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To cite this article: Dwi Marlina Syukri *et al* 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **305** 012067

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Preliminary Phytochemical, Antioxidants, and Antibacterial Properties of *Eucalyptus* Aqueous Leaf Extract Against *Streptococcus pyogenes*

Dwi Marlina Syukri^{1*}, Ozioma Nwabor², and Rani Ismiarti Ergantara³

¹Medical Faculty, Malahayati University, Lampung, Indonesia, Jl. Pramuka No 27 Kemiling, Bandar Lampung 35153

²Department of Microbiology, Prince of Songkla University, Thailand

³Departement of Environmental Engineering, Malahayati University, Lampung, Indonesia, Jl. Pramuka No 27 Kemiling, Bandar Lampung 35153

*Email: dmarlinas79@gmail.com

Abstract. *Streptococcus pyogenes* is one of the exclusive human pathogenic bacterial, it causes diverse diseases such as pharyngotonsillitis, impetigo, necrotizing fasciitis, bacteremia or sepsis, and often leads to complications such as acute rheumatic fever and glomerulonephritis. In this study, the antimicrobial activity of *Eucalyptus* aqueous leaf extract against 15 clinical isolates of *Streptococcus pyogenes* and reference strain DMST 17020 was evaluated using the standard microdilution technique. *Eucalyptus* showed antibacterial activity against all the isolates with minimum inhibitory and minimum bactericidal concentrations of 64 µg/ml and 64-256 µg/ml respectively. The extract further showed free radical scavenging ability as detected using DPPH and ABTS assays. Phytochemical analysis of the extract revealed the presence of bioactive compounds.

1. Introduction

Streptococcus pyogenes (group A streptococci), is an important clinical Gram-positive bacterium. It is one of the leading causes of nosocomial infection, and often implicated in throat and skin infections. *S. pyogenes* infections might progress to wide a variety of complications and systemic infections. The prevalence of *S. pyogenes* infections is high amongst children of 5 to 15 years of age, with very few cases in adult. Nowadays, penicillin is still used as the best treatment for this bacterium [1].

According to World Health Organization (WHO), some of the world's most common and potentially most dangerous infections are proving drug resistant. Therefore, there is a surge in research focusing on the search for new natural agent that is effective against various disease-causing microorganisms. Plant bioactive compounds have shown promising results as an alternative source of potent compounds that might resist the development of microbial resistance. The genus *Eucalyptus* belonging to the plant family Myrtaceae consists of about 900 species. Plants of this genus has proved to be one of such kinds of aromatic plant that is rich in bioactive compound such as monoterpenes, oxygenatedmonoterpen, sesquiterpenes, oxygenatedsesquiterpenes, often found in essential oil [2].

In addition, saponin, phenolic, and flavonoid are present in crude extracts of this plant. These bioactive compounds have ability to disrupt microbial cell walls, due to the presence of radical



hydroxyl (OH[•]) as a reactive oxygen species (ROS). This results in the alteration of the membrane permeability apparatus leading to an increase in intracellular osmotic pressure and subsequent swelling and lysis of the microbial cell [3]. Therefore, in this study focused on effectiveness of *Eucalyptus* aqueous extract against *S.pyogenes*.

2. Method

2.1. Preparation of *Eucalyptus* aqueous leaf extract

Twenty gram of *Eucalyptus* leaf powdered was mixed with 400 ml distilled water and heated at 85°C on heating mantle magnetic stirrer with 200 rpm for 60 min (Framo®-Gerätetechnik M21/1). The extract was immediately cooled on ice to room temperature then filtered using Whatman® No. 1 filter paper and freeze-dried for 48 h (CHRIST® ALPHA 1-4 LD plus) with drying chamber pressure of 0.2 mbar at -48°C and stored at refrigerator temperature until needed. The extract will be dissolved in distilled water for performing the *in vitro* antimicrobial assays.

2.2. Preliminary phytochemical analysis

Tannins; Two hundred micro gram plant materials in 10 ml distilled water were filtered then 2 ml filtrate was added with 2 ml FeCl₃. Blue-black precipitate indicated the presence of tannins. Saponins; Frothing test: 0.5 ml filtrate was added with 5 ml distilled water. Frothing persistence meant saponins were present. Phenolics/Terpenoids (Liebermann-Burchard reaction: Two hundred micro gram plant materials in 10 ml chloroform were filtered. Furthermore, 2 ml filtrate was added with 2 ml acetic anhydride and concentrated H₂SO₄. Blue green ring indicated the presence of terpenoids. Flavonoids Two hundred micro gram plant materials in 10 ml distilled water were filtered then 2 ml filtrate was added with concentrated HCl magnesium ribbon. Pink-tomato red color indicated the presence of flavonoids [5].

2.3. Determination of antioxidant activity

2.3.1. DPPH radical scavenging assay

Twofold dilution of extract will be added in distilled water in a sterile 96-wells micro titer plates to get the final concentration from 10 µg/ml to 0.08 µg/ml. Furthermore, 100 µl of DPPH solution (6 x 10⁻⁵ M) in absolute ethanol is added into each well and left the mixture at room temperature for 30 min in dark room. The absorbance of the resulting solutions was measured at 517 nm after 30 min. The well containing distilled water as a control and ascorbic acid 10 µg/ml to 0.08 µg/ml as standards. Experiment will be carried out in triplicate. The DPPH radical scavenging ability of essential oil extract was calculated according Equation:

$$\text{DPPH scavenging effect (\%)} = [(A_0 - A_t)/A_0] \times 100$$

Where A₀ is the control absorbance after 30 min, and A_t is the sample absorbance after 30 min. The antiradical scavenging activity was expressed as IC₅₀ value (µg/ml) [6].

2.3.2. ABTS radical scavenging assay

The extract and ascorbic acid as a standard (10 µg/ml to 0.08 µg/ml) will be allowed to react with ABTS radical cation solution for 5 min. The absorbance of the resulting solutions was measured at 750 nm [3].

2.4. Determination of Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of *Eucalyptus* aqueous leaf extract

A modified broth micro dilution method outlined by Clinical and Laboratory Standards Institute (CLSI 2017) will be performed. Twofold dilution of extract will be added in BHI in a sterile 96-wells micro titer plates to get the final concentration from 1024 to 8 µg/ml. Furthermore, 100 µl of the bacterial suspension (10⁶ CFU/ml) is added into each well and incubated at 37°C for 24 hours. The well

containing contains BHI as a negative control and penicillin G 4 to 0.125 µg/ml as positive control. The MIC is determined as the lowest concentration of the antimicrobial agent that producing a visible inhibition of bacterial growth. The MBC will be considered at the lowest concentrations required to kill bacteria through sub-culturing of MIC on MHA containing 5% red blood cells plates. Experiment will be carried out in triplicate.

3. Results and Discussion

3.1. Phytochemical assay

Phytochemical analysis of *Eucalyptus* aqueous leaf extract showed the presence of bioactive compounds including tannin, saponins, and phenolic compounds (Table 1). Previous research has reported the presence of these phytochemicals in *E. camaldulensis* and other *Eucalyptus* species [7] [8].

Table 1. Preliminary phytochemical results.

Qualitative test	Result
Tanin	Blue black precipitate (+)
Saponin	Frothing (+)
Phenolic	Blue green ring (+)

3.2. Antioxidant activity assay

The antioxidant activity of the plant extract evaluated using the DPPH and ABTS assay, showed free radical scavenging activity with IC₅₀ values of 3.44 µg/mL and 2.68 µg/mL respectively. Previous studies reported an IC₅₀ value of 6.26 ± 0.19, 6.14 ± 0.21 and 8.24 ± 0.26 µg/mL [9] for *Eucalyptus grandis*, *Eucalyptus urograndis*, and *Eucalyptus maidenii* respectively. Total antioxidant activity demonstrated using the ferric reducing antioxidant power (FRAP) revealed an antioxidant power of 19.32 µg Ascorbic acid equivalent/ mg extract. The remarkable antioxidant activity demonstrated by the extract is suspected to be as a result of the presence of biotiv phytochemicals such as tannin and phenolic compounds as previously shown in (Table 1).

Table 2. Antioxidant profile of *Eucalyptus* aqueous leaf extract

Assay	Extract	Ascorbic acid
2,2-diphenyl-2-picrylhydrazil radical scavenging activity (DPPH) (IC ₅₀ µg/ml)	3.44	2.53
2,2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay (ABTS) (IC ₅₀ µg/ml)	2.68	0.47
Ferric reducing antioxidant power (FRAP)	19.32	NA

3.3. The antibacterial activity of *Eucalyptus* aqueous leaf extract

The antibacterial activity of the extract against *S.pyogenes* the most demonstrated using the microdilution technique showed minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of 64 to 256 µg/mL and 256 to >512 µg/mL respectively. The presence of hydroxyl group might rupture the microbial cell membrane, causing a leakage of cellular component and subsequent cell death [3]. Previous researchers have reported the antimicrobial of *Eucalyptus* species against clinical important pathogens as well as food pathogens [10] [11] [12]. The effects of plant from the family Myrteceae against *S. pyogenes* has been reported [13] [14].

Table 3. Antibacterial activity of *Eucalyptus* aqueous leaf extract against *Streptococcus pyogenes*

Isolates	Extract MIC/MBC µg/mL	Penicillin G MIC/MBC µg/mL
DMST 17020	64/256	0.06 / 0.5
Clinical isolates (n=15)	64-256/256->512	0.03-0.25 / 0.50- 1.00

4. Conclusions

The results of this investigation confirmed the presence of bioactive phytochemicals in *Eucalyptus* aqueous extract. It further revealed that the extract possesses good antimicrobial and antioxidant properties. This work reports initial preliminary data of the activity of *Eucalyptus* extract on *S. pyogenes*.

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