# Introduction

## 1.1. Background

Acne is a common skin condition that occurs in approximately 9.4% of the world's population (Alanazi, Hammad & Mohamed, 2018). Acne manifests physically in an array of different forms ranging from red bumps to larger bumps filled with pus that is painful to touch. Aside from its physical impact, acne is also well known to be a contributor in the development of depression and low self-esteem (Revol, Milliez & Gerard, 2015). Acne can be caused by several factors, one of them being bacteria. The skin flora has multiple bacteria present which may be related to acne -- *Propionibacterium acnes, Staphylococcus epidermidis, Staphylococcus aureus, Streptococcus pyogenes*, and *Klebsiella pneumonia*e to name a few.

*Propionibacterium acnes* is a gram-positive anaerobic bacteria mainly known to feed off the sebum produced on the skin and trigger inflammation through immune responses (Komuro, 2017). On the other hand, *Staphylococcus epidermidis* is a facultative anaerobic bacteria that is able to form a biofilm which creates an anaerobic condition suitable for *Propionibacterium acnes* (Kumar et al., 2016). *Staphylococcus aureus* and *Streptococcus pyogenes* in the oropharynx of acne patients are higher in prevalence and resistance pattern compared to those without acne indicating the association between the bacteria and acne (Levy et al., 2003). Acne patients treated with long-term therapy of antibiotics, *Klebsiella pneumoniae*, a gram-negative bacteria, may exert virulence factors which produce papules and pustules (Damian et al., 2009). Although antibiotics such as erythromycin, clindamycin, doxycycline, and minocycline are used to treat acne by eliminating these bacteria, antibiotic resistance has developed in some acne causing bacteria. As a preventive measure, several natural compounds have been shown to have antibacterial properties against these bacteria.

*Diospyros nigra*, also commonly known as black sapote, is a tropical fruit belonging to the persimmon family and is native to Mexico, Central America, and Colombia. Trees of black sapote are evergreen with elliptic-oblong dark green glossy leaves (Lim, 2011). The unripe black sapote fruit has

an inedible olive skin and inedible white pulp, while the ripe fruit has a deep yellow-green skin with a pulp that resembles chocolate pudding in color, flavor, and texture. Fruit of black sapote is known for its antioxidant properties, while its bark and leaves have been used traditionally to alleviate leprosy, ringworm, and itchy skin (Lim, 2011). Phytochemicals found in black sapote play an important role in exerting its pharmacological properties. Choosing multiple solvents with varying polarity (n-hexane, ethanol, and water) enables extraction of a wider range of compounds.

Although the antimicrobial activity of black sapote is rarely studied, one study showed that the leaves of black sapote have shown some antimicrobial properties against bacteria such as *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa,* and *Staphylococcus aureus* (Dinda et al., 2006). Discovery of the antimicrobial activities make black sapote a promising candidate as a natural anti acne ingredient. Hence, the antimicrobial activity of black sapote fruit will be tested against *Staphylococcus aureus* and *Staphylococcus epidermidis.* Both bacteria are chosen for the antimicrobial activity testing as it is an acne related bacteria that can be cultured with relatively simple handling techniques.

### 1.2. Objectives

The objective of this research is to investigate the phytochemical content as well as the antimicrobial activity of n-hexane, ethanol, and water extracts of black sapote fruit *in vitro*.

### 1.3. Hypothesis

The stem and leaves extracts of black sapote have been shown to exert antimicrobial properties in previous studies against a wide range of bacteria (Dinda et al., 2006). It is hypothesized that extracts of black sapote fruit exerts antimicrobial activity against acne related bacteria.