+	-						
Control-	-	-					
20%	0.95	0.007	-				
40%	0.018	0.005	0.011	-			
60%	0.009	0.003	0.002	0.084	-		
80%	0.002	0.001	0.000	0.016	0.142	-	
90%	0.007	0.003	0.002	0.007	0.047	0.349	-

Table 4. Results of laboratory analysis of eucalyptus oil of MSME Sendang Arum Lamongan

Parameter	Indonesia National Standard	Result	Measuring Device
Specific gravity in 25C°(g/mL)	0.900-0.930	0.911	Pycnometer
The refractive indexin 20°C	1.450-1.470	1.465	Refractometer
Optical rotation (°)	-4°until 0°	-	Polarimeter
Solubility in ethanol	Clear	Clear	Ethanol 80%
1.8-cineol concentration (%)	Super : >60% Major : 55% - 60% Primary : 50% - <55%	72.30	Gas chromatography
Color	Colourless, greenish/yellowish and clear	Clear and greenish	Visual
Odor	Typical eucalyptus odor	Typical eucalyptus odor	Sense of smetl

RESULTS AND DISCUSSIONS

In this study, the inhibition ability as an antimicrobial of eucalyptus oil against *Candida albicans* can be seen in Table 1.

According to the Clinical and Laboratory Standards Institute (CLSI) (Table 2), Nystatin 100 IU positive control has a sensitive interpretation if an inhibition zone of ≥ 15 mm is formed, intermediate if an inhibition zone of 10-14 mm is obtained, and resistant if the inhibition zone formed is ≤ 10 mm.^{22,23}

In this study, the average diameter of the inhibition zone of the 100 IU Nystatin disk was 19.3 mm. Therefore, the *Candida albicans* specimen used in this study can be considered sensitive to Nystatin, so the positive control in this study can be reliable. In this study, all concentrations had a higher inhibition diameter than Nystatin 100 IU.

The results of the obtained inhibition zone diameters were processed using IBM SPSS statistics 26 software. The first statistical test employed was the Shapiro-Wilk test for normality and homogeneity. The normality test obtained a p-value greater than 0.05, indicating that the data were normally distributed. However, a p-value less than 0.05 was obtained in the homogeneity test, indicating that the data was not homogeneous. Since data was not normally distributed, the analysis continued using the Kruskal-Wallis non-parametric test. In the Kruskal-Wallis test, a p-value less than 0.05 was obtained. These results indicate that the different concentrations of eucalyptus oil produced by MSMEs in Lamongan inhibit *Candida albicans* growth.

Further post-hoc test was required using the Dunnet T3 test to determine the significant difference in the average diameter of the inhibition

zone between positive and negative control variables against several concentrations of eucalyptus oil. There was a significant difference ($p < 0.05$) in the negative control against eucalyptus oil concentrations of 20%, 40%, 60%, 80%, and 90%. In the positive control, there was a significant difference ($p < 0.05$) in the concentration of eucalyptus oil 40%, 60%, 80%, and 90%.

Based on several previous studies, eucalyptus oil of Sendang Arum MSMEs can inhibit the growth of *Escherichia coli*. In this study, the content of 1.8-cineole in eucalyptus oil amounted to 72.30%. The identification results obtained from the PERHUTANI Laboratory can be seen in Table 4.

Eucalyptus oil is a herbal plant spread across various islands in Indonesia.²³ Eucalyptus oil contains 32 active compounds, with the main compound being 1.8-cineole, which has antifungal abilities.^{8,23} The compound 1.8-cineole contained in eucalyptus oil at high concentrations can induce ROS-dependent cell death and inhibit hyphal morphogenesis; at lower concentrations, the compound 1.8-cineole can inhibit biofilm formation of *Candida albicans*.²⁰ Eucalyptus oil also can increase the effectiveness of the drug Fluconazole against *Candida albicans*.²⁴

Candida albicans is highly resistant to the antifungal drug Fluconazole at 25-35.3%.^{24,25} This resistance is caused by genetic and phenotypic mutations.²⁶ Genetic mutations occur in several genes, such as the MDR1, ERG11, and CDR1 genes.²⁷ Over expression of the MDR1 gene disrupts membrane transport in *Candida albicans*.²⁸ Meanwhile, mutation of the ERG11 gene causes failure in ergosterol biosynthesis in the *Candida albicans* cell wall.²⁹ In the research of Keereedach *et al.*, the mixture of eucalyptus oil with Fluconazole was able to reduce the Minimum Inhibitory Concentration (MIC) of Fluconazole-resistant *Candida albicans* by 8 times the MIC of Fluconazole by decreasing the expression of the MDR1 gene.²¹

In this study, eucalyptus oil demonstrated antifungal activity as an antimicrobial against *Candida albicans*. At a concentration of 20%, eucalyptus oil had an average inhibition zone diameter of 26.36 mm, greater than the inhibition zone produced by Nystatin (19.63 mm), the positive control. Additionally, other concentrations also exhibited larger inhibition zone diameters. This result indicates that eucalyptus oil produced by Lamongan MSMEs was more effective than Nystatin 100 IU disks at concentrations above 20% in inhibiting the growth of *Candida albicans*.

The results of non-parametric data analysis through the Kruskal-Wallis test showed the effect ($p < 0.05$) of eucalyptus oil in inhibiting the growth of *Candida albicans*. In the Dunnet T3 follow-up test, there was no significant difference ($p > 0.05$) between 100 IU Nystatin disk and 20% concentration of eucalyptus oil in the ability to inhibit the growth of *Candida albicans*. However, 40%, 60%, 80%, and 90% at higher concentrations had significant differences ($p < 0.05$) against the positive control Nystatin 100 IU. This finding could be due to increased levels of the active compound 1.8-cineole, which potently inhibits the growth of *Candida albicans*. Therefore, eucalyptus oil at this concentration effectively inhibited the growth of *Candida albicans*.

This study's results align with the research of Naomi *et al.*, which found an inhibition zone of 26.33 mm at a concentration of 100% eucalyptus oil.³⁰ The differences in results may be attributed to variations in the subspecies of eucalyptus used and the geological factors influencing eucalyptus plant growth, which can cause differences in the content of eucalyptus oil. The content of the 1.8-cineole compound in eucalyptus oil produced by MSME Sendang Arum Lamongan is 72.30%, higher than the 1.8-cineole content of 5.2% reported in Naomi's research.

In the research conducted by Zhang *et al.*, the results showed eucalyptus oil's inhibitory ability (MIC) at a concentration of 62.5 $\mu\text{g/mL}$. This study used a positive control (Fluconazole) with a MIC of 6.25 $\mu\text{g/mL}$. Zhang's study used an antifungal dilution test, while this study employed an antifungal diffusion test. Additionally, the positive control used in Zhang's study differed from the positive control in this study. Therefore, the inhibitory ability of eucalyptus oil against *Candida albicans* between the two studies cannot be compared significantly. Zhang's study stated that terpinene-4-ol (36.85%) was the main compound in eucalyptus oil, while the content of 1.8-cineole was 4.61%. In contrast, this study found that 1.8-cineole (72.30%) was the main compound present in eucalyptus oil.¹⁹

The differences in the content of compounds in eucalyptus oil can be influenced by the climate and soil conditions in which eucalyptus plants grow. The growth of eucalyptus plants is affected by the phosphates and cation exchange capacity levels in the growing media used. Eucalyptus thrives in tropical areas with high rainfall (1.300-1.750 mm) and is well-suited to grumosol, latosol, and regosol soil types.³¹ However, the compound 1.8-cineole with terpinen-4-ol has similarities as a class of oxide monoterpene compounds commonly found in

various essential oils.³² Several compounds in eucalyptus oil as an antimicrobial exhibit antibacterial and antifungal properties. Among them, 1,8-cineole has the strongest ability to damage cell walls by disrupting membrane permeability, while terpinen-4-ol is the most effective in inhibiting *Candida albicans* biofilm formation.^{33,34}

CONCLUSIONS AND SUGGESTIONS

Eucalyptus oil produced by MSME Sendang Arum Lamongan can inhibit the growth of *Candida albicans*. Therefore, eucalyptus oil has great potential as an alternative or adjunctive therapy for treating *candidiasis* caused by *Candida albicans*. In addition, using eucalyptus oil is expected to reduce *Candida albicans*' resistance rate to other antifungal drugs.

This study was only conducted to determine the antifungal effect of eucalyptus oil against *Candida albicans*, and it is suggested that future studies could conduct antifungal tests of eucalyptus oil against several other pathogenic fungi or bacteria.

REFERENCES

- Hakim RI, Wilson W, Darmawati S. Uji aktivitas antibakteri ekstrak ethanol daun kayu putih (*Melaleuca leucadendron* L.) terhadap pertumbuhan Methicillin Resistant *Staphylococcus aureus* (MRSA). Pros Semin Nas Mhs Unimus, 2019; 2: 109-15.
- Ismanto AW. Ekstraksi minyak atsiri dari daun kayu putih (*Melaleuca leucadendra* Linn.) dengan metode microwave hydrodistillation dan solvent-free microwave extraction. Thesis Dep Tek Kim ITS. 2018.
- Gayuh Wilujeng S, Purwanta M, Fathul Qorib M, Retnowati W. Antibacterial effects of eucalyptus oil (*Melaleuca leucadendra*) made in Lamongan against *Escherichia coli* bacteria in vitro study. Int J Res Publ, 2022; 110: 353-61.
- Robbani G, Retnowati W, Dwi Wahyu Widodo A, Hasanatuludhhiyah N. The potential of cajuput oil (*Melaleuca leucadendra*) Small and Medium Enterprises (SME) in Lamongan against *Salmonella typhi* bacteria. Int J Res Publ, 2023; 137: 139-46.
- Constantia Lidwina Targanski, Wiwin Retnowati, Mohammad Fathul Qorib, Manik Retno Wahyunitisari, Wilda Mahdani. In vitro antibacterial activity of eucalyptus (*Melaleuca leucadendra*) oil against Methicillin-Resistant *Staphylococcus aureus* (MRSA). Maj Biomorfologi, 2023; 33: 59-67.
- Zaky Tiara Wibowo N, Retnowati W, Hasanatuludhhiyah N, Dwi Wahyu Widodo A. Antibacterial activity of cajeput oil (*Melaleuca leucadendra*) from UMKM Lamongan against *Pseudomonas aeruginosa*. Int J Res Publ, 2023; 137: 102-9.
- Monzote L, Scherbakov AM, Scull R, Satyal P, Cos P, et al. Essential oil from *Melaleuca leucadendra*: Antimicrobial, antikinoplastid, antiproliferative and cytotoxic assessment. Molecules, 2020; 25.
- Efruan GK, Martosupono M, Rondonuwu FS. Review: Bioaktivitas senyawa 1,8-sineol pada minyak atsiri. Seminar Nasional Pendidikan dan Saintek, 2016; 2016: 2557-533.
- Moghimi R, Aliahmadi A, Rafati H. Ultrasonic nano emulsification of food grade trans-cinnamaldehyde: 1,8-Cineol and investigation of the mechanism of antibacterial activity. Ultrason Sonochem [Internet]. 2017;35:415-21. Available from: <http://dx.doi.org/10.1016/j.ultsonch.2016.10.020> (accessed October 10, 2024).
- Parham S, Kharazi AZ, Bakhsheshi-Rad HR, Nur H, Ismail AF, et al. Antioxidant, antimicrobial and antiviral properties of herbal materials. Antioxidants, 2020; 9: 1-36.
- Priscila Barbosa J. Anti-*Candida* activity of essential oils from eucalyptus species. A preliminary study. Adv Dent Oral Heal, 2018; 8.
- Talapko J, Juzbaši M, Matijevi T. *Candida albicans*-the virulence factors and clinical manifestations of infection. J Fungi, 2021; 7: 1-19.
- Ghaddar N, Anastasiadis E, Halimeh R, Ghaddar A, Dhar R, et al. Prevalence and antifungal susceptibility of *Candida albicans* causing vaginal discharge among pregnant women in Lebanon. BMC Infect Dis, 2020; 20: 1-9.
- Nugraha AP, Ernawati DS, Parmadiati AE, Soebadi B, Triyono EA, et al. Prevalence of *Candida* species in oral candidiasis and correlation with CD4+count in HIV/AIDS patients at Surabaya, Indonesia. J Int Dent Med Res, 2018; 11: 81-5.
- Pal M. Morbidity and mortality due to fungal infections. J Appl Microbiol Biochem, 2018; 01: 2-4.
- Bhattacharjee P. Epidemiology and antifungal susceptibility of *Candida* species in a tertiary care hospital, Kolkata, India. Curr Med Mycol, 2016; 2: 0-0.
- Houšť J, Spížek J, Havlíček V. New antifungal drugs. Metabolites, 2020; 10: 2-16.
- Kaur R, Dhakad MS, Goyal R, Kumar R. Emergence of non-*Candida albicans* species and antifungal resistance in intensive care unit patients. Asian Pac J Trop Biomed [Internet]. 2016;6:455-60. Available from: <http://dx.doi.org/10.1016/j.apjtb.2015.12.019> (accessed October 13, 2024).
- Zhang J, Wu H, Jiang D, Yang Y, Tang W, Xu K. The antifungal activity of essential oil from *Melaleuca leucadendra* (L.) grown in China and its synergistic effects with conventional antibiotics against *Candida*. Nat Prod Res [Internet]. 2019;33:2545-8. Available from: <http://doi.org/10.1080/14786419.2018.1448979> (accessed October 13, 2024).
- Shahina Z, Homsy R Al, Price JDW, Whiteway M, Sultana T, Dahms TES. Rosemary essential oil and its components 1,8-cineole and α -pinene induce ROS-dependent lethality and ROS-independent virulence inhibition in *Candida albicans* [Internet].

- PLos One,2022; 17: 1-31 Available from: <http://dx.doi.org/10.1371/journal.pone.0277097> (accessed October 13, 2024).
21. Keereedach P, Hrimpeng K, Boonbumrung K. Antifungal activity of Thai cajuput oil and its effect on efflux-pump gene expression in fluconazole-resistant *Candida albicans* clinical isolates. *Int J Microbiol*, 2020;4.
 22. Monsen RE, Kristoffersen AK, Gay CL, Herlofson BB, Fjeld KG, *et al.* Identification and susceptibility testing of oral candidiasis in advanced cancer patients. *BMC Oral Health*, [Internet]. 2023;23:1-9. Available from: <https://doi.org/10.1186/s12903-023-02950-y> (accessed March 15, 2025).
 23. Astuty E. Antibacterial potential of endemic medicinal plants of the Maluku islands, Indonesia, against urinary tract infection pathogens antibacterial potential of endemic medicinal plants of the Maluku islands. *Folia Medica Indones*, 2024; 60.
 24. Turan D. Increased antifungal resistance due to variability in *Candida* species: Experience from the Central Mycology Laboratory. *Haydarpasa Numune Train Res Hosp Med J*, 2023; 63: 457-64.
 25. Sobel JD. Resistance to fluconazole of *Candida albicans* in vaginal isolates: A 10-year study in a clinical referral center. *Antimicrob Agents Chemother*, 2023;67: 23-6.
 26. Khalaf RA, Fattouh N, Medvecky M, Hrabak J. Whole genome sequencing of a clinical drug-resistant *Candida albicans* isolate reveals known and novel mutations in genes involved in resistance acquisition mechanisms. *J Med Microbiol*, 2021; 70.
 27. Maenchantrarath C, Khumdee P, Samosornsuk S, Mungkornkaew N, Samosornsuk W. Investigation of fluconazole susceptibility to *Candida albicans* by MALDI-TOF MS and real-time PCR for CDR1, CDR2, MDR1 and ERG11. *BMC Microbiol* [Internet]. 2022; 22:1-13. Available from: <https://doi.org/10.1186/s12866-022-02564-4> (accessed October 17, 2024).
 28. Dhasarathan P, AlSalhi MS, Devanesan S, Subbiah J, Ranjitsingh AJA, *et al.* Drug resistance in *Candida albicans* isolates and related changes in the structural domain of Mdr1 protein. *J Infect Public Health*, [Internet]. 2021;14:1848-53. Available from: <https://doi.org/10.1016/j.jiph.2021.11.002> (accessed October 17, 2024).
 29. Zare-Bidaki M, Maleki A, Ghanbarzadeh N, Nikoomanesh F. Expression pattern of drug-resistance genes ERG11 and TAC1 in *Candida albicans* clinical isolates. *Mol Biol Rep*, 2022;49: 11625-33.
 30. Tsuji M, Kobayashi N, Yukizawa Y, Oishi T, Takagawa S, Inaba Y. Effect of flurbiprofen and s-flurbiprofen patches on multimodal pain management after total knee arthroplasty: A prospective randomized controlled trial. *J Arthroplasty*, 2020; 35: 2033-8.
 31. Sadono R, Soeprijadi D, Wirabuana PYAP. Effect of chemical soil properties on the growth of cajuput (*Melaleuca leucadendron* L.) stand. *J Penelitian Kehutanan Wallacea*, 2019; 8:1.
 32. Arisandi R, Pujiarti R, Lukmandaru G, Mulyana B. Chemical constituents of *Melaleuca leucadendron* linn. leaf essential oils quality under different collecting times in KPH Yogyakarta, Gunung Kidul, Indonesia. *Indones J For Res*, 2023; 10: 195-205.
 33. Shariati A, Didehdar M, Razavi S, Heidary M, Soroush F, Chegini Z. Natural compounds: A hopeful promise as an antibiofilm agent against *Candida* species. *Front Pharmacol*, 2022; 13: 1-20.
 34. Merghni A, Belmamoun AR, Urcan AC, Bobiş O, Lassoued MA. 1,8-cineole (eucalyptol) disrupts membrane integrity and induces oxidative stress in methicillin-resistant *Staphylococcus aureus*. *Antioxidants*, 2023; 12.