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Antibacterial Activity Of Crude Ethanolic Leaves Extract Of *Artocarpus Heterophyllus* Lam Cultivated in Toba Region Against *Staphylococcus Aureus*

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Abstrak

Jackfruit leaves (Artocarpus heterophyllus Lam.) was tested for its antibacterial activity against a human pathogenic bacterium, Staphylococcus aureus in vitro. Jackfruit leaves were collected from a forest region in the Toba Regency, North Sumatra and air-dried for five days. The dried leaves were crushed into powder to obtain simplicia and macerated using EtOH. The crude extracts were concentrated in vacuo using a rotary-evaporator and diluted into various concentrations (25, 50, 75, 100%) for antibacterial assay. The results showed that the antibacterial activity of extracts followed a concentration-dependent manner with the highest inhibition as expressed in the diameter of inhibitor zone reached 19 mm. The MIC_{50} value generated from a linear regression analysis between diameter of inhibition zone and concentration was determined at 10% of extract to display its effective concentration. The findings may be further investigated through phytochemical analysis and the selection of different organic solvents to maximize the bioactivity of jackfruit from this region.

Keywords : Keywords: Antibacteria, Disk Diffusion, Jackfruit, North Sumatra, Toba.

INTRODUCTION

Due to the fact that natural products frequently contain specific and a cocktail of bioactive compounds with potential therapeutic benefits, they have been exploited as sources of medications to treat human ailments. The World Health Organization (WHO) estimates that 70% of the world's population utilizes these herbal products to receive a range of treatments (Septama & Panichayupakaranant, 2015). Infectious diseases are a significant issue worldwide, especially in the majority of developing nations. In order to mitigate the incidence and onset of a disease, the pharmaceutical industries have created numerous commercial antibiotics. This has provided numerous motivation for researchers to search for and identify novel naturally derived antimicrobial substances. The therapeutic potential of antibacterial compounds derived from plants is enormous, and many have been shown to be efficient for treatment with fewer side effects than chemicals derived from synthetic sources (Christudas et al., 2012).

The use of medicinal plants in traditional medicine has been on the rise over the past ten years, generally in tandem with the growth of the traditional or herbal medicine industries. Typically, medicinal plants are used in the form of simplicia, which can refer to either fresh plants or dried plants or plant parts. *Artocarpus heterophyllus* Lam. (Moraceae) is a perennial tropical-to-subtropical perennial tree species, popularly known as jackfruit or *lamasa/ malasa/ nangka/ noongka* (Bahasa) in Indonesia (Baliga et al., 2011). In the past, the species has been well-documented in the Ayurvedic and Egyptian writing as traditional remedies (Saxena et al., 2009). According to pre-clinical studies, jackfruit has anti-inflammatory, antibacterial, antifungal, anticariogenic, anti-antioxidant, anticarcinogenic, antioxidant, hypoglycemic, wound-healing, anticarcinogenic, and antineoplastic properties. It also temporarily reduces sexual activity. Clinical studies have also demonstrated that the leaf decoction has hypoglycemic effects in both healthy people and diabetic patients who are not insulin-dependent (Baliga et al., 2011).

Various jackfruit parts have been utilized and investigated, but empirical studies on the bioactivity of fruits, stems, and barks predominated (Ajiboye et al., 2016; Devi et al., 2019; Ragasa et al., 2004). Depending on the plant species, leaves are one of the simplest simplicia materials to prepare, obtain, and store numerous type and yield of secondary metabolites (Figueiredo et al., 2008). Despite being studied from specimens grown abroad, Artocarpus heterophyllus has a well-established history of antibacterial activity (Khan et al., 2003). The purpose of this study is to investigate the effectiveness of *Artocarpus heterophyllus* leaf extract against *Staphylococcus aureus* for future research, especially that which is grown in the Toba region.

RESEARCH METHODS

This study was conducted in February 2020 at the Laboratory of Microbiology, STIKES Arjuna, Laguboti District, Toba Regency, North Sumatra. Leaves of *Artocarpus heterophyllus* were sampled from the forest region of Toba Regency with the characteristics of healthy specimens such as being fresh, green, papyraceous, and exhibiting no sign of disease. Duplicate specimen was collected and authenticated at Herbarium MEDA, Universitas Sumatera Utara, Medan, Indonesia. A total of 2 kg fresh leaves was collected from the site and air-dried for one week. The dried leaves (500 g) were immersed in a bottle filled with 96% (v/v) of EtOH and shaken vigorously for five days. After maceration, the macerates were collected and pooled from thrice filtering using the same solvent. The crude EtOH extracts were concentrated *in vacuo* using a rotary-evaporator and stored for further experimentation. The clinical strain, *Staphylococcus aureus* was pre-grown in Nutrient Agar (NA) medium. Direct suspension method was used to prepare the bacterial solution by swabbing the colonies using sterile swab cotton into a physiological saline and homogenized. Disk diffusion assay was used to evaluate the antibacterial activity of extracts against *S. aureus* using sterile paper disks (Bauer et al., 1959. A sterile cotton swab was dipped into bacterial suspension and swabbed entirely on Mueller Hinton Agar (MHA) medium to produce a bacterial lawn. Disks containing a series of extract concentration (0, 25, 50, 75, 100%) in Dimethyl Sulfoxide (DMSO) was prepared and placed on top of the bacterial lawn. The assay was conducted in five replicates. The plates were incubated at 37^{o}C for 24 hr. Any clear zone appeared around disk indicate the antibacterial activity of extracts and measured using a caliper in mm (Figure 1). Data were analyzed using ANOVA and tested if significant using Tukey's test for multiple comparison at $p \leq 0.05$. A linear regression test was used to determine the Minimum Inhibitory Concentration (MIC_{50}) based on the diameter of inhibition zone versus concentration of extracts.

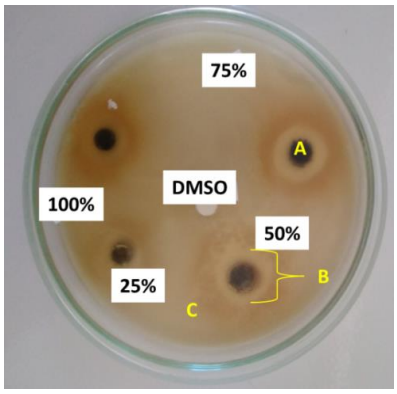

Figure 1. Disk diffusion test of crude EtOH extract and DMSO (negative control) of *A. heterophyllus* leaves against *S. aureus*. A. Disk, B. Inhibitory zone, C. Bacterial lawn.

RESULTS AND DISCUSSION

Maceration of *A. heterophyllus* leaves yielded a crude extract using EtOH as solvents. The extracts were diluted into various concentration (25%, 50%, 75%, 100%, w/v) for testing against *S. aureus* using disk diffusion method. The result is presented in Figure 1. The highest antibacterial activity was obtained in the 100% extract with a diameter of inhibitory zone (DIZ) of 19.88 mm, followed by 75% (17.40 mm), 50% (14.66 mm), and 25% (11.38 mm). The results demonstrated that the inhibition was concentration-dependent, meaning that a higher concentration contributed to a higher level of activity. The MIC_{50} value of *A. heterophyllus* leaves extract was determined to be 10.36%, which may be considered the most effective concentration to produce antibacterial activity without using an excessive amount of extract (Figure 3).

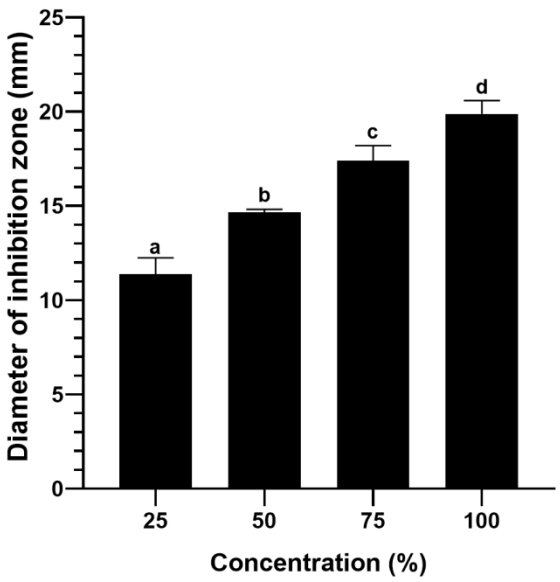

Figure 2. Mean diameter of inhibition zone of crude EtOH extracts of *A. artocarpus heterophyllus* leaves against *S. aureus* (*N* = 5). Different letters denote statistical differences based on ANOVA and Tukey's test at 95%.

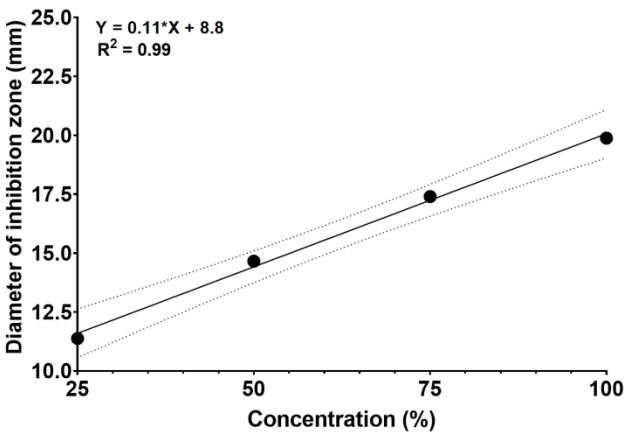

Figure 3. Linear regression curve illustrating activity of various concentrations of EtOH extract of *A. artocarpus* leaves against *S. aureus*.

Antibacterial activity of A. heterophyllus leaves has been documented, with Khan et al. (2003) utilizing Papua New Guinea specimens. According to their findings, the antibacterial activity against S. aureus ranged from 10 to 14 mm depending on the diameter of the inhibitory zone and the solvent used, which included butanol, chloroform, ethyl acetate, methanol, and petrol.

Loizzo et al. (2010) also demonstrated bactericidal activity of Sri Lankan jackfruit leaf extract in water and ethyl acetate solvents ranging from 10 to 15 mm. Siahaan et al. (2019) employed jackfruit EtOH extract from Deli Serdang Regency, North Sumatra, to generate inhibitory activity against S. aureus ranging from 7.5 to 10.8 mm. Based on the findings, it is known that the antibacterial activity of jackfruit varies between specimens and accessions depending on where it was harvested. Furthermore, the organic solvents used determine the antibacterial efficiency against the target bacterium. Loizzo et al. (2010) also added that the bioactivity of jackfruit may be influenced by the phenol content and its derivatives. Since many plant phenolics have been found to be responsible for several biological properties, including antimicrobial properties, and researchers have also reported that phenolic compounds from different plant sources may inhibit various human pathogens, it was expected that the antimicrobial activity of *A. heterophyllus* would be related to its phenolic content (Cueva et al., 2010). More research is needed to evaluate the use of different organic solvents to maximize the activity of our local jackfruit extract. Furthermore, the phytochemical compounds found in the most active extract must be profiled in order to possibly gain new knowledge about the relevant phytocompound(s).

CONCLUSION

Based on the inhibitory zone and low MIC50 value (10%), EtOH extract from Toba Regency jackfruit leaves exhibits high antibacterial action against Staphylococcus aureus. The potential can be investigated further in order to validate these findings.

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