Chapter 1

Introduction

1.1 Background

Research on organic substances present in plants and fruits has become more important, especially in light of the rise of bacteria that are resistant to antibiotics. In order to battle the priority illnesses listed by the World Health Organization (WHO, 2021), new antibacterial agents must be developed as antibiotics become less and less efficient against these bacteria. In the fight against antibiotic resistance, plant-based phytochemicals such terpenoids, phenolics, and alkaloids present viable substitutes for antibiotics (Divekar et al., 2022; Ninkuu et al., 2021).

Due to its delectable flavor, simplicity of production, and adaptability, black sapote (*Diospyros digyna*) has grown in popularity in Indonesia. Although earlier research concentrated on identifying its components and traits, more pharmacological study is required to investigate its possible medicinal uses. Sinapic acid is a key phenolic component in black sapote and has anti-inflammatory, anticarcinogenic, and antibacterial activities (Mannino et al., 2022; Singh et al., 2022). Black sapote is also high in carotenoids, vitamins, and phenolic compounds. Additionally, anthocyanins, water-soluble pigments found in black sapote, have powerful anti-inflammatory and antioxidant effects on both gram-positive and gram-negative bacteria (Ma et al., 2019).

Black sapote fruit undergoes changes as it ripens, including an increase in carotenoids and a decrease in phenolic and chlorophyll content (Zulfikar et al., 2020; Singh et al., 2015; Saini et al., 2017). By causing oxidative stress and harming bacterial cell membranes, carotenoids, such as beta-carotene and lycopene, have considerable antibacterial action (Seymour et al., 2020; Fiedor and Burda, 2014). By exerting bactericidal or bacteriostatic effects and interfering with critical bacterial processes, these carotenoids efficiently inhibit the development of bacterial strains including *E. coli* and *S. aureus* (Zhang et al., 2019; Tan et al., 2021).

The technique of extraction and solvent selection are important variables impacting the activity of phytochemical substances. Utilizing high-frequency ultrasonic waves, ultrasonic-assisted extraction (UAE) is a cutting-edge method that improves extraction effectiveness and shortens extraction time (Zhou et al., 2019). UAE is a valuable extraction technology for usage in the food, pharmaceutical, and cosmetic sectors since it has been effectively employed to extract a variety of bioactive chemicals from various plant sources (Salehi et al., 2022). The choice of the solvent is also important since it affects how the plant material and extracted phytochemicals interact with the solvent's biochemical and physicochemical properties. Hydrophilic solvents, as opposed to hydrophobic ones, have been proven to produce better extraction outcomes for flavonoids and phenolic chemicals, for instance. Although hydrophobic solvents have also shown efficacy (Tungmunnithum et al., 2018).

Microdilution tests are a reliable and affordable way to assess a bioactive compound's antibacterial potential. This test can determine minimal inhibitory doses since it uses the 96-well microplate technology, which uses less extract and yields reliable findings (Eloff, 1998). In this work, the microdilution method was used to test the antibacterial

1.2 Objective

The aim of this project is to extract the unripe and ripe black sapote fruit using hydrophobic and hydrophilic solvents, to determine the phytochemical compounds of the extracts, and to investigate the antibacterial activity of the extracts.

1.3 Hypothesis

The black sapote fruit extract has the better antibacterial activity compared to the positive control against *P.aeruginosa* and *S.aureus*