THE SINGLE AND COMBINATION ANTIMICROBIAL EFFECTS OF *OCIMUM TENUIFLORUM*, *PLECTRANTHUS AMBOINICUS*, *AZADIRACHTA INDICA* AND *MURRAYA KOENIGII* AGAINST BACTERIA CAUSING RESPIRATORY TRACT INFECTIONS

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ABSTRACT

Nowadays, medicinal plants have gained an increased attention to be applied as alternatives to antibiotics for the treatment of various diseases. This is a result of the overuse and misuse of antibiotics which reduce the effectiveness of antibiotics against the diseases. *Ocimum tenuiflorum*, *Plectranthus amboinicus*, *Azadirachta indica*, and *Murraya koenigii* are among the medicinal plants which have been claimed to exhibit antimicrobial effect against *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *Pseudomonas aeruginosa* which cause different types of respiratory tract infections. In this study, the individual and synergistic antimicrobial activities of the above mentioned medicinal plants against the above mentioned bacteria were investigated using disc diffusion and agar well diffusion methods. Also, the antimicrobial effect of *A. indica* and *M. koenigii* was compared with that of antibiotics, namely penicillin, erythromycin and tetracycline, which was tested using disc diffusion method. In addition, the potential ability of *S. pneumoniae* and *S. pyogenes* to develop resistance against *A. indica* and *M. koenigii* was investigated using linear gradient plate method. In this study, different individual plant extracts exhibited different levels of antimicrobial effect against each bacterium. The combinatorial plant extract consisting of *A. indica* and *M. koenigii* was more effective than *A. indica* or *M. koenigii* alone against each bacterium. In addition, *A. indica* and *M. koenigii* had higher antimicrobial effect than the antibiotics against *S. pyogenes* and *P. aeruginosa* which was in contrast to their lower effect than tetracycline against *S. pneumoniae*. Moreover, *S. pneumoniae* and *S. pyogenes* were not able to develop resistance against *A. indica* and *M. koenigii*. In conclusion, *A. indica* and *M. koenigii* possess the potential to be applied as antimicrobial agents to treat respiratory tract infections due to *S. pneumoniae*, *S. pyogenes* and *P. aeruginosa*. Therefore, further studies are crucial to confirm their effectiveness and their resistance modifying activity.

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AIDS	acquired immunodeficiency syndrome
CLSI	Clinical and Laboratory Standards of Institute
cfu	colony-forming unit
CV-I complex	crystal violet-iodine complex
CF	cystic fibrosis
^0C	degree Celsius
EPI	efflux pump inhibitor
g	gram
H_2O_2	hydrogen peroxide
MRSA	methicillin-resistant *Staphylococcus aureus*
MSSA	methicillin-susceptible *Staphylococcus aureus*
µL	microliter
µm	micrometer
mg	milligram
mL	milliliter
mm	millimeter
MDR pump	multidrug resistance pump
psi	pounds per square inch
RMA	resistance modifying agent
RTIs	respiratory tract infections
rpm	revolutions per minute
WHO	World Health Organisation

CHAPTER 1

INTRODUCTION

Infectious diseases which account for the mortality of approximately 50,000 people every day have become the world's foremost cause of premature death (Namita & Mukesh, 2012). Bacterial pathogens such as *Shigella* sp., *Vibrio cholera*, enteropathogenic *Escherichia coli* (EPEC), *Pseudomonas* sp., *Staphylococcus aureus* and *Enterobacter* sp. are the most common causes of infectious diseases (Namita & Mukesh, 2012; Hema et al., 2013). In recent years, as a result of the misuse of antibiotics, there has been increased incidence in bacterial resistance to currently available antibiotics (Njoroge & Bussmann, 2006). This phenomenon of antibiotic resistance has been reported worldwide, thus making it a threatening health issue. The development in international trade and travel has allowed drug-resistant pathogens to rapidly spread worldwide (Ebrahim, 2010). Nowadays, we can come across many challenging drug-resistant pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant *Streptococcus pneumoniae* among Gram positive bacteria and ESBL-producing Gram negative bacteria (Lister et al., 2009). Besides, in some cases, 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 (Namita & Mukesh, 2012).

In order to help in overcoming antibiotic resistance and the side effects of antibiotics, there is a need for alternative antimicrobial agents. Currently, there is an increased interest to search for potential antimicrobial compounds from medicinal plants to develop new antimicrobial drugs to fight against antibiotic-resistant bacteria (Morobe et al., 2012; Mohammed Arifullah et al., 2014; Philip et al., 2009). This is because medicinal plants offer many advantages such as comparatively less expensive, better tolerance in patients, less adverse side effects and easily available in nature (Hema et al., 2013). Futhermore, resistance to plant extracts could be much less than resistance to antibiotics since plant extracts have been reported to possess resistance modifying activities (Chovanova et al., 2013). Many plant extracts are sold

individually as powders or in tablet form, and have different effects on various bacteria. Hence, the combination of these extracts could have a more potent effect on clinically important bacteria (Ncube et al., 2012).

The development of new antimicrobial drugs from medicinal plants is a great advantage to Malaysia because among the 20,000 plant species available, almost 2000 or more plants are found to have medicinal value (Mohammed Arifullah et al., 2014). In Malaysia, many traditional health care systems have employed these medicinal plants to treat various diseases (Philip et al., 2009). *Azadirachta indica, Ocimum tenuiflorum, Plectranthus amboinicus* and *Murraya koenigii* which are originated from India and are available in Malaysia are among the medicinal plants that have been applied as traditional medicines. These medicinal plants have been proved to have promising antimicrobial effect against a number of microbes, thus suggesting their use in modern medicine, either individually or synergistically with other plants or antibiotics, to cure diseases caused by those microbes.

This study has been designed to examine the antimicrobial effect of *A. indica, O. tenuiflorum, P. amboinicus* and *M. koenigii* against pathogens causing respiratory tract infections (RTIs), namely *Streptococcus pneumoniae*, *Streptococcus pyogenes* (Gram positive bacteria) and *Pseudomonas aeruginosa* (Gram negative bacterium) by disc diffusion and agar well diffusion methods. Also, this study investigates the synergistic effects of these plant extracts against the above mentioned bacteria. The potential ability of these bacteria to develop resistance against these plant extracts will also be investigated. Furthermore, the effectiveness of these plant extracts against the bacteria will be observed by comparing their antimicobial effect with that of antibiotics.

CHAPTER 2

LITERATURE REVIEW

2.1 MEDICINAL PLANTS

The field of traditional medicine has been growing progressively worldwide in the last few decades. In 2005, World Health Organisation (WHO) revealed that nearly 60-80% of the world's population were using traditional remedies to treat common diseases (Samy Abdel et al., 2012). Medicinal plants are available all over the world, but tropical countries have the most plentiful repository of these precious commodities. Various plant species that have medicinal properties have been identified, researched on, and have been used traditionally to cure diseases (Mousavi et al., 2014). Currently, the increased incidence of microbial resistance to the available antibiotics as a consequence of the misuse and overuse of the antibiotics has resulted in the trend of utilising herbal drugs as an alternative treatment for various human illnesses (Meghashri et al., 2011; Harikumar & Manjusha, 2013; Sharma et al., 2012).

Furthermore, herbal drugs exhibit minimal side effects and less toxicity compared to the most commonly used pharmaceutical drugs such as erythromycin and tetracycline (Sharma et al, 2012; Ravi et al., 2012). India which is designated as the "Botanical Garden of the world", is popular for its considerable number and variety of medicinal plants (Kumar et al., 2013). *O. tenuiflorum*, *P. amboinicus*, *A. indica*, and *M. koenigii* are examples of these medicinal plants which are available and commonly used in India. These four medicinal plants have been claimed to exhibit promising antimicrobial effect against various microbes as shown in Table 2.1.

2.1.1 *Ocimum tenuiflorum*

Ocimum tenuiflorum (Figure 2.1), also called Shyama tulsi, is among the species of the genera *Ocimum* and belongs to the family Lamiaceae (Sharma et al, 2012). It is commonly found in the tropical countries, including Malaysia and India and it is applied as a traditional medicine in these two countries (Mousavi et al., 2014). It is an

erect, tall subshrub with branches and green leaves (Ravi et al., 2012). In traditional medicine, the cure of many ailments, including skin diseases, cough, vomiting, cold, fever, and swelling involves the application of various parts of *O. tenuiflorum* such as flowers, leaves and stem (Ravi et al., 2012). The leaf extracts of *O. tenuiflorum* have been reported to possess several bioactive compounds, including alkaloids, saponins, cardiac glycosides, flavonoids, steroids, and tannins, all of which have contributed to its antimicrobial effect on a range of microbes (Sermakkani & Thangapandian, 2011). In addition, it has been proven to exhibit antioxidant activity, anti-diabetic activity, cardiac activity, and anti-cancer activity (Ravi et al., 2012).

2.1.2 *Plectranthus amboinicus*

Plectranthus amboinicus (Figure 2.2) which belongs to the family Lamiaceae commonly occurs in Africa, America, East Indies and other Asian countries (Sathasivam & Elangovan, 2011; Gurgel et al., 2009). It is an aromatic perennial medicinal plant commonly called Indian borage, Indian oregano and locally called *bangun-bangun*, *bebangun*, *sedingin* or *hati-hati hijau* (Bhatt & Negi, 2012; Erny Sabrina et al., 2014). It has a thick green stem as well as thick, succulent and juicy leaves (Sathasivam & Elangovan, 2011). *P. amboinicus* is used as a folk medicine in the treatment of various disorders, including asthma, cold, cough, fever, headache, inflammation, respiratory infection, fungal and bacterial infections (Bhatt & Negi, 2012; Erny Sabrina et al., 2014; Goncalves et al., 2012; Gurgel et al., 2009). There have been studies reporting the antibacterial, antimicrobial, antioxidant and anti-inflammatory activities of the leaf extracts from *P. amboinicus* (Sathasivam & Elangovan, 2011). The essential oil extracted from its leaves has been confirmed to be effective against several bacteria and fungi as a consequence of the presence of phytochemicals such as carvocrol, thymol, and p-cymene in them (Grace et al., 2012).

2.1.3 *Azadirachta indica*

Azadirachta indica (Figure 2.3), also known as neem tree, is an evergreen tree which belongs to the mahogany family Meliaceae (Mohammad Asif, 2012). It is indigenous to India and Burma and can be found in the tropical and semi-tropical countries. In India, people have applied neem tree in traditional medicine to cure numerous human

ailments for thousands of years such as leprosy, intestinal helminthiasis, respiratory disorders in children, fever, nausea, and so on (Mohammad Asif, 2012). It has been demonstrated by some early studies to have multiple therapeutic properties in nearly every part of the plant, including seeds, roots, leaves, trunk, bark, and branches (Mishra et al., 2013). The antibacterial effect of leaves of *A. indica* has been claimed by some authors (Lall et al., 2013; Mishra et al., 2013; Raja Ratna Reddy et al., 2013). Raja Ratna Reddy et al. (2013) reported that the leaf extract of *A. indica* was most effective against *P. aeruginosa* compared to the bark and seed extracts. Furthermore, *A. indica* is known to possess antifungal, antiviral, antimalarial, antihyperglycaemic, anticarcinogenic, antioxidant and anti-inflammatory activities (Lall et al., 2013).

2.1.4 *Murraya koenigii*

Murraya koenigii (Figure 2.4), also known as "curry leaf plant", belongs to the family Rutaceae (Baskaran et al., 2011). It is indigenous to India, Sri Lanka and other south Asian countries (Harish et al., 2012). It is an aromatic, more or less deciduous shrub or tree with pinnate leaves and small, white, sweet-smelling flowers (Kumar et al., 2013). It is a medicinal plant with different parts possessing different medicinal properties, thus it is widely used in the traditional medicine. Its leaves, roots and bark are tonic, stomachic and carminative. The leaves are useful in the treatment of skin eruption, digestion problems, inflammation and dysentery as well as the stop of vomiting (Kumar et al., 2013; Harish et al., 2012). Antioxidant, hypoglycaemic, antibacterial, anti-dysentery and hepatoprotective properties have been proven in the leaves (Baskaran et al., 2011). Baskaran et al. (2011) claimed that ethanol extract from the leaves of *M. koenigii* was effective against *P. aeruginosa* and *Klebsiella pneumoniae*. The juice of the root is able to relieve kidney-related pain and the branches to strengthen gums and teeth (Harish et al., 2012).

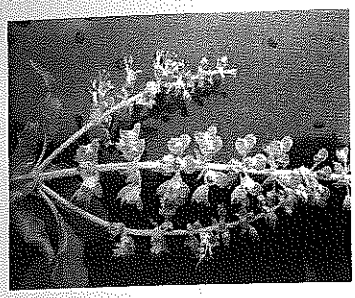

Figure 2.1 Leaves of *O. tenuiflorum.* (Ravi et al., 2012)

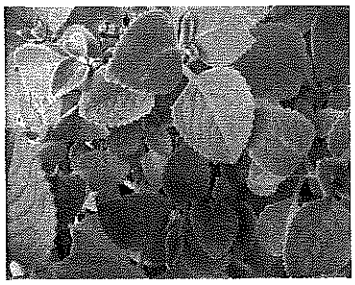

Figure 2.2 Leaves of *P. amboinicus.* (Khare et al., 2011)

Figure 2.3 Leaves of *A. indica.* (Csurhes, 2008)

Figure 2.4 Leaves of *M. koenigii.* (Harish et al., 2012)

Table 2.1 Range of microbes susceptible to antimicrobial effect of *O. tenuiflorum*, *P. amboinicus*, *A. indica* and *M. koenigii.*

Scientific name of medicinal plant	Microorganisms	Reference
O. tenuiflorum	*Pseudomonas aeruginosa*, *Proteus vulgaris*, *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pyogenes*, *Candida albicans*	(Meghashri et al., 2011), (Sermakkani & Thangapandian, 2011), (Sharma et al., 2012)
P. amboinicus	*E. coli*, *S. aureus*, Methicillin resistant *S. aureus*, *P. aeruginosa*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*	(Sathasivam & Elangovan, 2011), (Grace et al, 2012), (Gurgel et al., 2009), (Erny Sabrina et al, 2014)
A. indica	*E. coli*, *Bacillus subtilis*, *S. aureus*, *P. aeruginosa*, *Aspergillus fumigates*	(Raja Ratna Reddy et al., 2013), (Lall et al., 2013), (Mishra et al, 2013)
M. koenigii	*S. aureus*, *P. aeruginosa*, *E. coli*, *K. pneumoniae*, *Aspergillus niger*	(Baskaran et al, 2011), (Kumar et al., 2013)