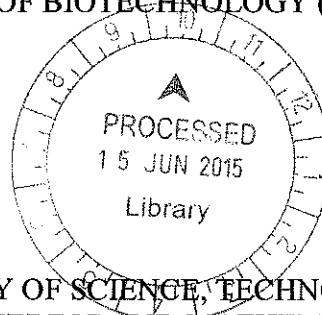


INDIVIDUAL AND SYNERGISTIC ANTIMICROBIAL PROPERTIES OF *Plectranthus amboinicus*, *Murraya koenigii*, *Ocimum sanctum* and *Azadirachta indica* AGAINST ACNE CAUSING BACTERIA

YAP KE XIN

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



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YAP
2015

FACULTY OF SCIENCE, TECHNOLOGY,
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INTI INTERNATIONAL UNIVERSITY
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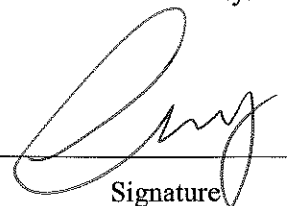
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ACKNOWLEDGEMENT

The journey of writing this dissertation has been a tough one and I will not be able to achieve the outcomes of my project without the support and guidance from several people. I would like to take this opportunity to express my appreciation and gratitude to them.

First and foremost, I would like to express my sincere gratitude to my academic supervisor, Dr. Geetha Subramaniam, who gave me unconditional support, guidance and advices throughout the research project even though she had a very tight schedule. Special thanks to Dr Choong Chieh Wean as well who helped me to take nice photographs of the results in my project. A warm gratitude to Ms. Lalita, Dr. Choong, Ms. Emily and Dr Wong for taking their time to conduct dissertation writing workshops. I have learnt so much from them.

I am also thankful to the laboratory staff, Ms. Quah Hui Hsien and Mr. Ng Peng Wah, who assisted me throughout the project by providing me guidance on the operation of the laboratory equipment and necessary laboratory apparatus or chemicals whenever I needed them. They have also provided me useful suggestions and input for my project.

A warm thanks to my coursemate, Lim Jia Xin who shared her laboratory apparatuses and chemicals with me for the completion of the project.

Lastly, I would like to thank my family and friends who have been there for me throughout the whole journey. Their support and encouragement are the driving energy for me to complete my degree.

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.

TABLE OF CONTENT

	PAGE
DECLARATION	ii
ACKNOWLEDGEMENT	iii
ABSTRACT	iv
TABLE OF CONTENTS	v
LIST OF TABLES	viii
LIST OF FIGURES	ix
LIST OF ABBREVIATIONS	xi
CHAPTER	
1. INTRODUCTION	1
2. LITERATURE REVIEW	3
2.1 Antibiotics resistance and issues with modern drugs	4
2.2 Traditional herbs	4
2.2.1 Brief history of traditional herbs as therapeutics	5
2.2.2 Traditional herbs of interest	6
2.2.2.1 <i>Plectranthus amboinicus</i>	6
2.2.2.2 <i>Murraya koenigii</i>	6
2.2.2.3 <i>Azadirachta indica</i>	6
2.2.2.4 <i>Ocimum sanctum</i>	7
2.2.3 Therapeutic properties of <i>Plectranthus amboinicus</i> , <i>Murraya koenigii</i> , <i>Azadirachta indica</i> and <i>Ocimum</i> <i>sanctum</i>	8
2.3 Acne	10
2.3.1 What is acne	11
2.3.2 Causative agent of acne	12
2.3.3 Treatment options for acne	13
3. MATERIALS AND METHODS	15
3.1 Preparation of agar	15
3.1.1 Preparation of agar (Nutrient agar)	15
3.1.2 Preparation of broth (Nutrient broth)	15
3.1.3 Preparation of agar (Mueller Hinton agar)	15
3.1.4 Preparation of agar (Mannitol Salt agar)	15
3.2 Bacterial cultures	16
3.2.1 Preparation of pure bacteria cultures	16
3.2.2 Preparation of inoculum for disc diffusion assay	16

3.2.3	Preparation of bacteria cultures from acne	16
3.2.4	Preparation of anaerobic bacterial cultures	16
3.3	Preparation of traditional herbs	17
3.3.1	Plant sources	17
3.3.2	Crude plant extracts preparation	17
3.4	Confirmation test	19
3.4.1	Gram staining	19
3.4.2	Differentiation of <i>Staphylococcus aureus</i> and <i>Staphylococcus epidermidis</i> using catalase test	20
3.4.3	Differentiation of <i>Staphylococcus aureus</i> and <i>Staphylococcus epidermidis</i> using differential agar	20
3.5	Antimicrobial effect of crude plant extracts	20
3.5.1	Inoculum preparation (pure culture)	20
3.5.2	Inoculum preparation (acne sample culture)	20
3.5.3	Preparation of disc with plant extracts	21
3.5.4	Disc diffusion technique (pure culture)	21
3.5.5	Disc diffusion technique (acne sample culture)	22
4.	RESULTS	24
4.1	Confirmation test	24
4.1.1	Gram staining	24
4.1.1.1	Gram staining on pure culture (<i>Staphylococcus aureus</i> and <i>Staphylococcus epidermidis</i>)	24
4.1.1.2	Gram staining on acne sample culture	25
4.1.1.3	Gram staining on acne sample culture grown under anaerobic condition	25
4.1.2	Catalase test	26
4.1.3	Confirmation on the strain of microbes by using differential agar	27
4.2	Antimicrobial test	29
4.2.1	Preparation of pure culture of inoculum	29
4.2.2	Preparation of acne sample culture inoculum	29
4.2.3	Antimicrobial test on pure bacterial culture with individual crude plant extracts	30
4.2.4	Antimicrobial test on pure bacterial culture with combination of two or more crude plant extracts	31
4.2.5	Antimicrobial test on acne samples collected from different volunteers against individual crude plant extracts	33
4.2.6	Antimicrobial test on acne samples collected from different volunteers against crude plant extracts in different combination	33

5. DISCUSSION	36
5.1 Confirmation of strain of bacteria	36
5.1.1 Determination of <i>Staphylococcus aureus</i> , <i>Staphylococcus epidermidis</i> and <i>Propionibacterium acnes</i> using Gram staining	36
5.1.2 Catalase test to confirm the presence of <i>Staphylococcus aureus</i> and <i>Staphylococcus epidermidis</i>	36
5.1.3 Mannitol Salt agar differentiate between <i>Staphylococcus aureus</i> and <i>Staphylococcus epidermidis</i>	37
5.2 Antimicrobial efficacy of crude plant extracts	37
5.2.1 Disc diffusion technique	37
5.2.2 Antimicrobial efficacy of individual crude plant extracts against pure bacteria culture	38
5.2.3 Antimicrobial efficacy of crude plant extracts in combination against pure bacteria culture	39
5.2.4 Antimicrobial efficacy of individual crude plant extracts against acne samples	40
5.2.5 Antimicrobial efficacy of crude plant extracts in combination against acne samples	41
6. CONCLUSIONS AND RECOMMENDATIONS	44
REFERENCES	45
APPENDICES	52

LIST OF TABLES

Table		Page
1	Range of bacteria susceptible to antimicrobial activity of <i>Plectranthus amboinicus</i> , <i>Azadirachta indica</i> , <i>Ocimum sanctum</i> and <i>Murraya koenigii</i>	8
2	Content for respective filter paper disc in plates with pure bacteria culture.	22
3	Content for respective filter paper disc in plates with cultured acne sample.	23
4	Results of gram staining for <i>Staphylococcus aureus</i> and <i>Staphylococcus epidermidis</i>	24
5	Figures of inoculated Mannitol Salt agar	28
6	Ratio for inoculum dilution for pure cultures	29
7	Ratio for inoculum dilution for acne samples	29
8	Antimicrobial test of crude extracts of <i>P. amboinicus</i> , <i>M. koenigii</i> , <i>A. indica</i> and <i>O. sanctum</i> against pure bacterial cultures	30
9	Antimicrobial test of crude extracts of <i>Plectranthus amboinicus</i> , <i>Murraya koenigii</i> , <i>O. sanctum</i> and <i>Azadirachta indica</i> in different combination against pure bacterial cultures	31
10	Figures of the formation of inhibitory zones on Mueller Hinton agar	32
11	Antimicrobial test of crude extracts of <i>Plectranthus amboinicus</i> , <i>Murraya koenigii</i> and <i>Azadirachta indica</i> against bacteria in acne samples collected from 5 volunteers	33
12	Antimicrobial test of crude extracts of <i>Plectranthus amboinicus</i> , <i>Murraya koenigii</i> and <i>Azadirachta indica</i> in various combinations against bacteria in acne samples collected from different volunteers	34
13	Figures showing the formation of inhibitory zones on Mueller Hinton agar	35

LIST OF FIGURES

Figure		Page
2.1	Leaves of medicinal plants used in this study A: <i>Plectranthus amboinicus</i> (adapted from Pillai et al., 2011) B: <i>Murraya koenigii</i> , (adapted from Noolu et al., 2013) C: <i>Azadirachta indica</i> (adapted from Csurhurs, 2008) D: <i>Ocimum Sanctum</i> (adapted from Mohan et al., 2011)	5
2.2	Biotherapeutics available on market A: Tulsi extracts (adapted from Sandhus, 2015) B: Forskolin extracts (adapted from Bioganix, 2015) C: Neem extracts (adapted from The Himalaya Drugs Company, (n.d.))	9
2.3	The formation of acne on human's skin (adapted from Ho and Friedlander, 2012)	10
2.4	The development of acne from <i>Propionibacterium acnes</i> proliferation (adapted from Gupta, 2009)	11
2.5	A: gram staining of <i>S. epidermidis</i> at 100x magnification (adapted from Sahay, 2013) B: gram staining of <i>S. aureus</i> at 100x magnification (adapted from Tanbe, 2005) C: Gram staining of <i>P. acnes</i> (adapted from Strong, 1972)	12
3.1	Flow chart depicting the preparation of crude plant extracts	18
3.2	Flow chart depicting the procedure of gram staining	19
4.1	Gram stain of <i>S. aureus</i> under oil immersion objective	24
4.2	Gram stain of <i>S. epidermidis</i> under oil immersion objective	24
4.3	Gram stain of an acne sample under oil immersion objective	25
4.4	The gram stain of acne sample grown under microoxic condition	26

4.5	Catalase test A and B: Bubbles formed on <i>S. aureus</i> and <i>S. epidermidis</i> respectively after addition of H ₂ O ₂ C: No bubbles formed on <i>S. pneumonia</i> culture after addition of H ₂ O ₂	26
4.6	Colony formation of <i>S. epidermidis</i> and <i>S. aureus</i> on Mannitol Salt agar	28
4.7	Colony formation of cultured acne sample No. 1 on Mannitol Salt agar	28
4.8	Colony formation of cultured acne sample No. 2 on Mannitol Salt agar	28
4.9	Zone of inhibition formed around disc impregnated with plant extracts on <i>S. epidermidis</i> culture.	32
4.10	Zone of inhibition formed around disc impregnated with plant extracts on <i>S. aureus</i> culture.	32
4.11	Zone of inhibition formed around disc impregnated with plant extracts on <i>S. epidermidis</i> culture.	32
4.12	Zone of inhibition formed around disc impregnated with plant extracts in cultured acne sample culture No. 5.	35
4.13	Zone of inhibition formed around disc impregnated with plant extracts in cultured acne sample No. 4.	35

LIST OF ABBREVIATIONS

°C	degree Celsius
µg	microgram
µg/mL	microgram per milliliter
µL	microlitre
µm	micrometer
mm	millimeter
g	grams
rpm	revolutions per minute
nm	nanometer
H ₂ O ₂	Hydrogen peroxide
<i>S. aureus</i>	<i>Staphylococcus aureus</i>
<i>S. epidermidis</i>	<i>Staphylococcus epidermidis</i>
<i>P. acnes</i>	<i>Propionibacterium acnes</i>
<i>S. pneumonia</i>	<i>Streptococcus pneumoniae</i>
<i>P. amboinicus</i>	<i>Plectranthus amboinicus</i>
<i>M. koenigii</i>	<i>Murraya koenigii</i>
<i>O. sanctum</i>	<i>Ocimum sanctum</i>
<i>A. indica</i>	<i>Azadirachta indica</i>

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, Ayurvedic 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 *Staphyococcus 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