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The synergistic antimicrobial effects of crude plant extracts from Plectrantus amboinicus, Azadirachta indica and Murraya koenigii on Staphylococcus sp.

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***Abstract*—** Antibiotic resistance in clinically important bacteria is an increasing problem worldwide. Alternative therapeutic agents such as medicinal plants should be sourced to overcome this problem.
Acne is a common skin disorders in many people and can have a debilitating effect on the self-esteem and confidence of an individual. *Staphylococcus aureus* and *Staphylococcus epidermidis*. The aim of this study was to determine if crude plant extracts from medicinal plants could be used as a suitable alternative to antibiotics for the treatment of acne.

Crude extracts were obtained from three medicinal plants: *Plectrantus amboinicus, Azadirachta indica* and *Murraya koenigii* using an ethanol extraction method. The extracts were tested individually and in combination against two common causative agents of acne: *Staphylococcus aureus* and *Staphylococcus epidermidis*. The antimicrobial activity was indicated by the zone of inhibition obtained in the disk diffusion assay.

All the plant extracts exhibited antimicrobial activity when tested individually against the acne-causing bacteria, with the greatest activity exhibited by *Murraya koenigii*. The combination of plant extracts from *Murraya koneigii* and *Azadirachta indica* showed a synergistic activity against *Staphylococcus aureus* and *Staphylococcus epidermidis*. The combination of *Murraya koneigii* and *Plectrantus amboinicus* showed an antagonistic effect whereby the inhibition of bacterial growth was suppressed.

This study shows that the combination of *Murraya koneigii* and *Azadirachta indica* is a good potential alternative to antibiotics for the treatment of acne.

***Keywords*—** plant extracts, *Staphylococcus aureus, Staphylococcus epidermidis,* acne, antimicrobial properties

I. INTRODUCTION

Infectious diseases which account for the mortality of approximately 50,000 people ever day become the world's foremost cause of premature death. (1) Bacterial etiologic agents such as *Shigella sp.*, *Vibrio cholera*, pathogenic *Escherichia coli*, *Pseudomonas sp.*, *Staphylococcus aureus* and *Enterobacter sp.* are the most common cause of infection diseases. (1,2) In recent years, as a result of the overuse and misuse of antibiotics,(3) there has been increased incidence of the bacterial resistance to currently available antibiotics reported worldwide, thus making it a threatening health issue. (1,2). The development in international trade and travel has allowed drug-resistant pathogens, including methicillin-resistant *Staphylococcus aureus*, multidrug-resistant *Streptococcus pneumonia*, and ESBL-producing *Escherichia coli* and *Pseudomonas aeruginosa* to rapidly spread worldwide. In addition, some patients who take antibiotics develop adverse effects, including hypersensitivity, reduction of beneficial gut and mucosal microorganisms and immunosuppression which make the treatment of infectious diseases problematic (3).

Therefore, in order to overcome the antibiotic resistance of bacteria and the side effects of antibiotics in the treatment of infectious diseases, looking for other antimicrobial drugs as an alternative to antibiotics becomes necessary. Nowadays, there is an increased interest to search for potential antimicrobial compounds from medicinal plants for the purpose of developing new antimicrobial drugs to fight against antibiotic-resistant bacteria (1,2,3,6,7,8) since medicinal plants offer many advantages such as comparatively less expensive, better tolerance of patient, less adverse side effects and being reproducible in nature. (2) Futhermore, resistance to plant extracts has been shown to be much less than resistance to antibiotics. Plant extracts are expected to be effective against antibiotic-resistant bacteria as their target sites on the bacteria vary from those used by antibiotics. (6) Since the late 19th century, documentation of scientific experiments performed to investigate the antimicrobial properties of plants and their components has enabled the accumulation of a great deal of evidence showing the promising potential of medicinal plants in curing various human diseases. (2)

In 2005, World Health Organization (WHO) revealed that there was nearly 60-80% of the world's population using traditional remedies to treat common diseases. (9) The development of new antimicrobial drugs from medicinal plants is a great advantage to Malaysia because Malaysia is one of 12 countries in the world that has mega biodiversity, especially in terms of its flora. (7) It has been reported to have more than

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20,000 plant species in the wild (7). Among 20,000 plant species, almost 2000 or more plants are found to have medicinal value. (7) In Malaysia, many traditional health care systems have employed these medicinal plants to treat various diseases. (8)

Usually, the ingredients from several different medicinal plant species are used to prepare traditional remedies in order to generate synergistic effects which have been demonstrated to have better therapeutic efficacy than a single constituent plant or a single isolated active compound of same dose (7, 8) due to the different mechanisms of actions produced by diverse classes of compounds. Hence, when the interaction between two or more compounds leads to the more significant potentiation of each other's effect than a single plant or single active compound, synergism take places. (9)

In this study, we investigated the antimicrobial activity of four medicinal plants *Azadirachta indica, Ocimum tenuiflorum, Plectranthus amboinicus* and *Murraya koenigii* against clinically important bacteria causing acne, both individually and in combination.

II. Methodology

A. Preparation of bacterial inocula

Bacterial cultures were screened using Manitol salt agar to distinguish between *s. aureus* and *S. epidermidis.* Other confirmatory tests performed included Gram stain and Catalase test. Single colonies of pure bacterial cultures were inoculated in Nutrient broth and incubated overnight at 37^0C. The overnight cultures were diluted to a concentration similar to 0.5 McFarlands standard (ThermoFisher Scientific) to ensure appropriate amount of bacterial culture was used.

B. Preparation of crude plant extracts

Fresh leaves, 100 g each, of *Plectrantus amboinicus, Azadirachta indica and Murraya koneigii* were obtained and dried for two days. The leaves were then washed with ethanol , rinsed with water and dried to remove all traces of liquid. The dried leaves were ground to a coarse powder and subjected to ethanol extraction (11). The crude extracts were filter sterilized and stored in sterile Universal bottles at room temperature until used in the disc diffusion assay.

C. Disc diffusion assay

Antimicrobial activity of the crude plant extracts were tested using the disc diffusion assay. Briefly, pure cultures of *S. aureus* and *S. epidermidis* were lawned onto Mueller-Hinton agar plates. Sterile filter discs impregnated with crude plant extracts of *Murraya koneigii, Azadirachta indica* and *Plectrantus amboinicus* (single and in combination) were placed on the plates and incubated overnight.

D. Detection of antimicrobial activity

Antimicrobial activity of the three medicinal plants against *S. aureus* and *S. epidermidis* were determined by measuring the resulting zones of inhibition produced in the disc diffusion assay. The results were tabulated and analyzed.

III. Results and Discussions

The confirmation tests for *S. aureus* and *S. epidermidis* were clearly able to distinguish between both bacteria (Figure 1A and B).

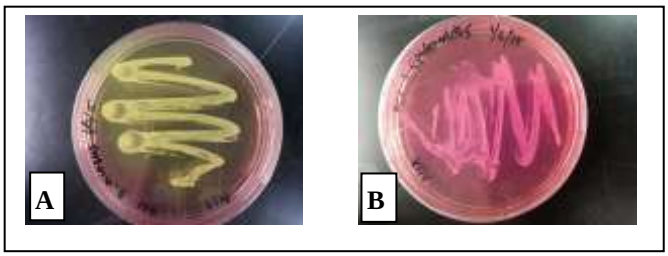

Figure 1. *S. aureus* and *S. epidermidis* cultured on Manitol Salt Agar

The disc diffusion results obtained showed the crude plant extracts from all three medicinal plants, *Plectrantus amboinicus, Azadiractha indica and Murraya koenigii* exhibited antimicrobial activity against both *S. aureus* and *S. epidermidis.*

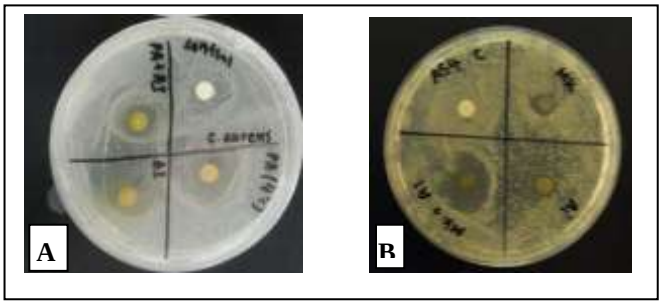

Figure 2. Antimicrobial effects of plant extracts from *A. indica, M. koneigii* and *P.amboinicus* on *S. aureus.*

The combination of plant extracts showed interesting and varied effects. When *Murraya koneigii* and *Azadirachta indica* were combined with *Plectrantus amboinicus*, there was a reduction in the zone of inhibition compared to when these plant extracts were used individually. This showed that *Plectrantus amboinicus* exhibited an antagonistic effect against *S. aureus* and *S. epidermidis* when combined with either *M. koneigii* or *A. indica*.

However, when *M. koneigii* and *A. indica* were combined, the zone of inhibition produced was significantly larger than in individual applications. This indicated a synergistic effect between *M. koneigii* and *A. indica* against *S. aureus* and *S. epidermidis*, making these extracts more potent when combined than when used individually.

The results indicated that certain plant extracts can be used in combination for more effective treatment of Staphylococcal infections whereas certain plant extracts, like *Plectrantus amboinicus*, should be used on their own as they are effective antimicrobial agents but show antagonism when combined

with other plants. However, this data is preliminary and more studies have to be conducted agains a wider range of Staphylococal species before a more definitive conclusion can be made.

Bacterial infections pose a serious threat globally as these infections can result in morbidity and mortality. Indiscriminate use of antibiotics has resulted in the evolution of new and more virulent strains of *S. aureus* resulting in more severe forms of infections in adults and children alike.

Traditional methods of treating infections are becoming increasingly popular. *A. indica, M. koneigii* and *P. amboinicus* are widely used in India for the treatment of several clinically important bacteria. In this study, *A. indica, M. koneigii* and *P. amboinicus* showed significant antimicrobial activity when tested individually. When tested in combination, *P. amboinicus* showed an antagonistic effect with both *M. koneigii* and *A. indica*, as indicated by the smaller zone of inhibition. In contrast, *M. koneigii* and *A. indica* showed significant synergism against *S. aureus* when combined s in different health care facilities.

About 75,000 patients are estimated to carry hospital associated Staphylococcal infections (0.5% per 100 admissions) in Malaysia. If a minimum of three days of hospital admission per person costs about RM1000 to the hospital (Antibiotic-resistant *S. aureus* or *S. epidermidis* infections require high dose, more toxic and expensive antimicrobial treatment), then approximately RM75 million was spent on controlling or screening these infections in Malaysia. Therefore, alternative antimicrobial therapy which is more effective in controlling these infections should be sought..

IV. Conclusion

The preliminary results from this study indicate that the combination of *M. koneigii* and *A. indica* can be used synergistically to design a biotherapeutic agent for the treatment of *S. aureus* infections. In addition, *P. amboinicus* shows good antimicrobial activity against *S. aureus* but should not be used in combination with either *M. koneigii* or *A. indica* as it retards the efficacy of these plants as therapeutic agents.

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