INDIVIDUAL AND COMBINATORIAL ANTIBACTERIAL PROPERTIES OF Plectranthus amboinicus, Murraya koenigii, Acorus calamus and Azadiractha indica AGAINST ACNE CAUSING BACTERIA Staphylococcus aureus, Propionibacterium acnes and Staphylococcus epidermidis

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ABSTRACT

Bacteria have developed resistance against various antibiotics. The aim of this study is to test for antibacterial properties of *Murraya koenigii*, *Plectranthus amboinicus*, *Azadirachta indica* and *Acorus calamus* crude extracts against clinically important bacteria *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Propionibacterium acnes*. A preliminary test was done to test individual and also combinatorial ethanolic extract formulas using the disk diffusion assay and agar diffusion assay against the pure bacterial cultures and also acne samples that had been obtained with the consent of volunteers. The disk diffusion assay carried out allowed less amount of extracts of *M. koenigii*, *P. amboinicus*, *A. indica* and *A. calamus* compared to the agar diffusion assay. The negative control used was 80% ethanol to confirm that the ethanol in the extraction preparation did not affect the antibacterial activity. It was noticed that all four extracts had antibacterial activity. However, in combination with *A. calamus*, there is an antagonistic effect that decreases the diameter of the zone of inhibition produced. The combination of *M. koenigii* and *A. indica* showed promising results and was further tested against pure bacterial cultures and acne sample cultures and provided a rather satisfying zone of inhibition for several sample especially Acne sample 5. Each combination of extract had different effect on different bacteria. ANOVA analysis also showed the mean difference is significant at the 0.05 level for *P. acnes*, Acne sample 5, 6, and 7 where else no significance was generated at 0.05 level for *S. aureus*, *S. epidermidis* and Acne sample 4. The tests also suggested that for topical application of these antibacterial agents, the crude plant extracts are best individually and not in combination.
# TABLE OF CONTENT

NON-PLAGIARISM DECLARATION  
ACKNOWLEDGEMENT  
ABSTRACT  
TABLE OF CONTENT  
LIST OF TABLES  
LIST OF FIGURES  
LIST OF ABBREVIATIONS  

CHAPTER

1. INTRODUCTION 1

2. LITERATURE REVIEW 3

2.1 MEDICINAL PLANTS: A BRIEF HISTORY 3
   2.1.1 Azadirachta indica 4
   2.1.2 Murraya koenigii 5
   2.1.3 Plectranthus amboinicus 8
   2.1.4 Acorus calamus 9

2.2 THE MICROBIOME OF THE SKIN: Staphylococcus aureus, Staphylococcus epidermidis and Propionibacterium acnes 11

2.3 THE BACTERIAL RESISTANCE AGAINST SYNTHETIC ANTIBIOTIC AND MEDICINAL PLANTS 15

2.4 THE SYNERGISTIC EFFECT OF MEDICINAL PLANTS 15

3. MATERIALS AND METHODS 17

3.1 PREPARATION OF MEDIA 17
   3.1.1 Autoclave method 17
   3.1.2 Preparation of Nutrient Agar 17
   3.1.3 Preparation of Nutrient Broth 17
3.2 PREPARATION OF BACTERIAL INOCULA FOR THE DISC DIFFUSION ASSAY

3.2.1 Streaking of Pure Bacterial Cultures 18
3.2.2 Preparation of Inoculum 19

3.3 PREPARATION OF PLANT CRUDE EXTRACTS 19

3.4 CONFIRMATION TESTS 20
3.4.1 Gram Staining 20
3.4.2 Catalase Test 21
3.4.3 Mannitol salt agar 21

3.5 DISC DIFFUSION ASSAY 21

3.6 AGAR DIFFUSION ASSAY 23

3.7 CLINICAL SAMPLE TESTING 24
3.7.1 Inoculum preparation of acne sample 24
3.7.2 Confirmation test on acne samples 24
3.7.3 Agar Diffusion method 24

3.8 STATISTICAL ANALYSIS 24

4. RESULTS 25

4.1 CONFIRMATION TESTS 25
4.1.1 Gram staining 25
4.1.1.1 Gram staining on pure cultures of P. acnes, S. aureus and S. epidermidis 25
4.1.1.2 Gram staining on acne sample culture 27
4.1.2 Catalase test 27
4.1.3 Confirmatory test on Mannitol salt agar 29

4.2 ANTIMICROBIAL TEST 31
4.2.1 Preparation of Pure culture Inoculum 31
4.2.2 Preparation of Acne Sample Inoculum 31
4.2.3 Antimicrobial Test on Pure Bacterial Culture with Individual and Combinatory Crude Plant Extracts by Disk Diffusion Assay 31

4.2.4 Antimicrobial Test on Pure Bacterial Culture and Acne Samples with Individual and Combinatory Crude Plant Extracts of Murraya koenigii and Azadirachta indica by Agar Diffusion Assay 37
4.2.5 Graphs of antibacterial activity 41
5. DISCUSSION
5.1 CONFIRMATION TEST
  5.1.1 Gram staining of bacterial pure cultures and acne samples
  5.1.2 Catalase test
  5.1.3 Mannitol Salt Agar as a Differential media
5.2 ANTIMICROBIAL ACTIVITY OF CRUDE PLANT EXTRACTS
  5.2.1 Disk Diffusion Technique for Individual Crude Plant extracts against
        Pure Bacterial Culture
  5.2.2 Disk Diffusion Technique for Combinatorial Crude Plant extracts
        against Pure Bacterial Culture
  5.2.3 Antimicrobial Test on Pure Bacterial Culture and Acne Samples
        with Individual and Combinatory Crude Plant Extracts of Murraya
        koenigii and Azadirachta indica by Agar Diffusion Assay

6. CONCLUSION AND RECOMMENDATIONS
REFERENCES
APPENDICES
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1.1</td>
<td>The background or taxonomy of <em>Azadirachta indica</em></td>
<td>4</td>
</tr>
<tr>
<td>2.1.2</td>
<td>Chemical constituents of <em>Azadiractha indica</em> (Neem) and its corresponding therapeutic properties in providing activity</td>
<td>5</td>
</tr>
<tr>
<td>2.1.3</td>
<td>Background and taxonomy of <em>Murraya koenigii</em></td>
<td>6</td>
</tr>
<tr>
<td>2.1.4</td>
<td>Parts of the <em>Murraya koenigii</em> and few contributions medicinally</td>
<td>7</td>
</tr>
<tr>
<td>2.1.5</td>
<td>The maximum zone of inhibition obtained as a result of antibacterial activity of <em>P. amboinicus</em> against <em>S. aureus</em>, <em>P. aeruginosa</em> and <em>E.coli</em></td>
<td>8</td>
</tr>
<tr>
<td>2.1.6</td>
<td>The scientific background of <em>Plectranthus amboinicus</em></td>
<td>8</td>
</tr>
<tr>
<td>2.1.7</td>
<td>The scientific background of <em>Acorus calamus</em></td>
<td>10</td>
</tr>
<tr>
<td>2.1.8</td>
<td>Active chemical constituents and pharmacological properties against several clinically important bacteria and also test subjects that include models such as mice and humans.</td>
<td>10</td>
</tr>
<tr>
<td>2.2.1</td>
<td>Susceptibility of <em>Propionibacterium acnes</em> to several plant extracts and a positive control antibiotic (clindamycin) that correspond to their respective zone of inhibition</td>
<td>13</td>
</tr>
<tr>
<td>2.2.2</td>
<td>Susceptibility of <em>Staphylococcus epidermidis</em> to several plant extracts and a positive control antibiotic (clindamycin) corresponding to their respective zone of inhibition</td>
<td>14</td>
</tr>
<tr>
<td>2.4.1</td>
<td>Curcumin combined with either antibiotics or elements of the periodic table to result in certain antimicrobial property for synergistic effects</td>
<td>16</td>
</tr>
<tr>
<td>3.3.1</td>
<td>Weights of each medicinal plant leaf powder and the amount of 80% ethanol added</td>
<td>20</td>
</tr>
<tr>
<td>3.5.1</td>
<td>Filter paper disks impregnated with respective plant extracts, 80% ethanol and antibiotic discs placed on Mueller Hinton agar according to its labelled position in the quadrant</td>
<td>22</td>
</tr>
</tbody>
</table>
3.5.2 The appropriate amounts of each extract added to the filter paper disks for individual extracts and combinatorial extracts

3.6.1 Wells pipetted with respective plant extracts, 80% ethanol and antibiotic discs placed on Mueller Hinton agar according to its labelled position in the quadrant on pure bacterial culture and acne samples

4.1.1 Gram staining images of P. acnes, S. aureus and S. epidermidis and their morphological description according to the viewed image

4.1.2 Catalase test images of bubble formation in all three bacterial cultures after addition of H₂O₂

4.1.3 Images of each bacterial pure culture and the acne sample on mannitol salt agar

4.2.1 The mean diameter of the zone of inhibition in millimeters (mm) of each plant extract individually and combinatorial in triplicates with 80% ethanol as a negative control

4.2.2 Images of zone of inhibition of all three bacteria against the crude plant extracts

4.2.3 Mean zone of inhibition of pure cultures against crude plant extracts of M. koenigii and A. indica individually and combinatorically with 80% ethanol as negative control

4.2.4 Raw data of the zone of inhibition in millimeters (mm) of pure cultures against crude plant extracts of M. koenigii and A. indica individually and combinatorial

4.2.5 Images of agar diffusion assay to test antimicrobial activity of M. koenigii and A. indica against pure culture inoculum and acne samples

4.2.6 Comparison of effectiveness of the positive controls against S. aureus, S. epidermidis and P. acnes
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figures</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Neem seeds, leaves and branch</td>
</tr>
<tr>
<td>2.2</td>
<td>The bipinnate leaves of <em>Murraya koenigii</em> hosting 11 to 25 leaves per branch B: seeds of <em>Murraya koenigii</em> that are green in colour, 11 mm long and 8 mm in diameter C: flowers of <em>Murraya koenigii</em> that are white in colour bearing up to 90 flowers.</td>
</tr>
<tr>
<td>2.3</td>
<td>Image of <em>Plectranthus amboinicus</em></td>
</tr>
<tr>
<td>2.4</td>
<td>Rhizomes of <em>Acorus calamus</em> or by its common name “sweet flag”</td>
</tr>
<tr>
<td>4.1</td>
<td>Gram stain of <em>P. acnes</em> under oil immersion objective lenses</td>
</tr>
<tr>
<td>4.2</td>
<td>Gram stain of <em>S. aureus</em> under oil immersion objective lenses</td>
</tr>
<tr>
<td>4.3</td>
<td>Gram stain of <em>S. epidermidis</em> under oil immersion objective lenses</td>
</tr>
<tr>
<td>4.4</td>
<td>Gram stain of an acne sample under 1000× magnification in oil immersion showing purple stained cocci and rods</td>
</tr>
<tr>
<td>4.5</td>
<td>Bubble formation with <em>P. acnes</em> after addition of H₂O₂</td>
</tr>
<tr>
<td>4.6</td>
<td>Bubble formation with <em>S. aureus</em> after addition of H₂O₂</td>
</tr>
<tr>
<td>4.7</td>
<td>Bubble formation with <em>S. epidermidis</em> after addition of H₂O₂</td>
</tr>
<tr>
<td>4.8</td>
<td>Colony formation of <em>S. aureus</em> on Mannitol salt agar</td>
</tr>
<tr>
<td>4.9</td>
<td>Colony formation of <em>S. epidermidis</em> on Mannitol salt agar</td>
</tr>
<tr>
<td>4.10</td>
<td>Colony formation of <em>P. acnes</em> on Mannitol salt agar</td>
</tr>
<tr>
<td>4.11</td>
<td>Colony formation of bacterial culture AS6 on Mannitol salt agar</td>
</tr>
<tr>
<td>4.12</td>
<td>Colony formation of bacterial culture AS7 on Mannitol salt agar</td>
</tr>
<tr>
<td>4.13</td>
<td>Zone of inhibition of <em>S. aureus</em> against I, II, I &amp; II and NC</td>
</tr>
<tr>
<td>4.14</td>
<td>Zone of inhibition of <em>S. epidermidis</em> against I, II, I &amp; II and NC</td>
</tr>
</tbody>
</table>
4.15 Zone of inhibition of *P. acnes* against I, II, I & II and NC

4.16 Zone of inhibition of *S. aureus* against III, IV, I & III and NC

4.17 Zone of inhibition of *S. epidermidis* against III, IV, I & III and N

4.18 Zone of inhibition of *P. acnes* against III, IV, I & III and NC

4.19 Zone of inhibition of *S. aureus* against I & IV, II & III, II & IV and NC

4.20 Zone of inhibition of *S. epidermidis* against I & IV, II & III, II & IV and NC

4.21 Zone of inhibition of *P. acnes* against I & IV, II & III, II & IV and NC

4.22 Zone of inhibition of *S. aureus* against III & IV, I, II & III and NC

4.23 Zone of inhibition of *S. epidermidis* against III & IV, I, II & III and NC

4.24 Zone of inhibition of *P. acnes* against III & IV, I, II & III and NC

4.25 Zone of inhibition of *S. aureus* against I, II & IV, II, III & IV and NC

4.26 Zone of inhibition of *S. epidermidis* against I, II & IV, II, III & IV and NC

4.27 Zone of inhibition of *P. acnes* against I, II & IV, II, III & IV and NC

4.28 Zone of inhibition of *S. aureus* against I, III & IV, I, II, III & IV and NC

4.29 Zone of inhibition of *P. acnes* against I, III & IV, I, II, III & IV and NC

4.30 Zone of inhibition of *S. epidermidis* against I, III & IV, I, II, III & IV and NC

4.31 Zone of inhibition from agar diffusion of I, II, I & II and NC against *S. aureus*

4.32 Zone of inhibition from agar diffusion of I, II, I & II and NC against *S. epidermidis*
4.33 Zone of inhibition from agar diffusion of I, II, I & II and NC against *P. acnes* 38

4.34 Zone of inhibition from antibiotic disks that serve as a positive control against *S. aureus* 38

4.35 Zone of inhibition from antibiotic disks that serve as a positive control against *S. epidermidis* 38

4.36 Zone of inhibition from antibiotic disks that serve as a positive control against *P. acnes* 38

4.37 Zone of inhibition by I, II, I & II and NC against acne culture sample 4 39

4.38 Zone of inhibition by I, II, I & II and NC against acne culture sample 5 39

4.39 Zone of inhibition by I, II, I & II and NC against acne culture sample 6 39

4.40 Zone of inhibition by I, II, I & II and NC against acne culture sample 7 39

4.41 The diameter of zone of inhibition (mean ± SD; mm) by crude plant extracts in response to *S. aureus* 41

4.42 The diameter of zone of inhibition (mean ± SD; mm) by crude plant extracts in response to *S. epidermidis* 42

4.43 The diameter of zone of inhibition (mean ± SD; mm) by crude plant extracts in response to *P. acnes* 42

4.44 The diameter of zone of inhibition (mean ± SD; mm) by crude plant extracts in response to acne sample 5 (AS 5) 43

4.45 The diameter of zone of inhibition (mean ± SD; mm) by crude plant extracts in response to acne sample 6 (AS 6) 43

4.46 The diameter of zone of inhibition (mean ± SD; mm) by crude plant extracts in response to acne sample 7 (AS 7) 44
LIST OF ABBREVIATIONS

°C             degree Celsius
CFU/mL         colony forming per unit millilitre
Rpm            revolutions per minute
H₂O₂           Hydrogen peroxide
S. aureus      Staphylococcus aureus
S. epidermidis Staphylococcus epidermidis
P. acnes       Propionibacterium acnes
P. amboinicus  Plectranthus amboinicus
E. coli        Escherichia coli
B. subtilis    Bacillus subtilis
S. typhi       Salmonella typhi
M. koenigii    Murraya koenigii
A. indica      Azadirachta indica
A. calamus     Acorus calamus
AS 4           Acne sample 4
AS 5           Acne sample 5
AS 6           Acne sample 6
AS 7           Acne sample 7
CHAPTER 1

INTRODUCTION

Before the use of conventional drugs, man relied on natural ways to treat diseases such as topical application of ground leaves on wounds. Medicinal plants have been known to embody very promising antimicrobial agents according to Bhagat et al. (2015). In Malaysia, the diversity of plants that provide therapeutic properties has been astonishing. With about 2000 or more have been proven to have medicinal purposes based on Arifullah et al. (2014). Traditional practices are still carried out in Malaysia and the use and effects of medicinal plants give enhancement to it. The main reason of using medicinal plants for therapeutic purposes is because modern medication contains too much chemicals and there also has been an over exploitation of antibiotic usage reported by Kazemipoor et al. (2012). According to Njoroge & Bussmann, (2006), the misuse of antibiotics has led to increased occurrence of bacterial resistance. The continuation of this has led to a build-up of resistance in bacteria and thus proving conventional drugs are beginning to not work anymore. Therefore, the use of medicinal plants have justified that phytochemicals present in plants are just as good as accomplishing antimicrobial activities as conventional drugs.

Plant based therapeutics are less expensive, easily biodegradable, harmless to the environment and have also been proven as non-narcotic (Fullerton et al., 2011). Medicinal plants not only provide antibacterial activities but have been proven to show other activities as well such as anti-cancer, anti-ulcer, antioxidant, anti-inflammatory, anti-septic and anti-pyrogenic (Bhatt, 2014). According to Moghadamtousi et al. (2014), due to rise in drug resistant microbes, there is a need to study the antimicrobial activity in a combination of the plants constituents. Since individual plant extracts have been proven to show certain antimicrobial effects, the synergistic properties is posed to have more promising effects on clinically vital bacteria (Ncube et al., 2012).

The present study has been intended to test for antimicrobial activity of plants valued to Indians and found abundantly in Malaysia. This research is to test antimicrobial properties of value added plants namely *Azadirachta indica*, *Ocimum
*Helichrysum tenuiflorum, Murraya koenigii* and *Acorus calamus*. The antimicrobial test is determined by disk diffusion method against bacteria causing acne namely *Propionibacterium acnes*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. This study also investigates the effects of a combination of plant extracts against the bacteria causing acne. The tendency to carry out antimicrobial activities by the plant extracts are also compared to that of antibiotics namely penicillin, tetracycline and erythromycin as positive controls. These extracts will be assayed using the disk diffusion method and agar diffusion method.