ANTIVIRAL ACTIVITY OF Cymbopogon Nardus Methanol Fractions Against Measles Disease Virus

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A thesis submitted in fulfillment of the requirement for the Degree of Master of Science (Virology)

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DECLARATION

I hereby declare that no portion of the work contained in this thesis has been submitted in support of any application for any other degree or qualification of this or any other university or institution of higher learning.

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Antiviral activity of Cymbopogon nardus methanol fractions against measles disease virus

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ABSTRACT

Measles disease virus has been a major concern since it has not been able to be fully eradicated. Vaccine was introduced and surveillances were conducted for the disease. The disease still claims the life of thousands of children especially in developing countries where vaccine and drugs are not easily accessible to the people. Active constituents from medicinal plant products were studied and their bioactivities were identified. The chemical structure allows researchers to better understand the mechanisms of infection, hence enable mass production of the compound either naturally or synthetically activity-enhanced compound. Methanol fractions and subfractions of Cymbopogon nardus, serai wangi, were tested for antiviral activity against measles virus and their cytotoxicity against ovarian cancer cell line, Caov3 compared to Vero cell line were evaluated. The assays were carried out based on bioassay-guided fractionation and the antiviral mechanism was evaluated. The fraction C2 showed the highest activity at post-infection assay and its subfraction C2.1 was the most active. The subfraction was run on thin-layer chromatography plate to separate the constituents, yielding four individual spots where the second spot, C2.1b has the active antiviral and highly cytotoxic toward Caov3 cell line. The GC/MS analysis revealed that it contained octadecanoic acid, methyl ester, a fatty acid. The findings showed that the methanol fraction of C. nardus possessed potential antiviral activity and selective toxicity against ovarian cancer cell. Further study on active compounds of the C. nardus against different
viruses, human cell lines and also other bioactivities such as antimicrobial should be carried out.
Aktiviti antivirus fraksi – fraksi metanol *Cymbopogon nardus* terhadap penyakit virus measles

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<tr>
<td>µl</td>
<td>microliter</td>
</tr>
<tr>
<td>µL/g</td>
<td>microliter per gram</td>
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<tr>
<td>µm</td>
<td>micrometer</td>
</tr>
<tr>
<td>DMEM</td>
<td>Dulbecco’s Modified Eagle Medium</td>
</tr>
<tr>
<td>FBS</td>
<td>Fetal Bovine Serum</td>
</tr>
<tr>
<td>g/L</td>
<td>Gram per liter</td>
</tr>
<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Inhibition concentration at 50% inhibition</td>
</tr>
<tr>
<td>LC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Lethal concentration to cause 50% cell death</td>
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<tr>
<td>mg</td>
<td>Miligram</td>
</tr>
<tr>
<td>ml</td>
<td>Milliliter</td>
</tr>
<tr>
<td>MOH</td>
<td>Ministry Of Health Malaysia</td>
</tr>
<tr>
<td>MV</td>
<td>Measles virus</td>
</tr>
<tr>
<td>NaCl</td>
<td>Sodium chloride</td>
</tr>
<tr>
<td>NaOH</td>
<td>Sodium hydroxide</td>
</tr>
<tr>
<td>OD</td>
<td>Optical density</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffer saline</td>
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<tr>
<td>Rf</td>
<td>Retention factor</td>
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<tr>
<td>SLAM</td>
<td>Signaling Lymphocyte Activating Molecule</td>
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<tr>
<td>USDA</td>
<td>United States Department of Agriculture</td>
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<td>WHO</td>
<td>World Health Organization</td>
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CHAPTER 1

INTRODUCTION

Plants have existed since the early age and mankind has mainly collected them as food source even before development of agricultural techniques. When mankind has learned that plants have medicinal properties, the plants became their valuable possession. Their usages as alternative medicine are still being practiced by healers that reside in rural areas. The knowledge on the consumption of certain plants, either consumed directly or boiled down that could cure some illnesses such as sore throat, headache, diarrhea and others are passed down from generation to generation. Through advancement of science and technology, these properties were found to be contributed by active compounds, mainly the secondary metabolites found in plants (Wink, 2010).

Nowadays, people opt for alternative medicine compared to synthetically developed drug due to safety issues in consumption. In United States, alternative medicine played major role as an option over the counter drugs and people have spent $33.9 billion on them in 2007 (Nahin et al., 2009). The advantages of exploiting alternative medicine from natural resources are mainly the economic impact it has on farmers, governments and institutions. Governments and institutions would provide programmes to farmers to help cultivate the plants by introducing latest technological knowledge to increase production of the plants hence increase the livelihood quality (Phondani et al., 2011). Global trade of import and export of herbal drugs has increased to $ 63,000 million a year and industries based on natural products have increased as well. However the industries are not able to exploit the full potential of plants as there are little study done on plants in terms of bioactivities, toxicity and effectiveness
compared to conventional drugs. Countries with vast biodiversity meant that there are resources of potential medicinal plants and they could gain benefits in terms of growing industries, technological development and advancement in research from technological transfer involving drug development (Shyun & Ali, 2004). Technological development would involve processing and quality control of active compounds together with research and development to produce safer and efficient natural drugs (Liu, 2010).

Different compounds can be extracted by different techniques such as using non-polar and polar solvents such as hexane and methanol, respectively. By determining the active compounds, synthetic drugs that are based on the molecular structure of active compound can be mass produced and this would lower the cost to develop new drugs from scratch. Nearly 70% of the modern medicines in India are derived from bioactive compounds obtained from natural products and the public are now aware of the potency and side effect of conventional/synthetic drugs (Verma & Singh, 2008). Researchers are now focusing in developing drugs to combat new emerging as well as endemic diseases. Once a species of a plant is known to contain potential medicinal properties, the other species of the same genus would be investigated in order to discover other active compound that may not be found in that previous species (Hudson, 1990).

South East Asia countries have long been practicing medicine using plants. Each country has come out with their own policies and guidelines regarding the practices (Gaitonde & Kurup, 2005). Other country such as India has implemented programme such as Home Garden Herbals that encourages people to plant herbs with known application to treat common diseases as their home remedies (Hariramamurthi et al., 2007).
In Malaysia, the practice of using medicinal plants have a long history especially in rural areas that are still using plants to cure boils, headache, parasitic worm infection, diarrhea and other common diseases (Lin, 2005). Malaysia had come out with policies that emphasize on using plants as economy resource such as the Biotechnology Policy (MOSTI, 2005). In the policy which stated that researches should focus to diversify the usage of plants in the area of health instead only for food consumption. There are various government bodies that are established to do research on plants regarding to their medicinal properties such as antimicrobial, antifungal and antiviral. The Malaysian herbal medicine market has experienced rapid growth with production of various products and introduction to health care. The progress of the research on medicinal plants is encouraged with the development and advancement of technology including bioassay and isolation techniques. However there is a lot of study need to be carried out in terms of toxicology, biological, immunological and also pharmakinetics before active compound is developed into new drugs for clinical trials (Jantan, 2004).

The increase of various health products provide patients with alternative treatments. Malaysian patients commonly use herbs products while taking prescribed drugs without knowing the possible danger of herbs-drug interactions that could produce undesired side effects. The patients usually do not disclose their herbs products usage as they deem it is unnecessary to inform the doctors. Most of the patients think that doctors would disapprove the usage of herbal products together with prescribed drugs and doctors seldom ask their patients if they consume herbal products while on prescribed drug treatments (Kennedy et al., 2008).
Upon the exploitation of biodiversity for commercial and industrial purposes (bioprospecting) that are unregulated; Convention of Biological Diversity was created. This convention provides guidelines and frameworks to parties involved in bioprospecting. This would specifically ensure the indigenous people with the traditional knowledge are not left out from the profit obtained (Afreen & Abraham, 2009). Some companies and institutes did not obtain illegal permit and share information/genetic access of the medicinal plant with country of origin, hence the importance of the convention guidelines (Christoffersen & Mathur, 2005). By adhering to the guidelines, indigenous people, organizations/governments and institutes could share knowledge and allow legal patenting (intellectual property rights, IPR) other than economic impacts (Pushpangadan & Nair, 2005).

Emerging diseases around the globe has been the important focus for researchers to produce new and enhanced drugs to eliminate or control the spread. Diseases such as measles disease have been able to spread far from their origins as the development of the means of transportation. For example, the spread of measles disease in Middle Eastern was caused by European exploration (Moss et al., 2009). When people began to travel due to the ability to fly and drive to their destinations, a higher potential to spread diseases presented. These travelling activities provided opportunity for measles disease transmission especially in area of crowded population as well as in developing countries that faced sanitary and malnutrition problems. The criteria of diseases to be considered for global elimination are if the diseases threaten human health and the availability of treatments. The diseases should be acute, self-limiting and infectious for short period of time to be considered for elimination. These criteria fit with measles disease for disease eradication as it has been found to be very infectious (Andrus et al., 2011). Steps have been taken to control vectors that transmit diseases. Non-human animal reservoir could be controlled through vaccination, removal of wild or stray...
animals and allocate them to a quarantine area. Some diseases may not be considered for global elimination due to several reasons. One of the reasons is the availability of vaccine but with limited supply and has adverse effect to the recipients. Some areas may also have limited access with vaccine distribution due to geographical location (Trying, 2005).

Measles disease virus (MV) has become global issue where this disease was reported to have caused millions of death especially among infants and young children. The children has higher risk because their undeveloped immune system due to several reasons. The children were not given early vaccination and the vaccine coverage is insufficient especially in third world countries. Measles disease has been a serious health threat especially among children and it was estimated to cause 130 million children below 6 years of age died annually before the introduction of vaccine in 1964. The disease is common in developing countries such as Africa where there are many immunocompromised individuals and low coverage of vaccine distribution. Vaccination programme has since being implemented to control the disease (WHO, 2009).
1.1 Significance of study

Plants’ secondary metabolites, SM play important role in new drug discoveries although little development of new compound to treat diseases. By fully utilizing plant biodiversities with various medicinal properties, potential new compound from SM would produce substitutes to synthetic drug can be developed. Although the interest had declined, researchers are back on developing drug from plant’s SM due to the high rate of synthetic drug failure and side effects (Verpoorte, 1998; Aneesh et al., 2009).

In Malaysia, *Cymbopogon nardus* is valued as one of important plant especially among traditional practitioners. There are various bioactivity of *C. nardus* such as antifungal, antibacterial (Williamson, 2007) and it has been also known to have mosquito repellent property (Jantan & Zaki, 1996).

A proven antiviral and anticancer activity of *C. nardus* would encourages more researches to be carried out in these particular bioactivity of the plant other than antimicrobial, antifungal, anti-inflammatory. Screening for other compound of the plant that could have bioactivity against other viruses, cancer types, microbes, fungal and other applications would be studied into discovering the potential of the plant.

By understanding on the mechanism of the active SM of *C. nardus* against measles virus and ovarian cancer, synthetic drug based on the compound structure could be mass produced and further studied with enhanced bioactivities potentials such as antioxidants, antimicrobial and others.
1.2 Objectives of study

The study for alternative solution has encouraged more research on the capabilities of plant fractions that contribute to medicinal properties. Isolating main bioactive compound will enable the production of synthetic analogs to the fraction with improved properties and more comprehension of the mechanisms involved in viral processes that was affected.

Hence, the objectives of this study are:

- To determine the toxicity level of *Cymbopogon nardus* fractions and subfractions on Vero and ovarian cancer, Caov3 cell
- To determine whether *C. nardus* fractions and subfractions exhibit antiviral action at low toxicity and in combined treatment with ribavirin against MV
- To determine the antiviral course of action of fractions; either providing protection or disrupting viral processes
- To identify the most active compound that contributes to the bioactivity via GC/MS
CHAPTER 2

LITERATURE REVIEWS

2.1 Measles

Measles disease has been known to be a problem among young children in developing countries especially the third world countries. The disease caused high death rates annually and claimed 164,000 lives of children under 5 years old during 2008 period (WHO, 2009). The WHO has taken preventive measures by introducing vaccine which managed to reduce the death rates. Although with the introduction of vaccine for nearly 50 years, the death rates are still high due to the lack of distribution of the vaccine in certain developing countries such as Africa. The Malaysian Ministry of Health started elimination strategy and surveillance in order to control the disease spread that proven to be a successful reduction in 2004 to 2008 period (Saraswathy et al., 2009).

2.1.1 Characteristics and structure

Measles disease virus belongs to the *paramyxoviridae* family along with the parainfluenza, mumps, Newcastle virus and several other viruses. The measles virus is very sensitive to heat and its infectivity reduced to one half after an exposure of heat at 37°C for 2 hours. It can be inactivated through several conditions such as 56°C within 30 minutes or exposed to ultra violet light. It remained stable at the pH of 5 – 10.5 and optimum condition at 7.27. The measles virus is in helical nucleocapsid and surrounded by lipid envelope where the HN
(hemagglutinase-neuraminidase) and F (fusion) glycoprotein protrudes. It contains negative-sense single stranded RNA as its genome (Kettering, 1999; Halsey, 2002).

The MV RNA genome consists of 16000 nucleotides and the genome encodes eight proteins for its structural makeup (Moss et al., 2009). Hemagglutinin protein is vital as its interaction with the fusion protein and the SLAM (CD150) receptor will form complexes that are involved in the infection processes of MV onto host cells. Fusion protein not only involved in the fusion process but directly interact with SLAM receptor in process (Zhang et al., 2005). SLAM receptors are expressed on immune cells and CD46 receptors are another alternative receptor for MV vaccine attachment (Yanagi et al., 2009). CD46 receptor is sensitive to ligand interaction and external stimuli and functions to regulate inflammatory response by suppressing or inducing. CD46 induced proinflammatory in multiple sclerosis patients (Choileain & Astier, 2010).

There is no antigenic shift in the MV because there is only one main antigenic type (Kettering, 1999). Thus an infection is enough to provide long term immunity. With this property, high efficiency was achievable in reducing the spread of the disease with the introduction of the vaccine.

### 2.1.2 Viral replication process

The MV replicates firstly in the respiratory tract and moved to lymphoid tissue where further growth processes occurs (Kettering, 1999). Since MV is a negative-single stranded RNA, during replication it needs to be converted into positive strand RNA using the host