INDIVIDUAL AND SYNERGISTIC ANTIMICROBIAL PROPERTIES OF Plectranthus amboinicus, Murraya koenigii, Ocimum sanctum and Azadirachta indica AGAINST ACNE CAUSING BACTERIA

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DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF BIOTECHNOLOGY (HONOURS)

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ABSTRACT

Antibiotic resistance is currently one of the major issues in the healthcare sector since the emergence of antibiotic resistant microorganisms. This has resulted in the ineffectiveness of the some of the available antibiotics to combat sickness. Hence, there is a need to find an alternative to replace the heavy usage of antibiotics. In this study, we have done a preliminary test to compare the individual and synergistic antimicrobial efficacy of the extracts of Plectranthus amboinicus (indian borage), Murraya koenigii (curry leaves), Ocimum sanctum (tulsi) and Azadirachta indica (Neem) against acne causing bacteria such as Staphylococcus aureus and Staphylococcus epidermidis. We extracted the crude extracts of these traditional herbs and tested their individual and synergistic antimicrobial efficacy in different combinations against pure cultures of S. aureus and S. epidermidis by using disc diffusion technique. We also obtained five acne samples from different volunteers and tested them with these traditional herbs for individual and synergistic antimicrobial effect. We have found that all the traditional herbs mentioned possessed antimicrobial effect against acne causing bacteria, except for the extracts of Ocimum sanctum. The combination use of Murraya koenigii and Azadirachta indica showed promising synergized antimicrobial efficacy whereas the combination of Plectranthus amboinicus with the other plant extracts such as Azadirachta indica resulted in an antagonistic effect. The results of our antimicrobial testing of plant extracts on bacteria causing acne showed promising results. However, further research on the antimicrobial efficacy of the plant extracts need to be done before we can suggest them as an alternative treatment for acne.
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<td>ºC</td>
<td>degree Celsius</td>
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<tr>
<td>µg</td>
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<td>µg/mL</td>
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CHAPTER 1

INTRODUCTION

Before the introduction and advancement in modern medicines, man relied on medicinal plants which were obtained from the nature to treat various ailments. Medicinal plants are the backbone of traditional medicine and are still widely used today by traditional medicine practitioners in less developed countries as their primary health care regime. This is because of the lower cost in obtaining the raw materials and also the belief that the *au natural* way of healing is better than modern medicines which used too much chemicals (Kazemipoor et al., 2012). Herbal medicine can be broadly classified into various groups based on geographical factors: Traditional Chinese Herbalism, Ayuverdic Herbalism and Western Herbalism.

There are several plants which show antimicrobial properties, for example *Plectranthus amboinicus, Ocimum sanctum, Azadirachta indica, Murraya koenigii* and many more. All these traditional herbs have a wide potential to be developed for conventional medicinal usage to replace the chemically-produced medicine due to their naturally acquired antimicrobial, antiviral, antifungal properties which are able to cure a range of microbial related diseases (Umadevi et al., 2013). There are also a few more advantages on the usage of medicinal herbs, which includes the relatively lower cost of production. This enables more people to be able to afford the medication. There is also an increasing problem with antimicrobial resistance. Hence, traditional herbs can be used as an alternative method to combat drug resistant pathogens. There are so many possibilities with the usage of traditional herbs and we should put more effort in researching the wonders that they are and what they can do.

In this project, we conducted a preliminary study on the individual and synergistic antimicrobial properties of traditional herbs, particularly *Plectranthus amboinicus, Ocimum sanctum, Azadirachta indica* and *Murraya koenigii* against causative agent of
acne, namely *Staphylococcus epidermidis* and *Staphylococcus aureus* using disc diffusion assay. We also compared and contrasted the antimicrobial properties of individual traditional herbs with the combination of these herbs.
CHAPTER 2

LITERATURE REVIEW

2.1 ANTIBIOTIC RESISTANCE AND ISSUES WITH MODERN DRUGS

Antibiotics are medication that either inhibit the growth of bacteria (bacteriostatic) or kill the bacteria (bacteriocidal). These biotherapeutic agents are usually prescribed by physicians to cure bacterial infections in humans (Shaban, Cruickshank & Christiansen, 2013). Antibiotics have been used to treat diseases such as pneumonia and throat infections for many years until the problem with antibiotic resistancy towards bacteria arose. Antibiotic resistance has become an alarming issue in the health care sector as bacteria started to show resistance towards antibiotics. One such example of antibiotic resistant strains are methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin resistant *pneumococci* (PRP) and the vancomycin resistant *enterococci* (VRE) (Suparmanto, 2005). The emergence of antibiotic resistant bacteria did not happened in a short period, it was due to the long term selection pressure caused by the indiscriminate usage of antibiotics which resulted in the evolution of bacteria to become resistant to antibiotics (Sosa, Byarugaba, Amabile-Cuevas, Hsueh, Kariuki & Okeke, 2010). Besides the usage of antibiotics to cure bacterial infections, scientists also use chemically derived drugs which can target to a wide range of bacteria. Chemically derived drugs which are based on antimicrobial peptides (AMP) have shown to be promising drugs to be used to treat bacterial infections as they target a wide spectrum of bacteria with rapid action. However, the development of drugs based on AMP has yet to be approved because clinical trials have shown that it has toxic side effects when consumed by humans. Furthermore, the production cost to manufacture chemically-derived drugs is too expensive (Fernebro, 2011).
The issues with antibiotic resistance and chemically derived drugs have caused the treatment of common ailments to become more complex and this could in turn, trigger more serious complications. Hence, there is a need to find other effective agents that can replace antibiotics in the treatment of bacterial infections. Traditional plants with antimicrobial properties against known bacteria might be a good potential alternative as they are natural, easily available, have shown to have lesser side effects. In addition they are cheaper to produce.

2.2 TRADITIONAL HERBS

2.2.1 Brief History of Traditional Herbs as Therapeutics

People back in time did not have sufficient information on the type of illness they had nor about the suitable medication to use to cure their sickness, therefore they relied on the traditional way which is the usage of medicinal plants. This has, since then, been proven to be very effective. Throughout the years, discovery of drugs from the leaves, barks and stems of medicinal plants has resulted in these drugs being widely used by traditional medicine practitioners in their prescription to treat various ailments. Currently with the continuous effort of scientist and apothecarists, the information on the medicinal plants is better characterized and can be used to cure more chronic illness in a definitive way (Petrovska, 2012). The use of traditional herbs is practiced today in many developing countries which rely on their indigenous form of traditional medicine in their daily healthcare regime (Chang and David, 2005). There is an increasing preference to use traditional herbs as therapeutic agents as people are beginning to be more aware of the benefits of au natural treatment which have reduced or even no side effects compared to modern drugs. Traditional herbs can also be administered in a more pleasant manner as we can mix the herbs with tea or food, rather than just swallowing pills. Furthermore, traditional herbs treatment does not target only one type of illness, they are usually prescribed to target several related illness. This effect could be enhanced by the synergistic use of various traditional herbs. There are quite a number of medicines which are derived from medicinal plants, such as morphine from Papaversomniferum sp. and Ephedrine from Ephedra vulgaris (Prakash and Gupta, 2004). The World Health