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# 2.3 Short Research Projects in Tropical Diseases Conducted by DAP&E Students 2010



# By the SEAMEO TROMPED Regional Centre for Microbiology, Parasitology and Entomology (SEAMEO TROPMED/ Malaysia), Malaysia

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Established in 1967, SEAMEO TROPMED/Malaysia is located at the Institute for Medical Research in Kuala Lumpur, Malaysia and conducts research for the prevention and control of diseases. The centre provides specialized training, diagnostic, consultative and advisory services. It also promotes health management as a collective responsibility of government, the private sector, non-governmental organizations, the community and individuals.

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## I. Abstract

SEAMEO TROPMED/Malaysia serves as the SEAMEO TROPMED Regional Centre for Microbiology, Parasitology and Entomology. The centre's main responsibilities are to research, identify, elucidate, control and prevent disease and investigate other health related issues in the country.

To achieve these objectives, two postgraduate academic programmes conducted by the Regional Centre place critical emphasis on health promotion to prevent the spread of disease, through training and research into tropical diseases prevalent within Malaysia.

Student assessments for both courses: the Diploma in Applied Parasitology and Entomology (DAP&E); and the Diploma in Medical Microbiology (DMM), include short research projects and written assignments respectively. This report presents summaries of research projects conducted by DAP&E students in 2010.

# **II. Project Description**

Tropical diseases are infectious diseases found predominantly in the tropics, where ecological and socioeconomic conditions facilitate their propagation. Climatic, social, and economic factors create environmental conditions that facilitate the transmission of these medical conditions.

Mosquitoes, flies and other organisms are the most common disease carriers, or vectors. These can either be mechanical or biological vectors. A mechanical vector, such as a housefly, picks up an infectious agent on the outside of its body and transmits it in a passive manner such as landing on food meant for human consumption. In contrast, biological vectors, such

as mosquitoes, harbor pathogens within their bodies and deliver pathogens to new hosts in an active manner, usually in the form of a bite.

The most prevalent tropical diseases in Malaysia include malaria, dengue fever, chikungunya, filariasis, and other vector-borne diseases of great medical importance. Every student on their respective courses were given specific research tasks linked to tropical diseases in order to partially complete the Diploma in Applied Parasitology and Entomology.

## 1. Project Objectives

- 1.1 To strengthen the skills of biomedical researchers in tropical diseases in the areas of laboratory and field techniques
- 1.2 To strengthen capabilities in project planning, management and evaluation in order to achieve successful implementation of the project and usage of funding
- 1.3 To strengthen human resources and to be able to apply research skills to everyday work in the students' respective countries

## 2. Methodology

Research projects were designed by faculty members and since they were only for a short period of time, they usually formed a section of the overall programme conducted by lecturers at the institute.

The students were taught research methodology, literature search, construction and design of research proposals and effective communication skills to fully prepare them for the successful completion of their respective projects. Programme support services, such as materials, assistance and guidelines for the successful completion of the overall project, were provided.



Vectrol control field trip of the DAP&E Students in 2010

## 3. Project Output

The total output was 14 diploma theses. An example is provided here for reference:

Anti-Plasmodia Activity and Cytotoxicity of Ocimum Basilicum (Selasih) Extract in Vitro.

Student: Ms. Om SovandaraDepartment: Haematology UnitCountry of Origin: National Institute of Public Health, Phnom Penh, Cambodia

Medicinal plants from tropical regions are considered to have many inherent properties for the treatment of a wide variety of ailments. Ocimum bacilicum is from the Lamiaceae family (mint family) and is one of more than 60 Ocimum species used for the treatment of fever in traditional folk medicine, a form of healing practice and health preservation known to a limited segment of the population in a particular culture, especially in Africa and Asia.

Properties from the Lamiaceae family are often used as flavouring in various food products (Sacchetti et al., 2004, Jirovetz et al., 2003). The common name for O. bacilicum is basil or sweet basil. O. basilicum L. is an important herbaceous plant species, which contains aromatic essential oils: eugenol; methyl eugenol; and caryophllin (Anon 1988). Since this species has properties that can be used in the treatment of fever, it was selected for in vitro screening of malaria parasites to determine the plant's potential as an antiplasmodial activity.

The malaria parasite, P. falciparum, continues to develop resistance to common antimalarial drugs. This is one of the greatest challenges facing malaria control today. Therefore, there is an urgent need to conduct a screening of medicinal plants available to assess their potential antiplasmodial activity. The aim of the present study was to determine the antiplasmodial activity of O. basilicum extract and the cytotoxicity activity of the extracts in vitro for their selectivity for further study.

#### **Objectives:**

- To determine the antiplasmodia activity of O. basilicum extract in vitro using SYBR Green assay
- 2. To determine the cytotoxicity activity of O. basilicum extract in vitro using MTT assay
- 3. To determine the Selectivity Index of the extract

## Methodology:

The antiplasmodial activity of O. bacilicum extract in vitro was carried out using SYBR Green assay (Bacon et al., 2007). Briefly, the malaria parasites were cultured according to the standard procedure of Trager and Jensen, 1996. When the parasitemia of the culture was high, they were quantified and the parasitemia was adjusted to 0.5 per cent, They were then exposed to a series of extract concentrations starting from 16-to-0.30  $\mu$ g/ml (final concentration).

The exposure lasted for 72 hours and the end point was measured by the SYBR Green assay (Bacon et al.,2007). The cytotoxicity assay was performed using the MTT assay (Mossman, 1983). The selectivity index was calculated at the ratio of IC50 of cytotoxicity assay: to IC50 of the biological activity, that is the SYBR green assay.

### **Results:**

The data was analyzed by HN-LONLIN (Harald et al., 2002). Briefly, the OD values were plotted in a pre-programmed curve-fitting spreadsheet called HN-NonLin. The software performed non-linear regression analysis with a polynomial model. Raw data from both types of drug sensitivity assay were plotted in a 96-well plate format, and the twofold drug concentrations used in the assays were entered manually.

The inhibitory concentration and does-response curves for individual isolates or strains were obtained directly. The IC50 value of O. basilicum against P. falciparum K1 and D10 were 16  $\mu$ g/ml and 0.25  $\mu$ g/ml respectively. The MTT assay showed that O. basilicum did not inhibit the growth of the MDBK cell in vitro. The SI calculated was 0.25 $\mu$ g/ml. This showed that the O. basilicum extract has good to moderate anti Plasmodia activity in vitro. The O. basilicum has IC50 0.25  $\mu$ g/ml, according to the malaria work flow it provides a good extract for antiplasmodial activity (Fidock et al., 2004). The selectivity index value was more than 10 for extracts, therefore it did not exhibit a harmful effect or toxicity to the cells. The data revealed the need to study the plant extracts more intensively.

### Conclusion:

The O. basilicum extract has good antiplasmodial activity in vitro with an IC50 value of 16  $\mu$ g/ml and 0.25  $\mu$ g/ml to P. falciparum K1 and D10 respectively.

## **III. Significant Impacts**

The Southeast Asia region is home to some of the most significant tropical diseases on the planet today. The research project has played a major role in capacity building of human resources in laboratory and field techniques which can be applied in the region. The use of the evidence-based approach in solving problems has contributed in many ways to the control and management of diseases. The research project has further enhanced the development of skilled human resources in techniques and capabilities in project planning, management and evaluation for application in different countries.

The project has made an important contribution to strengthening the Institute as the regional centre for Microbiology, Parasitology and Entomology.

## **IV. Success Factors**

#### 1. The importance of the project to the country and region

This project has opened the door to establishing linkages between institutions and promoting research capacity strengthening in regional countries.

#### 2. International R&D network in the region

The implementation of the project has created a closer working relationship between students and faculty members. It has also provided a wealth of opportunities for further collaborations internationally for research into life-threatening tropical diseases.



DAP&E students in 2010

