UNIVERSITI TEKNOLOGI MARA

ISOLATION AND CHARACTERIZATION OF PHYTOCHEMICALS FROM THE STEM BARKS OF *Lepisanthes rubiginosa* (Roxb.) AND ITS ANTIBACTERIAL ACTIVITY

SITI ZAFIRAH BINTI ZULKIFLI

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ABSTRACT

Lepisanthes rubiginosa, known as 'Pokok Mertajam' among Malaysian, is a plant traditionally used to treat various ailments and possesses a broad spectrum of antimicrobial activities. This study was purposely conducted to discover the phytochemicals of the stem barks of *L. rubiginosa* using a dereplication strategy for early chemical profile analysis prior to isolation. The stem barks of *L. rubiginosa* were extracted using hexane, dichloromethane, and methanol. Each extract was screened for its antibacterial activity against gram-positive (*Staphylococcus haemolyticus* and *Methicillin-resistant Staphylococcus aureus*) and gram-negative (*Klebsiella pneumonia, Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Shigella sonnei,* and *Escherichia coli*) bacteria. The hexane extract exhibited significant activity towards *S. haemolyticus*. Further examined using Gas-Chromatography Mass Spectrometry (GCMS), and has successfully identified palmitic acid as a major constituent with percent of abundance of 45%. Mass-based dereplication strategy using an online mass database revealed the presence of saponins, terpenoids, phenolic compounds, flavanols, butanolide, and amino acids in the dichloromethane and methanol extracts. Isolation and purification of methanol extract of *L. rubiginosa* led to the characterization of five saponins and one farnesyl glycoside. Four out of six are new compounds, namely 3-*O*-{*β*-D-glucopyranosyl-(1→2)-[*α*-D-xylopyranosyl(1→3)]-*β*-D-glucopyranosyl}oleanolic acid (lepiginoside A) (**128**), 3-*O*-{*β*-D-glucopyranosyl-(1→2)-[4-*O*-acetyl-*α*-D-xylopyranosyl(1→3)]-*β*-D-glucopyranosyl}oleanolic acid (lep iginoside B) (**129**), 3-*O*-{*β*-D-glucopyranosyl-(1→2)-[4-*O*-acetyl-*β*-D-xylopyranosyl (1→3)]-*β*-D-glucopyranosyl}-oleanolic acid (lepiginoside C) (**130**), and 1-*O*-{*α*-L-arabinopyranosyl(1→6)-[*β*-D-glucopyranosyl(1→3)-*α*-L-rhamnopyranosyl-(1→2)]-*α*-L -arabinopyranosyl(1→3)]-*β*-D-glucopyranosyl}-all-trans-farnes-1-ol (lepiginoside D) (**131**), whilst lepisantheside A (**19**) and gleditsoside C (**132**) are known compounds. All structures were elucidated using 1D- and 2D-NMR spectroscopy, supported by MS, UV and IR data. The isolated compounds were tested against the same bacteria strains previously used to screen the crude extracts. However, none of the compounds was effective against the bacteria.

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CHAPTER ONE
INTRODUCTION

1.1 Research Background

Natural products are major sources of organic substances that can be used to treat various diseases and provide active components for traditional and modern medicinal purposes (Chintoju *et al.,* 2015). They are produced as primary metabolites or biosynthesized as secondary metabolites for protection, competition, and species interactions (David & Gordon, 2012). For decades, natural plant products have been a vital source of therapeutic agents, and many of today's medications are still derived from plants. For example, quinine, an antimalarial and antipyretic drug derived from the quinoline alkaloid of *Cinchona ledgeriana,* while betulinic acid, an anticancer drug, was derived from the pentacyclic triterpenoid of *Betula pendula* (Singh & Sharma, 2019; Dehelean *et al*., 2021).

Statistics in 2020, infectious diseases account for seven out of every ten deaths in developing countries (Sang *et al*., 2022). The increasing prevalence of bacterial infections and their antibiotic resistance has raised awareness of the need to find alternative treatments. The necessity to constantly find antimicrobial agents has sparked a worldwide interest in traditional plant research. This enormous interest in plant-derived drugs is mainly due to the current widespread belief that herbal medicine is safer and more reliable, particularly in countries with limited pharmaceutical access. As a result, plants are still considered the bedrock of medicine, especially in the never-ending search for new antibacterial agents.

The preliminary screening of the crude extracts from *Lepisanthes rubiginosa* stem barks revealed potential antibacterial activity. This plant belongs to Sapindaceae family and is considered an important tropical plant species due to its medicinal and multiple usages (Hasan *et a.l,* 2017). Sapindaceae species are characterized by rich saponin content, some of which have been reported to possess a broad spectrum of antifungal and antimicrobial effects (Kuspradini *et al*., 2012). Additionally, phytochemicals of the *Lepisanthes* genus have been reported to have antioxidant, antibacterial, anticancer, antidiabetic, and cytotoxicity properties (Zulkifli *et al.*,