GROWTH CURVE INHIBITION OF *ENTAMOEBA HISTOLYTICA* AFTER EXPOSURE TO *ANDROGRAPHIS PANICULATA* NEES. (WHOLE PLANT), *EUPHORBIA HIRTA* L. (WHOLE PLANT) AND *LONICERA JAPONICA* THUNB. (FLOWERS AND LEAVES)

JINDA KUNGVANRATTANASIN

A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR
THE DEGREE OF MASTER OF SCIENCE (PUBLIC HEALTH)
MAJOR INFECTIOUS DISEASE
FACULTY OF GRADUATE STUDIES
MAHIDOL UNIVERSITY
2006

ISBN 974-04-7656-2

Thesis
Entitled

GROWTH CURVE INHIBITION OF ENTAMOEBA HISTOLYTICA AFTER EXPOSURE TO ANDROGRAPHIS PANICULATA NEES. (WHOLE PLANT), EUPHORBIA HIRTA L. (WHOLE PLANT) AND LONICERA JAPONICA THUNB. (FLOWERS AND LEAVES)

Jinda Kungvanrattanasin
Miss Jinda Kungvanrattanasin
Candidate

Pakpimol Mahannop
Assoc. Prof. Dr. Pakpimol Mahannop
Ph.D. (Tropical Medicine)
Major-Advisor

Nitaya Thammapalerd
Assoc. Prof. Nitaya Thammapalerd
M.Sc. (Biochemistry)
Co-Advisor

Prof. M.R. Jisnuson Svasti
Ph.D.
Dean
Faculty of Graduate Studies

Kulaya Narksawat
Assoc. Prof. Kulaya Narksawat
D.D.S.,M.P.H. (Urban Health)
M.Sc. (Medical Epidermiology)
Chair
Master of Science (Public Health)
Major in Infectious Disease and Epidemiology
Faculty of Public Health

Thesis
Entitled

GROWTH CURVE INHIBITION OF ENTAMOEBA HISTOLYTICA AFTER EXPOSURE TO ANDROGRAPHIS PANICULATA NEES. (WHOLE PLANT), EUPHORBIA HIRTA L. (WHOLE PLANT) AND LONICERA JAPONICA THUNB. (FLOWERS AND LEAVES)

Was submitted to the Faculty of Graduate Studies , Mahidol University
For the Degree of Master of Science (Public Health)
Major in Infectious Disease
On
10 March , 2006

Jinda Kungvanrattanasin
.....
Miss Jinda Kungvanrattanasin
Candidate

Pakpimol Mahannop
.....
Assoc. Prof. Dr. Pakpimol Mahannop
Ph. D. (Tropical Medicine)
Chair

Nitaya Thammapalerd
.....
Assoc. Prof. Nitaya Thammapalerd
M. Sc. (Biochemistry)
Member

Thareerat Kalambaheti
.....
Asst. Prof. Thareerat Kalambaheti
Ph. D. (Microbiology)
Member

.....
Prof. M.R. Jisnuson Svasti
Ph. D.
Dean
Faculty of Graduate Studies
Mahidol University

Phitaya Ch, MD
.....
Assoc. Prof. Dr. Phitaya Charupoonphol
M.D., Dip. Thai Board of Epidemiology
Dean
Faculty of Public Health
Mahidol University

ACKNOWLEDGEMENT

I would like to express my sincere gratitude and deep appreciation to Assoc. Prof. Dr. Pakpimol Mahannop my major advisor for her guidance, understanding and encouragement thoughout this study. I also wish to express my sincere thanks and deep appreciation to Assoc. Prof. Nitaya Thammapalerd my co-advisor for her recommendation, supervision, assistance encouragement, meaningful suggestions and valuable advice without her advice my thesis could not be completed.

I special thank to Department of Microbiology and Immunology, Faculty of Tropical Medicine, Mahidol University, for laboratory experimental facility; to Department of Nurse, Siriraj Hospital, for education support and also my colleague in ICU Neurosurgery and ICU Cardiovascular thorasic surgery for allowing me to study.

The successful completion of this thesis was made possible by the support and the kindness of many people. I would like to express my deepest gratitude to my parents and members of my family for their kind concern, love and insight throughout my study.

Jinda Kungvanrattanasin

GROWTH CURVE INHIBITION OF ENTAMOEBA HISTOLYTICA AFTER EXPOSURE TO ANDROGRAPHIS PANICULATA NEES. (WHOLE PLANT), EUPHORBIA HIRTA L.(WHOLE PLANT) AND LONICERA JAPONICA THUNB. (FLOWERS AND LEAVES)

JINDA KUNGVANRATTANASIN 4336225 PHPH/M

M.Sc. (PUBLIC HEALTH) MAJOR INFECTIOUS DISEASE

THESIS ADVISORS : PAKPIMOL MAHANNOP, Ph.D., NITAYA THAMMAPALERD, M.Sc.

ABSTRACT

The purpose was to study the effect of herbal aqueous extract of *A. paniculata Nees.*(whole plant), *E. hirta L.*(whole plant) and *L. japonica thunb.*(flowers and leaves) to the growth of two strains of *E. histolytica* (AP and S) and one strain of *E. moshkovskii* in vitro(xenic culture, biphasic media). The dried plants were extracted in boiled water. Each aqueous extract of herbs was sterilized by being passed through milipore and then mixed in aqueous media culture. Living amoebas were counted after exposure to herb extract for 24 hours, and calculated for percentage inhibition. Positive control was Metronidazole, 10 micrograms per milliliter.

The results revealed that undiluted herbal aqueous extract of *L. japonica Thunb.* can kill three strains of *Entamoeba* trophozoites, but undiluted herbal aqueous extract of *E. hirta L.* and aqueous extract of *A. paniculata Nees.* inhibits growth of three strains of *Entamoeba* trophozoites. Undiluted herbal aqueous extract of *E. hirta L.* had a growth inhibition effect on all three strains of *Entamoeba* trophozoites and was more effective than undiluted herbal aqueous extract of *L. japonica Thunb.*. Three kinds of herbal aqueous extracts had a growth inhibition effect on all three strains of *Entamoeba* trophozoites; wheseas, growth inhibition effect decreased as the aqueous extract was diluted.

The herbal aqueous extract of *L. japonica Thunb.*(flowers and leaves) can kill *Entamoeba* trophozoite; wheseas, aqueous extracts of *E. hirta L.* (whole plant) and *A. paniculata Nees.*(whole plant) can inhibit growth of *Entamoeba* trophozoites.

KEY WORDS : ENTAMOEBA HISTOLYTICA / ENTAMOEBA MOSHKOVSKII / ANDROGRAPHIS PANICULATA NEES./ EUPHORBIA HIRTA L./ LONICERA JAPONICA THUNB. / AQUEOUS EXTRACT

64 P. ISBN 974-04-7656-2

การยับยั้งการเจริญเติบโตของเชื้อบิดอะมีบาภายหลังได้รับสารสกัดจากฟ้าทะลายโจร,น้ำนมราชสีห์และสายน้ำผึ้ง (GROWTH CURVE INHIBITION OF ENTAMOEBA HISTOLYTICA AFTER EXPOSURE TO ANDROGRAPHIS PANICULATA NEES. (WHOLE PLANT),EUPHORBIA HIRTA L.(WHOLE PLANT) AND LONICERA JAPONICA THUNB. (FLOWERS AND LEAVES))

จินดา กังวานรัตนศิลป์ 4336225 PHPH/M

วท.ม. (สาธารณสุขศาสตร์) สาขาวิชาเอกโรคติดเชื้อ

คณะกรรมการควบคุมวิทยานิพนธ์ : พักตร์พิมล มหรรณพ, Ph.D., นิตยา ธรรมพาเลิศ, M.Sc.

บทคัดย่อ

วัตถุประสงค์ของการวิจัยนี้เพื่อ ศึกษาผลของสารสกัดสมุนไพร 3 ชนิดด้วยการต้มในน้ำเดือด คือ ฟ้าทะลายโจร(ทั้งต้น) , น้ำนมราชสีห์(ทั้งต้น) และสายน้ำผึ้ง(ดอก และ ใบ) ต่อเชื้อเอนตามีบา ฮิสโตลัยติกา 2 สายพันธุ์ คือ AP และ S และเชื้อเอนตามีบา มอสคอฟสกี สายพันธุ์ LA ในหลอดทดลอง โดยนำน้ำที่ได้จากการต้มทำให้ปลอดเชื้อโดยผ่านชุดกรองมิลลิพอร์ แล้วนำไปทดสอบกับเชื้อบิดอะมีบาในหลอดทดลองที่เลี้ยงแบบอยู่ร่วมกับแบคทีเรีย ในอาหารเลี้ยงเชื้อกึ่งแข็งกึ่งเหลวและนับจำนวนเชื้อบิดอะมีบาหลังได้รับสารสกัดสมุนไพร 24 ชั่วโมง เปรียบเทียบกับจำนวนเชื้อบิดอะมีบาในหลอดทดลองที่ไม่ได้รับสารสกัดสมุนไพร เป็นค่าร้อยละของผลการยับยั้งการเพิ่มจำนวนของอหะมีบา โดยใช้เมทโทรนิดาโซล 10 ไมโครกรัม ต่อ มิลลิลิตร เป็นตัวควบคุมบวกในการทดลอง

ผลการศึกษาพบว่า สารสกัดสมุนไพรที่ไม่ได้ทำให้เจือจางจากสายน้ำผึ้ง มีผลฆ่าเชื้ออะมีบาทั้งสามสายพันธุ์ แต่สารสกัดสมุนไพรที่ไม่ได้ทำให้เจือจางจาน้ำนมราชสีห์ และ ฟ้าทะลายโจร มีผลยับยั้งการเจริญเติบโตของเชื้อเอนตามีบาทั้งสามสายพันธุ์ และสารสกัดสมุนไพรที่ไม่ได้ทำให้เจือจางจากน้ำนมราชสีห์ มีผลยับยั้งการเจริญเติบโตของเชื้อเอนตามีบาทั้งสามสายพันธุ์ ได้ดีกว่าสารสกัดสมุนไพรที่ไม่ได้ทำให้เจือจางจาสมุนไพรฟ้าทะลายโจร ทั้งนี้สารสกัดสมุนไพรทั้งสามชนิด เมื่อถูกทำให้เจือจางมากขึ้น จะมีผลให้ประสิทธิภาพในการยับยั้งการเจริญเติบโตของเชื้อเอนตามีบาทั้งสามสายพันธุ์ลดลง

สารสกัดจากการต้มพืชสมุนไพรสายน้ำผึ้ง (ส่วนดอกและใบ) มีผลทำลายเชื้ออะมีบาได้ และสารสกัดจากการต้มพืชสมุนไพรน้ำนมราชสีห์ (ทั้งต้น) มีผลยับยั้งการเพิ่มจำนวนเชื้อบิดอะมีบามากกว่าสารสกัดที่ได้จาการต้มพืชสมุนไพรฟ้าทะลายโจร (ทั้งต้น)

64 หน้า ISBN 974-04-7656-2

CONTENTS

CONTENTS (Continued)

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF ABBRAEVIATION

AP	=	A strain of *Entamoeba histolytica*
b.i.d	=	twice a day
cu.cm	=	Cubic centiliter
cu.mm	=	Cubic mililiter, microliter
°C	=	Degree of celcius
g	=	Gram
hr	=	Hour
kg	=	Kilogram
LA	=	A strain of *Entamoeba moshkovskii*
LE	=	Locke Egg
mg	=	Milligram
ml	=	Microliter
MTZ	=	Metronidazole
PO.	=	by mouth
q.i.d.	=	Four times a day
rpm	=	Round per minute
S	=	A strain of *Entamoeba histolytica*
t.i.d.	=	Three times a day

CHAPTER I
INTRODUCTION

1.1 General Introduction

Amoebiasis is a parasitic infection of human gastrointestinal tract caused by *Entamoeba histolytica*. This parasite is capable of invading the intestinal mucosa and may spread to other organs, mainly the liver which usually leads to amoebic liver abscess. This infection remains a significant cause of morbidity and mortality worldwide (1).

E. histolytica infection occur worldwide, morbility and mortality are greatest in Central and South America, Africa and the Indian subcontinent (2). *E. histolytica* infection in the developing countries is a significant health problem in amebiasis-endemic areas with a significant impact on infant mortality (3).

Metronidazole is the the drug of choice widely used and recommended in the treatment of amoebiasis (4). It is less effective in the tissue than in gut lumen (5). In addition it can eradicate only up to 50 % of luminal infections and has no action on cysts. This suggests the combination of metronidazole with other drugs as diloxanide furoate or paramomycin to eliminate the parasite from the intestine and to cure *E. histolytica* carriers. However, this combination therapy is not fully effective (6).

At present, the ideal treatment for amoebiasis does not exist (7). The drugs used currently for the treatment of amoebiasis include agents that are mutagenic, antibacteria, carcinogenic to laboratory rodents (Metronidazole), cardiotoxic for human (Emetine) and have been associated with transient myopia, neuropathy and immunosuppresion. Because of their toxicity it is desirable, therefore, to find new amoebicides for a greater margin of safety and have amoebicidal activity with less toxicity for the host (8).

Developed and developing countries show a great interest in indigenous medicine, and many developing countries use traditional medicines at the primary

health care level. A large segment of the world's population relies on tradition remedies to treat a plethora of diseases. Medicinal herbs and herbal extracts are indispensable part of the traditional medicine practiced all over the world due to low cost, easy access and ancestral experience (9).

Research on plants used in traditional medicine as antiamoebic medication offers an alternative to the development of new drugs and/or validation of their use folk medicine. In addition, it is important because it is a common form of medicine as an alternative, or as the only available medicine (10).

Three kinds of Thai herbs were selected for this study ; *Andrographis paniculata Nees.*, *Euphorbia hirta L.* and *Lonicera japonica Thunb.* These are famous in Thailand and used for treatment on dysentary which is caused by bacteria or parasite. The aim of this study was to verify the possible antiamoebic activity of aqueous extracts from three herb plants used in Thailand.

It is important to emphasize that their activities are tested in aqueous extracts because teas or infusions from whole plant or their components are an extended practice for treatment of amoebiasis in folk medicine. This also suggests an inexpensive alternative for anti-amoebic drug.

Although the in vitro assays may not be directly related to in vivo activities in studies (11), it is still an important approach to activity screening, which may provide a firm base for improving basic community health care (12).

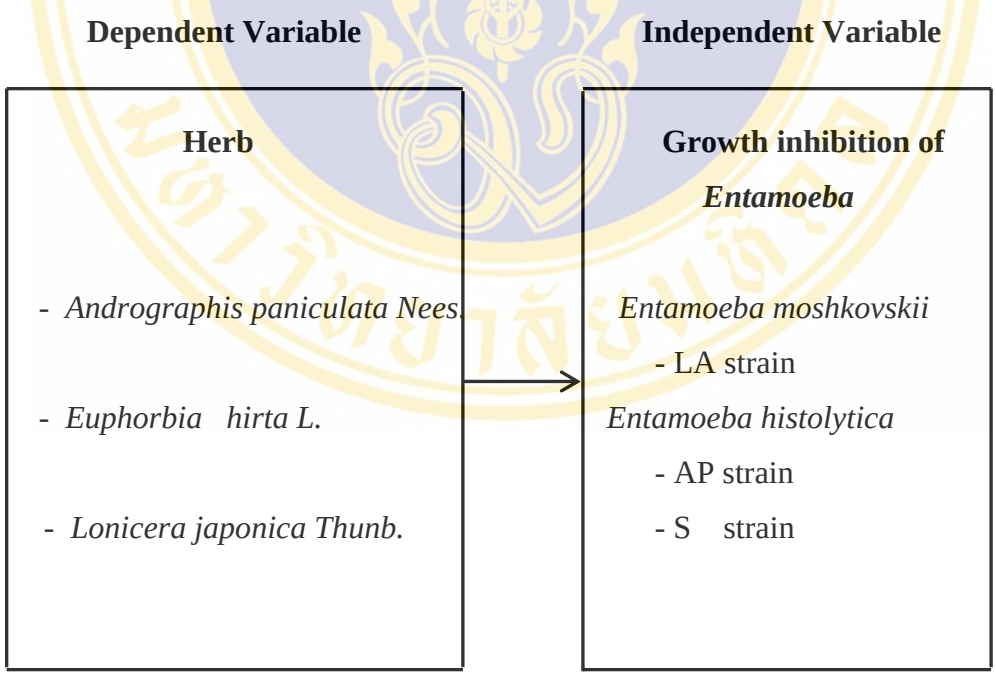
1.2 Objectives

1. To study growth inhibition action of herbal extracts from *A. paniculata Nees.* (whole plant), *E. hirta L.* (whole plant) and *L. japonica Thunb. (flowers and leaves)* on two strains of *Entamoeba histolytica* trophozoites (AP and S) and one strain of *Entamoeba moshkovskii* trophozoites (LA).
2. To compare the growth of two strains of *E. histolytica* trophozoites (AP and S) and one strain of *E. moshkovskii* trophozoites (LA) after exposure to *A. paniculata Nees.* (whole plant), *E. hirta L.* (whole plant) and *L. japonica Thunb.*(flowers and leaves) and select the one showing best killing effect for future uses.

1.3 Research Hypotheses

1. The growth of two strains of *E. histolytica* trophozoites (AP and S) and one strain of *E. moshkovskii* trophozoites (LA) can be inhibited by herbal extract (boil in water) from *A. paniculata Nees.* (whole plant).
2. The growth of two strains of *E. histolytica* trophozoites (AP and S) and one strain of *E. moshkovskii* trophozoites (LA) can be inhibited by herbal extract (boil in water) *Euphorbia hirta L.* (whole plant).
3. The growth of two strains of *E. histolytica* trophozoites (AP and S) and one strain of *E. moshkovskii* trophozoites (LA) can be inhibited by herbal extract (boil in water) from *L. japonica Thunb*. (flowers and leaves).

1.4 Conceptual Framework



1.5 Usefulness of Research

The usefulness of this research study is searching on and basic development for alternative drugs that could be suitable for use in preventing and treating amoebiasis caused *by Entamoeba histolytica* infection.

CHAPTER II
LITERATURE REVIEW

Entamoeba histolytica, the causative agent of intestinal amoebiasis, is responsible for estimately 40 thousand to 100 thousand deaths from amoebiasis occur worldwide annually which makes amoebiasis second only to malaria as a cause of death resulting from this protozoan parasite (3).

Amoebiasis is still one of the most widespread diseases worldwide. It is both cosmopolitan and tropical in distribution, and is common in areas with poor sanitation. Being that it is capable of thriving in a cosmopolitan environment such as those found in developed countries, it prevails among homosexual men, mental institutions, and among overseas travelers (13).

Agent ,Vector and Host

E. histolytica is ingested via water or food contaminated with human feces habouring *E. histolytica* four nucleated cysts of *E. histolytica.*

Hosts **:** Humans, several nonhuman primates, cats, dogs and rats are well documented hosts. Reports of infection in other mammals must be viewed with caution unless evidence of tissue invasion has been provided, and/or the parasite has been isolated and subjected to isoenzyme or other discriminatory analysis (13).

2.1 *Entamoeba histolytica*

Common name	:	Amoeba
Kingdom	:	Protista
Phylum	:	Protozoa
Class	:	Lobosea
Order	:	Amoebida

Family	:	Entamoebidae
Genus	:	*Entamoeba*
Species	:	*histolytica*

2.1.1 Morphological characters

Trophozoites ranging in size from 20 to 40 µm in diameter. They have rapid locomotion, gliding, by means of a single well defined pseudopodium, often extended explosively, without conspicuous differentiation between ectoplasm and endoplasm. Cytoplasm contains a single nucleus, often with ingested red blood cells, sometimes with leukocytes, or bacteria; rich in glycogen; with ribosomes arranged in helices which aggregate to form characteristically shaped elongate bars with rounded ends (chromatoid bodies). They have no classical mitochondria, rough endoplasmic reticulum, or golgi apparatus. Nucleus is vesicular, spherical, measuring 4 to 7 µm in diameter, consisting of a delicate achromatic membrane lined usually by a single layer of small chromatin granules, uniform in size, in contact or very close to each other. A small spherical karyosome (0.5 µm in diameter), to often centrally located, surrounded by an achromatic capsule-like structure. Cysts are spherical, measuring 10 to 16 µm in diameter; with four nuclei when mature, rarely up to eight; with glycogen in a distinct vacuole in the immature cyst, becoming more diffuse as the cyst matures; often with chromatoid bodies are found in the immature cyst, disappearing as the cyst matures. Nuclei shows morphology similar to those of trophozoites (13).

2.1.2 Incubation

The incubation period for amebic dysentery is usually 1- 4 weeks, but may be shorter or substantially longer (13).

2.1.3 Life cycle

Infection by *E. histolytica* occurs by ingestion of mature cysts in fecally contaminated food, water, or hands. Excystation occurs in the small intestine and

trophozoites are released, which migrate to the large intestine. The trophozoites multiply by binary fission and produce cysts, which are passed in the feces. Because of the protection conferred by their walls, the cysts can survive several days to weeks in the external environment and are responsible for transmission. Trophozoites can also be passed in diarrhoeal stools, but are rapidly destroyed once outside the body, and if ingested would not survive exposure to the gastric environment. In many cases, the trophozoites remain confined to the intestinal lumen (non-invasive infection) of individuals who are thus asymptomatic carriers and cysts passers. In some patients the trophozoites invade the intestinal mucosa (intestinal disease), or through the bloodstream, extraintestinal sites such as the liver, brain, and lungs (extra-intestinal disease), with resultant pathologic manifestations. It has been established that the invasive and noninvasive forms represent separate species, respectively as *E. histolytica* and *E. dispar*, which are morphologically indistinguishable. Transmission can also occur through fecal exposure during sexual contact in which case not only cysts, but also trophozoites could prove infective (13).

2.1.4 Signs and Symptoms

Approximately 10% of those infected develop an invasive *Entamoeba histolytica* infection, while the majority (90%) remain asymptomatic. The most common signs and symptoms of this dysentery include diarrhoea, nausea, abdominal pain, weight loss, and cramping. This corresponds to invasion of the intestine when active trophozoites invade and penetrate the intestinal muscular wall and simultaneously feed on red blood cells. Amoebic dysentery ensues when trophozoites continue to erode the intestinal epithelium, leading to the formation of flask-shaped ulcers. Thus, amoebae with ingested erythrocytes, although seen rarely, are a key diagnostic feature of *Entamoeba histolytica* when found in the stool of a person experiencing amoebic dysentery. The onset of these symptoms occurs over a period of several weeks. In rare instances, toxic megacolon occurs in 5% or less of patients suffering from amoebic colitis. Occasionally, trophozoites can invade and cause extensive damage to other organs, including the liver and brain, by gaining access to the portal vein and spreading through the circulatory system. Trophozoites also spread

to the lungs by direct extension from the liver and across the diaphragm, thus causing pulmonary infection. In hepatic infections, amoebic abscesses may form. In such hepatic infections, leukocytosis will develop, ranging from 15,000 to 35,000 WBC per microliter, but there is no characteristic differential count. Liver function panels will be abnormal, but are not specific for an amoebic abscess diagnosis. Finally, infection of the skin can occur, although rarely, and produces perianal ulcerations as well as genital infections (13).

2.1.5 Treatments

Tissue amoebicides (dehydroemitine, emetine, and metronidazole) act on amoebas in the bowel wall but not the lumen. Luminal amoebicides (diloxanide furoate, idioquinol and paromomycin) act on amoebae in the bowel lumen. Tetracycline inhibits bacterial growth in both the bowel wall and lumen. Metrodazole effects both the bowel wall and lumen but if given alone has a 50% fail rate as it needs a luminal amoebicide to augment its action. Asymptomatic patients are treated with idioquinol 30-40 mg/kg/24 hours (maximum 650 mg/dose) p.o. t.i.d. for 7 days. Diloxanide furoate is available only from the CDC for treatment of asymptomatic amoebiasis. Patients with invasive amoebiasis and symptoms are treated with metronidazole 30-50 mg/kg/24 hours (maximum 500-750 mg/dose) p.o. t.i.d. for 10 days. This amount of metronidazole often causes severe nausea that can be prevented by taking promethazine one hour prior to dosing. Some experts recommend adding a luminal amoebicide such as paromomycin. Patients with hepatic amoebiasis are treated with metronidazole 750 mg p.o. t.i.d. for 10 days or 500 mg intravenous every 6 hours for 10 days. Some references recommend diloxanide furoate or iodoquinol, followed by chloroquine. Follow up care includes examination of stools at 2-3 days intervals starting at 4 weeks and up to 3 months after the end of treatment (6).

2.2 Historical Background

This parasite was first established in culture by Boeck and Drbohlav in 1925 in a diphasic egg slant medium they had developed for isolation of intestinal flagellates (14). A modification of this medium (Locke-egg [LE]) is still in use today (Appendix B). The success refuted the Promethean view of *E. histolytica* as an obligate tissue parasite. Dobell and Laidlaw (15) introduced the use of rice starch as a carbohydrate source, which remains a component of all media for xenic cultivation to this day. If soluble sugars were used they would be metabolized rapidly by the bacteria, and this would prevent the necessary bacterium-amoeba balance from being reached in culture. Several other diphasic media were subsequently developed with serum, agar, or egg extracts in the slants (16). Monophasic media that have been developed include the egg yolk infusion medium of Balamuth (17), Jones's medium (18) and TYSGM-9 of Diamond (19). Currently the most widely used media for xenic cultivation of *E. histolytica* are the diphasic LE and Robinson's media (20) and the monophasic TYSGM-9 (19). These media can also be used for the cultivation of other species of *Entamoeba* and *Endolimax nana* with various degrees of success (14).

Monoxenic cultivation of *E. histolytica* with a single bacterium was first accomplished by Cleveland and Sanders (21) in a diphasic medium. The most widely used medium for this type of culture is the monobacterial modified Shaffer-Frye medium (MS-F)(22). Monoxenic cultivation with a trypanosomatid was first achieved using *Trypanosoma cruzi* in a diphasic medium (23).

Monoxenic cultivation is of limited use today except as a transitional stage between xenic and axenic cultures. *Crithidia fasciculata* and *T. cruzi* are the associated organisms of choice for monoxenic cultivation (24, 25). However, in at least some cases axenic culture media have worked well for monoxenic cultivation.

Axenic cultivation of *E. histolytica* was first accomplished by Diamond in 1961 (26). The diphasic medium used was complex—a serum-enriched nutrient agar slant overlaid with a broth supplemented with chicken embryo extract and vitamins. It was not until Diamond introduced the monophasic medium TP-S-1 in 1968 (25) that axenic cultures of *E. histolytica* started to be widely used. TP-S-1 was superseded by TYI-S-33 in 1978 (27) and this is currently the most widely used medium for axenic

cultivation of *E. histolytica* (28, 29). Diamond et al. later described YI-S as an alternative to TYI-S-33 (30) ; A homemade axenic culture medium (PEHPS) (31) made from liver and pancreas extracts has been described as an other alternative to the above media which rely on commercially produced products. Methods for obtaining cloned colonies in agar have also been published (32, 33), as have micromanipulation methods for cloning xenic (34) and axenic (35) cultured amebae.

The main components of the *E. histolytica* axenic culture media are a source of peptides and amino acids (Trypticase or casein digest peptone), nucleic acids (yeast extract), carbohydrate (glucose), lipids (serum), and vitamins. Most of these components are also part of the axenic culture media for the other parasites.

Because of its emerging importance, especially with respect to diagnosis, it is appropriate to mention *E. dispar*, earlier called "nonpathogenic *E. histolytica*" but now recognized as a distinct species (36), *E. dispar* can be grown in xenic culture just as easily as *E. histolytica*. However, most isolates grow poorly in monoxenic culture, and few have been reported in axenic culture (37, 38). It appears that *E. dispar* may be less able than *E. histolytica* to obtain its nutrients in a particle-free medium.

2.3 Medicinal herbs

Thai indigenous medicine is a unique blend of knowledge attained though centuries of practice together with the knowledge ‘adopted’ from other systems of medicine, mainly those of Indian and Chinese origins. Thai people in each region of the country have developed their own unique style of indigenous medicine ranging from the simple us of medicinal plants as ingredients in foods and drinks to sophisticated compound drugs formulated as specific remedies for certain ailments.

The art of herbal remedy is a complex one and involves many disciplines of science. It is remarkable that Thai people in the ancient times were able to acquire the knowledge on the medicinal properties as well as the toxicity of so many drug ingredients. With this knowledge, they were able to formulate specific cure for each ailment as well as to devise appropriate techniques for the detoxification of certain drug ingredients. The methods used to detoxify drug ingredients in traditional Thai

medicine include sun-drying, roasting, exsiccating, acidifying, fermenting, grilling, and mixing with drugs of opposite attributes.

In the past century, the use of herbal medicine in Thailand has been on the decline, due largely to the introduction of modern medicine which is more effective and easier to use. However, there is a worldwide trend toward the use of drugs of natural origin since they are believed to possess less harmful side-effects than synthetic drugs. This results in an increased public awareness of our rich and valuable heritage in traditional Thai medicine. There has also been a concerted effort by both the government and the private sector to develop Thai medicinal plants into safe and effective drugs.

Thailand, with its prestigious geographical location and advantageous terrain ranging from the cool mountainous areas of the North to the dense tropical rain forests and the vast expanses of coastlines of the South, boasts a large number of indigenous flora, of which over one thousand species are believed to possess medicinal properties. It is pertinent that this vast and untapped natural resource be developed into useful drugs.

The safety and quality of low medicinal plant materials and finished products depend on factors that may be classified as intrinsic (genetic) or extrinsic (environment, collection method, cultivation, harvest, post-harvest processing, transport and storage practices). Inadvertent contamination by microbial or chemical agents during any of the production stages can also lead to deterioration in safety and quality. Medicinal plants collected from the wild population may be contaminated by other species or plant parts through misidentification, accidental contamination or intentional adulteration, all of which may have unsafe consequences (39, 40).

The crude drug which reaches the pharmaceutical manufacturing line will have passed though various stages, all of which influence the nature and amount of Environmental Conditions, Cultivated and Wild Plant, Collection, Drying and Storage (39, 40).

2.3.1 Environmental conditions

Climatic conditions, for example, length of day, rainfall (water supply) and field temperature, significantly influence the physical, chemical and biological qualities of medicinal plants. The duration of sunlight, average rainfall, average temperature, including daytime and night-time temperature differences, also influence the physiological and biochemical activities of plants, and prior knowledge should be considered (39, 40).

Plant growth and development, and often the nature and quantify of secondary metabolites, are affected by temperature, rainfall, aspect, length of day (including the quality of light) and altitude. Such effects have been studied by growth particular plants in different climatic areas and observing variations (39, 40).

Temperature: Temperature is a major factor controlling the development and metabolism of plants. Although each species has become adapted to its own natural environment, plants are frequently able to exist in a considerable range of temperature. Many tropical and subtropical plants will grow in temperate regions during summer mounts, but lack frost resistance to withstand the winter. In general, the highest temperatures are experienced near the Equator, but as the temperature fall about 1°C for every 200 m of elevation (39, 40).

Rainfall: The important effects of rainfall on vegetation must be considered in relation to the annual rainfall, its distribution throughout the year, its effect on humidity and its effect coupled with the water-holding properties of soil. Variable results have been reported for the production of volatile oils under different conditions of rainfall and may in some instances be coupled with the development of glandular hairs. Continuous rain can lead to a loss of water-soluble substance from leaves and roots by leaching ; this is known to apply to some plants producing alkaloids, glycosides and even volatile oils. This could account for low yields of some active constituents in wet seasons from plants whose general condition appears to be good (39, 40).

Day-length and radiation characteristics: Plants vary much in both the amount and intensity of the light which they require. In the wild state the plant will be found where its shade requirements are met, and under cultivation similar shade must be

provided. In certain cases research has shown that light is a factor which helps to determine the amount of glycosides or alkaloids produced (39, 40).

The daily variation in the proportion of secondary metabolites is probably Light-controlled (please see more fully discussed under ‘Collection Times’). Many plants initiate flowers only in certain day-lengths, and where flowering is essential, this factor must be carefully considered before planting in a new region. Presence or absence of light, together with wave length range, have a marked effect on the secondary metabolite production of some plants in tissue culture (39, 40).

2.3.2 Culture and wild plants

For success in cultivation it is necessary to study the conditions under which the plant flourishes in the wild state and reproduce these conditions or improve on them. Small changes in ecology can affect plant products : thus, satisfactory rubber trees grow wild in the Amazon basin but cleared areas converted to rubber plantations have been a failure (39, 40).

Soils: Different plant species vary enormously in their soil and nutritive requirements, and this aspect has received considerable attention with medicinal plants. Three important basic characteristics of soils are their physical, chemical and microbiological properties (39, 40).

Variations in particle size result in different soils ranging from clay, via sand, to gravel. Particle size is one factor influencing water-holding capacity, and some plants which produce mucilage as a water-retaining material contain less mucilage when grown on soil with a high moisture content. Although particular grown on soil with a high moisture content. Although particular species have their own soil pH tolerances, no marked influence of pH value within the tolerance range has been demonstrated for essential oils and alkaloids. All plants require calcium for their normal nutrition but plants known as caliphobous plants cannot be grown on chalky soils, probably owing to the alkalinity. In other case different varieties of the same species may grow on different soils (39, 40).

The effect of nitrogen-containing nutrients on alkaloid production has received considerable study; generally nitrogen fertilizers increase the size of the plants and the

amounts of alkaloids produced but, as indicated elsewhere, the method of expressing the results of such experiments is important. The effects of nitrogen on glycoside and essential oil content appear variable; presumably in these cases the final result arises from the general effect of nitrogen on the plant's metabolism. The effect of potassium on alkaloid production shows no consistent trend, but an interesting example is the increase in putrescine production in barley grown on a potassium-deficient medium, where it is possible that the organic base has been formed to act as a substitute for potassium ions (39, 40).

2.3.3 Collection

Medical plants may be collected from wild or cultivated plants, and the task may be undertaken by casual, unskilled native labour or by skilled workers in a highly scientific manner (39, 40).

Medical plants should be harvested during the optimal season or time period to ensure the production of medicinal plant materials and finished herbal products of the best possible quality. The time of harvest depends on the plant part to be used. Detailed information concerning the appropriate timing of harvest is often available in national pharmacopoeias, published standards, official monographs and major reference books. However, it is well known that the concentration of biologically active constituents varies with the stage of plant growth and development. This also applies to non-targeted toxic or poisonous indigenous plant ingredients. The best time for harvest (quality peak season/time of day) should be determined according to the quality and quantity of biologically active constituents rather than the total vegetative yield of the targeted medicinal plant parts. During harvest, care should be taken to ensure that no foreign matter, weeds or toxic plants are mixed with the harvested medicinal plant materials (39, 40).

Cutting devices, harvesters, and other machines should be kept clean and adjusted to reduce damage and contamination from soil and other materials. They should be stored in an uncontaminated, dry place or facility free from insect, rodents, birds and other pets, and inaccessible to livestock and domestic animals (39, 40).

The season at which each drug is collected is usually a matter of considerable importance, as the amount, and sometimes the nature, of the active constituents is not constant throughout the year (39, 40).

The age of plant is also of considerable importance and governs not only total quantity of active constituents produced but also the relative proportions of the components of the active mixture (39, 40).

Generally speaking, leaves are collected as the flowers are beginning to open flowers just before they are fully expanded, and underground organs as the aerial parts die down. Leaves, flowers and fruits should not be collected when covered with dew or rain. Any which are discolored or attacked by insects or slugs should be rejected. Even with hand - picking, it is difficult, certainly expensive, to get leaves, flowers or fruits entirely free from other parts of plant. Similarly, with roots and rhizomes a certain amount of aerial stem is often collected and is permitted in the case of senega root (39, 40).

Barks are usually collected after a period of damp weather, as they then separate most readily from the wood. For the collection of gums, gum resins, etc., dry weather is obviously indicated and care should be taken to exclude vegetable debris as far as possible (39, 40).

Underground organs must be freed from soil. Shaking the drug before, during and after drying, or brushing it, may be sufficient to separate sandy soil , but in the case of a clay or other heavy soil washing is necessary (39, 40).

All large organs, should be sliced to facilitate drying. Before gentian root is dried, it is made into heaps and allowed to ferment. Seed are extracted from mucilaginous fruits, are washed free from pulp before drying (39, 40).

2.3.4 Drying

If enzymic action is to be encouraged, slow drying at a moderate temperature is necessary. If enzymic action is not desired, drying should take place as soon as possible after collection. For these reasons, drying apparatus and stills should be situated that freightage is mush reduced, as many fresh drugs contain a considerable amount (60-90%) of water (39, 40).

Drying by artificial heat is more rapid than open-air drying and is often necessary in tropical countries. Alternatively heat may be applied by means of open fires, stoves or hot-water pipers. In all drying sheds there must be a space of at least 15 cm between superimposed trays, and air must circulate freely (39, 40).

Rapid drying helps flowers and leaves to retain their color and aromatic drugs their aroma, but the temperature used in each case must be governed by the constituents and the physical nature of the drug. As a general rule, leaves, herbs and flowers may be dried between 20 and 40°C, and barks and roots between 30 and 65°C. Exactly how far drying is to be carried is a matter fort practical experience. If leaves and other delicate structure are over dried, they become vary brittle and tend to break in transit. Drug such as aloes and opium may require further drying after importation (39, 40).

2.3.5 Storage

All medical plants used at harvest should be kept clean and free from contamination by previously harvested medicinal plants and other foreign matter. If plastic containers are used, particular attention should be paid to any possible retention of moisture that could lead to the growth of mould. When containers are not in use, they should be kept in dry conditions, in an area that is protected from insects, rodents, birds and other pets, and inaccessible to livestock and domestic animals (39, 40).

Any mechanical damage or compacting of the raw medicinal plant materials, as a consequence, for example, of overfilling or stacking of sacks or bags, that may result in composting or otherwise diminish quality should be avoided. Decomposed medicinal plant materials should be identified and discarded during harvest, post-harvest inspections and processing, in order to avoid microbial contamination and loss of product quality (39, 40).

Medical plants stored in the usual containers-sack, bales, wooden cases, cardboard boxes and paper bags- reabsorb about 10 - 20 % or more of moisture. They are then termed ‘air-dry’. Plastic sacks will effectively seal the contents. The combined effects of moisture and temperature on humidity and the subsequent water condensation when the temperature falls, must be considered in drug storage. They

may be kept in sealed containers with a dehydrating agent. For large quantities the bottom of a case may be filled with quicklime and separated from the drug by a perforated grid or sacking. If the lime becomes moist, it should be renewed. Volatile oils should be stored in sealed, well-filled containers in a cool, dark place. Similar remarks apply to fixed oils, particularly codliver oil. In the latter case the air in the containers is sometimes replaced by an inert gas. Air-dry drugs are always susceptible to the attack of insects and other pests, so they should be examined frequently during storage and any showing mould or worminess should be rejected (39, 40).

In order to undesirable microbial contamination and to prevent the development of other living organisms, some plant materials may require sterilization before storage (39, 40).

2.4 Preparation of traditional Thai medicine

Nowadays the acquisition of drug ingredients is made easy by the existence of retail tradition drug stores where a vast array of crude drugs is in stock. In rural areas, traditional herbalists may grow a number of the more common medicinal plants while others may be collected from trees growing in the forests. Once all the ingredients have been acquired, they may be further processed by pulverization if the drug is to be made into pills or tablets. Grinders made of stone or metal alloy may be utilized in the pulverization process and the powdered drug is then passed through a sieve being before being shaped into the desired form.

There are several dosage forms in which traditional drugs may be prescribed. Hence, include solid dosage forms, such as pills, tablets, snuff, suppository and liquid dosage forms such as fluid extracts, alcoholic extracts, teas and expressed juices. These drugs may be taken internally, applied externally, used as snuff, an inhalant or as a poultice. Of the dosage forms previously mentioned only 5 or 6 are commonly in use and these are described below (39, 40).

2.4.1 Fluid Extracts

To prepare an extract, a handful of the ingredients is placed in a clay pot shaped like a gourd. The crude drugs may be coarsely chopped or tied into a sheaf the size of one's palm prior to being transferred to the pot. Water is then added to the ingredients until it just covers the drugs. The mixture is boiled for 10 – 30 minutes, after which the supernatant is decanted and drunk when luke–warm (39, 40).

2.4.2 Infusions and Herb Teas

The gradients used in herb tea are usually cut into fine strips and then air-dried. Occasionally, the ingredients may be roasted to give an agreeable aroma. One part of the drug mixture is placed in a container with a lid, into which ten parts of boiling water are added. This is then put aside to brew for 15 – 20 minutes (39, 40).

2.4.3 Alcoholic Macerates

In most cases, air-dried ingredients are roughly pounded and wrapped in a piece of muslin before being placed in a glass jar containing rice whiskey for a period of 7 – 10 days. The resultant macerate is then decanted and taken as directed (39, 40).

2.4.4 Pills

Fresh herbs are sliced and left to dry in the sun. After the drying process, the herbs are pulverized while they are still warm. Two parts of this powered drug are thoroughly mixed with one part of honey or syrup and allowed to stand for 2 hours. The mixture is then shaped into round balls with a diameter of approximately 0.2 – 1 centimeter. The finished pills are laid out in the sun to dry and heat treatment process repeated after a fortnight to prevent fungal infestation (39, 40).

2.4.5 Expressed Juices

To prepare the above dosage form, fresh herbs are pounded to a pulp, to which a small amount of water may be added. The juice is then squeezed out and taken as directed (39, 40).

2.4.6 Poultices

Fresh herbs are used for this form of treatment. The pounded herbs, to which a small amount of water or spirit may be added, are made into a thick paste and the paste applied to the affected areas. The drug is kept moist and the dressing changed 2 - 3 times a day (39, 40).

The above mentioned forms drugs are the most commonly prescribed form in traditional Thai medicine, though other dosage forms are still in use. In the preparation of these drugs a number of vehicles may be used; these include water, spirit, syrup, honey and lime water. In general, they serve to make the drugs soluble and in some case they may enhance the action of the major drugs (39, 40).

As a safety precaution, finite periods of shelf life are recommended for various drug products. Thus, a powered drug consisting entirely of leaves has a shelf of 3 to 6 months while a drug containing heartwoods may be stored for as long as 6 to 8 months. In general, pills and tablets store better than powders, and pills consisting of roots or rhizomes are considered to be safe for use for up to one and a half years. Therefore, knowledge of the drug ingredients, appropriate methods of preparation as well as safety aspects is essential in the formulation of traditional Thai drugs (39, 40).

2.5 *Andrographis paniculata Nees*.

Botanical Name : Andrographis Paniculata

Scientific Name : *Andrographis paniculata Nees.*

Other Common Name : Andrographis, Chuan Xin Lian, Kan Jang (Chinese), Kalmegh (Bengali, Hindi), King of Bitters

(See picture at Appendix D)

Taxonomy

Kingdom : Plantae

Subkingdom : Tracheobionta

Division : Angiosperms

Class : Dicotyledonae

Subclass : Gamopetalae

Order : Personales

Family : Acanthaceae

Genus : *Andrographis*

Andrographis paniculata has been used in traditional Indian and Chinese herbal medicine. The most common reported uses were for digestive problem, snakebite and infections ranging from malaria to dysentery (41, 42). For over twenty years, the effectiveness, safety, and composition of the Asian medicinal herb. Andrographis paniculata has been the subject of scientific research. Traditionally, Chinese, Indian, and Thai practitioners have used this herb to treat numerous infectious and chronic diseases, especially upper respiratory tract infections, liver ailments, and diarrhea.

Over the last decade, *andrographis paniculata* has become popular, particularly in Scandinavia, as a treatment for colds, and is now available in the United States (43).

Chemical composition

Chemically the drug contains flavones and lactone. Among diterpene lactones known as Andrographolide is the main constituent and it also active principle of the plant. Andrographolide has been isolated in pure form and it has shown various pharmacological activity (44). These bitter constituents are believed to have immune stimulating, anti-inflammatory, fertility decreasing, liver protective and bile secretion stimulating actions (45).Though some older studies suggested andrographis was antibacterial, modern research has been unable to confirm this finding (46). Andrographis has has proven helpful in combination with antibiotic for people with dysentery, a severe form of diarrheoa (47). It has also show preliminary benefit for people with chronic viral hepatitis (48).

Biological Activities

Andrographolide: Antibacterial, Antidysentary, Antiinflammatory, Antihepatoxic, Antitonsilitic, Hepatoprotective, Antitubercular, Antipneumonic, anti-HIV, etc (49).

Neoandrographolide: Antibacterial, Antidysenteric, Antioxidant, Antisecretory, etc (49).

Pharmacology

It increases biliary flow and liver weight in rat. Andrographolide produces a significant dose dependent choleretic effect, as evidence by increase in bile flow, bile salt and bile acids in conscious rats and anaesthetised guinea pigs. It shows hepatoprotective action. It improves non-specific immune response. Although both *andrographis* and beta glucan effective immune enhancers, Extracts of andrographis, have been shown to stimulate powerful immune responses in living creatures. The immune response may be specific directed at a microbial invader already present in the body, or generally, strengthening the immune system in preparation against future

infections. *Andrographis* strongly stimulates phagocytosis and the production of specific antibodies (43).

Uses

Andrographis is used for prophylactic and symptomatic treatment of upper respiratory infections such as the common cold, as well as for uncomplicated sinusitis, pharyngotonsillitis, pneumonia, and bronchitis. Although there are other, less commonly reported uses for andrographis, such as colic pain and eczema, there is little scientific data supporting claims regarding these conditions. Highly recommended for excess fire or heat conditions, colds and influenza, pneumonia, upper respiratory infections, acute bacterial sinusitis, fever, inflammation and any "pus forming" conditions of the skin. It is also used for intestinal disturbances and parasitic conditions, hepatitis, infected wounds and as a uterine stimulant. Research shows possible anti-cancer activity (43). Uses supported by clinical data were possible prevention of the common cold , symptomatic treatment of the common cold and pharyngotonsillitis (50, 51, 52).

Side-effects

Andrographis paniculata Nees. rarely causes negative side-effects. It does have antifertility and pregnancy-terminating effects so women who are pregnant or wish to become pregnant should not use it. Some people have experienced dizziness and heart palpitations. Allergic reactions, as with nearly any substance, have also occurred (43, 53).

2.6 *Euphorbia hirta L.*

Botanical Name : Euphorbia hirta

Scientific Name : *Euphorbia capitata , Euphorbia pilulifera L., Euphorbia hirta L.*

Other Common Name : Australian asthma weed (Engl.), Cat's hair (Engl.), Asthma weed, Queensland asthma weed, Garden spuge

(See picture at Appendix D)

Taxonomy

Kingdom	:	Plantae
Subkingdom	:	Tracheobionta
Division	:	Maqnoliophyta
Class	:	maqnoliopsida
Subclass	:	Rosidae
Order	:	Euphorbiaceae
Family	:	Euphorbiaceae
Genus	:	*Euphorbia L.*

Euphorbia is an annual herb with a slender hairy stem and lanceolate opposite-toothed leaves. Small yellow flowers occur in dense clusters in the leaf axils and produce small reddish wrinkled seeds. The plant produces a milky latex which is irritating to the mucous membranes. *Euphorbia* is indigenous to India and most tropical countries (53, 54).

Harvest

Plants are cut when flowering (or fruiting) and dried for use in infusions, liquid extracts, and tinctures; plant used fresh for juice (53). The aerial parts of the plant are harvested when in flower during the summer and can be dried for later use (54).

Chemical composition

There are flavonoids (including quercetin), triterpenoids, glycosidal substance (0.4%), choline, tannin, fatty acids, phorbic acid, sterols, euphosterol, jambulol, melissic acid, sugars, and possible alkaloid in some samples (0.1%). Most spurges contain diterpene esters which are carcinogenic, highly irritant and purgative. *E. hirta*, however, is ester-free and considered a safe remedy in Traditional Chinese Medicine (TCM) in the treatment of dysentery (55, 56,57,58,59).

Biological Activity

Inositol : Antialopecic, Anticirrhotic, Antidiabetic, Antineuropathic, Cholesterolytic, Lipotropic, Sweetener, Ubiquiot, etc (49).

Linoleicacid : Antiacne, Antianaphylactic, Antialopecic, Antiarthritic, Antiandrogenic, Antiinflammatory, Nematicide, Propecic, Insectifuge, etc (49).

Alphaamyrin: Antiedemic, Antiinflammatory, Antinociceptive, Antitumor, Insectifuge, Cytotoxic, etc (49).

Beta – amyrin : Antiedemic, Antiinflammatory, Antinociceptive (49).

Beta-sitosterol : Androgenic, Anorexic, Antiadenomic, Antiandrogenic, Antibacterial, Anticancer(Breast), Antiedemic, Antiinflammatory, Antiviral, Candidicide, etc (49).

Betulin : Anti HIV, Anticarcinomic, Antiinflammatory, Antiviral, etc (49).

Pharmacology

Euphorbia has a relaxing action upon the smooth muscle of the lungs and is of great value in the treatment of asthma and bronchitis. It will also relieve spasms in the larynx, helping nervous coughs, and will help to relieve upper respiratory catarrh. It is a useful remedy in hayfever. It also has a specific action in destroying the organisms that cause amoebic infections in the intestines. Additional Comments: Chinese physicians use this herb for athlete's foot and other skin conditions (53, 54).

The whole plant is decocted and used in the treatment of athlete's foot (54), dysentery (59), enteritis and skin conditions. It has been used in the treatment of syphilis. The sap is applied to warts in order to destroy them (54).

Medicinal applications

Euphorbia has used against asthma, bronchitis (60), worm infestation (60), conjunctivitis (60), antiprotozoa (61), antibacterial (62,63), antifungus (63), reduce inflammation (64,65), antidiarrheoa (59), bloody diarrheol (66), etc. The latex of the plant is used for warts and cuts. It also has lactogenic properties (60).

Uses

Folkloric uses: Enteritis, dysentery; dermatitis, eczema, pruritus; decreased milk secretion after delivery. Infusion or tea of the plant, Four glasses daily, for bronchits and labored breathing, asthma, chronic dysentery. Decoction of dry plant used for skin disease. Decoction of fresh plant used as gargle for the treatment of thrush. Decoction of the root used to allay vomiting, chronic diarrheas, and fevers. The same root decoction as an enema for constipation (54, 55).

Caution

Although this is one of the few members of the spurge family not poisonous to humans, large doses may cause nausea and vomiting (55).

A physiological study of *Euphorbia pilulifera* to the conclusion that the active principle acts directly upon the heart and respiration; that it is not an irritant to the skin, but in large dose it is to the gastric mucous membrane (55, 56).

2.7 *Lonicera japonica Thunb*.

Botanical Name	:	Lonicera japonica
Scientific Name	:	*Lonicera japonica*
Synonym(s)	:	Lonicera japonica var. chinensis (P.W. Wats.) Baker, Nintooa japonica (Thunb.) Sweet
Other Common Name	:	Chinese honeysuckle, Japanese honeysuckle, jin yin hua

(See picture at Appendix D)

Taxonomy

Kingdom	:	Plantae
Subkingdom	:	Tracheobionta
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Subclass	:	Asteridae
Order	:	Dipsacales
Family	:	Caprifoliaceae
Genus	:	*Lonicera L.*

The Japanese honeysuckle (*Lonicera japonica Thunb*.) is used in traditional Chinese medicine. It has fragrant, yellow-white flowers and black berries. Although the Chinese most commonly use the bud of the flower in their medical practice, in other countries it is mostly the flowers and leaves that are used for their healing properties. Japanese honeysuckle works well as a detoxifier, and is best used for acute infections and inflammations. As an alterative, which cleanses and purifies the blood, and an antipyretic, which reduces fever with its cooling properties, Japanese honeysuckle is best used for such ailments as sore throats, swollen eyes, headaches, etc (67).

Honeysuckle has long been used in Chinese medicine to reduce heat and relieve toxicity, especially that of such inflammatory diseases as rheumatoid arthritis, abscesses, sores, inflammation of the breast and dysentery (57).

Modern laboratory research has confirmed that the flowers have an inhibitory effect on the bacteria that cause salmonella, staphtococus, streptococus, pseudomonas, and tuberculosis infections. In the west called this herb is antibiotic and most of them grow in Shan Gong Province of China (67).

Chemical composition

In flower, it contains luteolin, inositol, chlorogenic acid and isochlorogenic acid (46). Despite the sweetness of its fragrance and nectar, the medicinal parts of the plant are bitter, due to the saponin in its stem, the 8% tannin in the leaves and the 1% insitol in its flowers (54).

Biological Activity

Inositol: Antialopecic, Anticirrhotic, Antidiabetic, Antineuropathic, Cholesterolytic, Lipotropic, Sweetener, Ubiquiot etc (49).

Luteolin: Antiallergic, Antihistaminic, Antiinflammatory, Antibacterial, Diuretic, Hepatoprotective, Antimutagenic, etc (49).

Tannin: Antibacterial, Antidiarrheic, Anthelmintic, Antidysenteric, Anticariogenic, Antihepatotoxic, Anticancer, AntiHIV, etc (49).

Parts Used

The dried flowers, harvested just before the bud opens. Some products used to treat boils and abscesses include honeysuckle bark. Japanese honeysuckle (*Lonicera japonica*) blooms in the spring from April to May, with fragrant white flowers touched

with a shade of purple that fade to yellow as they mature. The species of honeysuckle is found in North America, the United Kingdom and western Asia (54).

Different parts of the honeysuckle are used to treat different conditions. The flower buds of honeysuckle are gathered in the beginning of summer, then dried in the shade before being used in herbal remedies. They can be used raw, or after being fried or distilled (52). The flowers relieve coughs and act as antispasmodic used in the treatment of asthma (57). The bark has diuretic properties and taken to relieve gout, kidney stone and liver problems (57). The leaves are astringent and make a good mouthwash and gargle for sore throats or canker sore (57).

General use

In traditional Chinese medicine, honeysuckle flower has sweet and cold properties, and is associated with the Lung, Stomach and Large Intestine meridians (47, 48). It is used to clear heat and remove toxins. Generally, honeysuckle flower has been employed to treat a variety of conditions, ranging from fevers, ulcers, inflammation and sore throats to skin infections. It is also used (in conjunction with coptis and pulsatilla) to treat diarrhea caused by toxic heat and bloody diarrheoa (68). Honeysuckle flower may be applied internally or externally (67).

Honeysuckle flowers act as natural antihistamine, although they are more useful for treating rashes and inflammation than for treating coughing and sneezing. Some Japanese health food products include honeysuckle for treating bloating, nausea, and vomiting caused by hepatitis C (67).

The honeysuckle stems and flowers are used together as medical infusion in the treatment of upper respiratory tract infections and dysentery. An infusion of the flower buds is used in the treatment of a wide range of ailments including syphilitic skin diseases and tumors, bacterial dysentery, colds and enteritis. Externally, the flowers are applied as a medicinal wash to skin inflammations, infections rashes and sores (69).

Precautions

Avoid when there is chronic diarrhea caused by cancer treatment, HIV, hepatitis C, or other chronic disease. Honeysuckle flower should be given with caution to patients diagnosed with cold in the stomach and spleen, or to patients related skin disorders. In addition, the leaves and stems of honeysuckle contain substances called saponins, which are poorly absorbed by the human body but can be dangerous if taken in extreme amounts. Symptoms of poisoning can include gastrointestinal discomfort and muscle cramps (67).

2.8 The study of *Entamoeba histolytica* after exposure to medicinal plants in Thailand

There were few studies, two were cited here.

The experiment was in vitro study of inhibitory concentration of three medicinal plant extracts namely *Psidium guajava Linn., Garcinia mangostana Linn.* And *Punica granatum Linn*. to *E. histolytica* trophozoites. Plants were extracted in water by vacuum rotory evaporator and tested in xenic culture. The efficicacy of plant extracts were determined at concentrations of 1, 5, 10 and 20 milligrams per millilitre, respectively. The minimal dose of amoebicidal activity was 20 milligrams per milliliter in all three medicinal plants (70).

The antiamoebic activity of chloroform, methanol and water extracts from 12 Thai medicinal plants (39 extracts) commonly used by AIDS patients in southern Thailand were screened, at concentration of 1,000 micrograms per milliliter, against *E. histolytica* strains HTH-56:MUTM and HM1:IMSS growing in vitro. The extracts were incubate with 20,000 *E. histolytica* trophozoites per milliliter of medium at 37°C under anaerobic conditions for 24 hours. The cultures were examined with an inverted microscope and scored (1-4) according to the appearance and numbers of the trophozoites. The extracts that caused inhibition were selected and retested using the same conditions but with concentrations that ranged from 31.25 to 1,000 micrograms

per milliliter using *E. histolytica* strain HM1:IMSS, and the IC 50 values for each extract were calculated. The chloroform extracts from *Alpinia galangal* (IC 50 55.2 micrograms per millilitre), *Barleria lupulina* (IC 50 78.5 micrograms per milliliter), *Boesenbergia pandurata* (IC 50 45.8 micrograms per millilitre), *Piper betle* (IC 50 91.1 micrograms per milliliter) and *Piper chaba* (IC 50 71.4 micrograms per milliliter) and the methanol extract from *B. pandurata* (IC 50 57.6 micrograms per milliliter) were all classified as “active”, i.e. with an IC 50 of less than 100 micrograms per milliliter, whereas those from *Murraya paniculata* (IC 50 116.5 micrograms per milliliter) and *Zingiber zerumbet* (IC 50 196.9 micrograms per milliliter) were classified as being “moderately”. The IC 50 of a standard drug, metronidazole, was 1.1 micrograms per milliliter (71).

CHAPTER III
MATERIALS AND METHODS

3.1 Amoeba trophozoites

Methods and maintenance and subculture of amoebae, the diphasic medium i.e. modified Boeck and Drbohlav's diphasic medium was used in this experiment. Trophozoites of amoeba were innoculated into the medium tubes and incubated in an incubator at 37°C for 24 hours.

3.1.1 Method of subculture

The method for subculturing many types of cultures is essentially the same. Cultures are chilled in an ice-water bath for 5 minutes to release trophozoites attached to the glass culture tube. Tubes are inverted several times to disperse the cells and a measured inoculum is passed aseptically to a culture tube containing fresh medium. The tubes are capped tightly and incubated at 36 to 37°C, at 5° to the horizontal .The old medium was replaced with 3 milliliters of new fresh medium (Ringer's solution 97 milliliters in the present of fresh bovine serum 3 milliliters) every 48 – 72 hours. Add 1 loop of starch and antibiotics (penicillin G sodium and streptomycin) 2 – 3 drops in each culture tube.

3.1.2 Method of growth inhibitory effect of three herbal aqueous extracts

Entamoeba trophozoites of age from 24 to 48 hours culture were utilized. The cells were harvested by chilling the tube on ice for 15 minutes to detach the trophozoite. The sediment of the culture was collected and then transferred in to test tubes and then centrifuged at 4°C, 1500 rpm, 10 minutes.

The supernatant was decanted and cells resuspended in Ringer's solution. The number of viable cells were calculated using a haemocytometer and 0.1% Trypan blue. The criteria for viability were motility and dye exclusion. Finally the amoebae were suspended in the same medium to yield approximately 200 trophozoites per microliter. 50 microliters of this suspension containing approximately 10,000 trophozoites, were inoculated into screw-capped culture tube which contained 3 milliliters of the freshly prepared medium. The culture tubes were maintained in the incubator at 37 °C for 24 hours.

Finally 0.5 milliliters herb solution of each concentration were added and negative controls consisted of adding appropriate amount of 0.5 milliliters of Ringer's solution and positive controls consisted of adding metronidazole (10 micrograms per milliliters). The cultures were maintained in the incubator at 37°C, 24 hours.

Each local strain of *Entamoeba* was tested separately and repeatly three times with the various concentration of herbs. The results were read after 24 hours exposure to each herb solution.

3.2 Preparation of herb extracts for growth inhibition test

Three medicinal herbs, *Andrographis paniculata Nees.*(whole plant), *Euphorbia hirta L.*(whole plant) and *Lonicera japonica Thunb.*(flowers and leaves) were used in this study.

Three dried plants were successively extracted separately with boiling water. Five hundred milliliters of distilled water were added to 50 grams of dried plant and boiled at 100°C for 30 minutes. The crude extract of herb was filtered through several thickness of surgical gauzes. About 300 milliliters of herbal aqueous extract were passed through milipore filter set. The sterile crude extracts of herb were being kept as stock solution. Stock solutions of herbs were diluted with Ringer's solution by two fold dilutions for growth inhibition test.

3.3 Minimum concentration of Metronidazole

Metronidazole, product by Pharmaceutical is used in the experimentals. The original concentration of MTZ is 500 milligrams per 100 milliliters. The concentrations was diluted to 500, 250, 125, 100, 50, 30, 20, 10, 5 and 3 micrograms per milliliter.

Approximately 10,000 *Entamoeba histolytica* trophozoites were inoculated in to screw-capped culture tube which contained egg slant and 3 milliliters of freshly prepared medium. The cultures were maintained in the incubator at 37 °C for 24 hours. Metronidazole was diluted in Ringer's solution and desired concentration in medium overlay were added after 24 hours incubate *Entamoeba histolytica* trophozoites. Only 0.5 milliliters of suspension containing drug was added to the culture media (3.5 milliliters total volume) and untreated control tube consisted of adding appropriated amount 0.5 milliliters of Ringer's solution. The culture were maintained in the incubator at 37 °C for 24 hours. The results were read after 24 hours exposure to Metronidazole. If amoeba multiplicated well in the untreated control tube but not in tested tube, the minimum effective (amoebiasis) concentration was determined.

Each local strain of amoebae was tested repeatly three times with various concentration of Metronidazole, starting from 500, 250, 125, 100, 50, 30, 20, 10, 5 and 3 micrograms per milliliter.

3.4 Method of counting amoebae

For counting the amoebae, hemocytometer was used in this study. This glass instrument is composed of two counting chambers separated by a horizontal grooved canal and bordered on each side by a similar vertical moat. The improved Neubauer ruling is preferred, which consists of a double line surrounding each group of 16 small squares. The distance between the bottom of the cover-glass and the surface of the chamber is 0.1 mm. This surface is ruled so that two specific areas are formed, one on each side of the horizontal trench. Each of these two largest ruled areas is square and

measures 3 mm on each side. Therefore each completely ruled chamber on each side of the transverse moat measures 3 x 3 x 0.1 mm, so has a volume of 0.9 cu.mm.

The number of tropzoites was counted after shaking the culture tube, which was made homogeneous. A sample of this suspension was taken out and put into an improved Neubauer haemocytometer. The characteristics of a properly filled counting chamber are the fluid filled the space beneath the cover glass entirely or almost entirely, and none of the fluid run over into the moat, and there are no bubble. The number of amoebae in all nine large squares are counted. Multiplication of this figure by 10/9 gives the number of amoebae per cubic millimeter (microliter).

Example: The number of amoebae in 9 large squares = A

The volume of 9 large squares = 9 x 1 x 1 x 0.1 mm

= 0.9 cu.mm.

So the amount of amoebae is (10/9) x A

= (10/9) x A per cu.mm.

= (10/9) x A x 1,000 amoebas per cu.cm.

The criterion for arriving at the amoebicidal end points was recorded on the presence or absence of living amoebae by direct observation under the microscope. A tube was recorded as “positive” if one or more active trophozoites were seen and the amoebae were counted in haemocytometer. The “negative” was when no amoebae was seen. Dead trophozoites of *E. histolytica* could be readily distinguished by their loss of movement, lowered contrast and discharge of cytoplasmic components. If there was doubtness in dead or living of trophozoites, especially if the trophozoites were non-motile, 0.1 trypan blue stain for 15-20 minutes was used for differentiation. If the amoebae are considered as dead trophozoites they takes on blue stain and the living trophozoites do not pick up the blue stain.

3.5 Calculation of percentage growth inhibition rate

$$\text{Growth inhibition rate } (\%) = 100 - \frac{100 \times \text{count / ml exposure to herb}}{\text{count / ml not treated}}$$

CHAPTER IV
RESULTS

The results of the testing of herb, including botanical names and parts of herbs extracts against *E. histolytica* trophozoites (AP and S strains) and *E. moshkovskii* trophozoites (LA strain) are summarised in **Table 4.1**.

Table 4.2 shows percentage of growth inhibitory of *E. histolytica* trophozoites (AP and S strains) and *E. moshkovskii* trophozoite**s** (LA strain), by herbal aqueous extracts.. M.I.C. of MTZ was found to be 10 micrograms per milliliter.

Herbal aqueous extracts of *A. paniculata Nees.* (whole plant) , *E. hirta L.* (whole plant) and *L. japonica Thunb.* (flowers and leaves) showed different growth inhibition effects on two strains of *E. histolytica* (AP and S) and one strain of *E. moshkovskii* (LA).

The growth inhibition effect of herbal aqueous extract of *L. japonica Thunb. (*flowers and leaves) was higher than those of herbal aqueous extract of *E. hirta L.* (whole plant) and herbal aqueous extract of *A. paniculata Nees.* (whole plant) whereas the growth inhibition effect of *A. paniculata Nees.* was lowest when compared at the same concentration of three kinds of herbs (**Table 4.2**).

Table 4.1 The numbers of viable *Entamoeba* trophozoites per microliter after 24 hours exposure to different concentrations of herbal aqueous extracts

HERB		LA	AP	S
Andrographis paniculata Nees. (whole plant)	undiluted	19	13	24
	1:2	16	14	23
	1:4	17	14	24
	1:8	17	17	24
	1:16	19	17	24
	1:32	20	19	27
Positive control	Metronidazole 10 micrograms/milliliter	0	0	0
Negative control	Not treated	24	19	33
Euphorbia hirta L. (whole plant)	undiluted	11	8	17
	1:2	14	10	17
	1:4	19	13	22
	1:8	22	17	26
	1:16	27	20	30
	1:32	28	22	33
Positive control	Metronidazole 10 micrograms/milliliter	0	0	0
Negative control	Not treated	29	22	49
Lonicera japonicaThunb. (flowers and leaves)	undiluted	0	0	0
	1:2	4	3	6
	1:4	7	6	8
	1:8	15	12	17
	1:16	16	15	24
	1:32	23	18	30
Positive control	Metronidazole 10 micrograms/milliliter	0	0	0
Negative control	Not treated	26	21	35

Table 4.2 Percentage of growth inhibitory effect of three herbal aqueous extracts on two xenic strains of *Entamoeba histolytica* trophozoites (AP and S) and one strain of *Entamoeba moshkovskii* trophozoites (LA)

HERB		**LA**	**AP**	**S**
Andrographis paniculata Nees. (whole plant)	undiluted	20.83	31.58	27.27
	1:2	33.33	26.32	30.30
	1:4	29.17	26.32	27.27
	1:8	29.17	10.53	27.27
	1:16	20.83	10.53	27.27
	1:32	16.67	0	18.18
Positive control	Metronidazole 10 micrograms/milliliter	100	100	100
Negative control	Not treated	0	0	0
Euphorbia hirta L. (whole plant)	undiluted	61.53	65	65.91
	1:2	50	55	65.91
	1:4	34.62	40	54.55
	1:8	23.08	25	47.73
	1:16	7.69	10	38.64
	1:32	3.85	0	31.82
Positive control	Metronidazole 10 micrograms/milliliter	100	100	100
Negative control	Not treated	0	0	0
Lonicera japonica Thunb. (flowers and leaves)	undiluted	100	100	100
	1:2	84.62	85.71	82.85
	1:4	73.08	71.42	77.14
	1:8	42.31	42.85	51.42
	1:16	30.77	28.57	31.43
	1:32	11.54	14.29	14.29
Positive control	Metronidazole 10 micrograms/milliliter	100	100	100
Negative control	Not treated	0	0	0

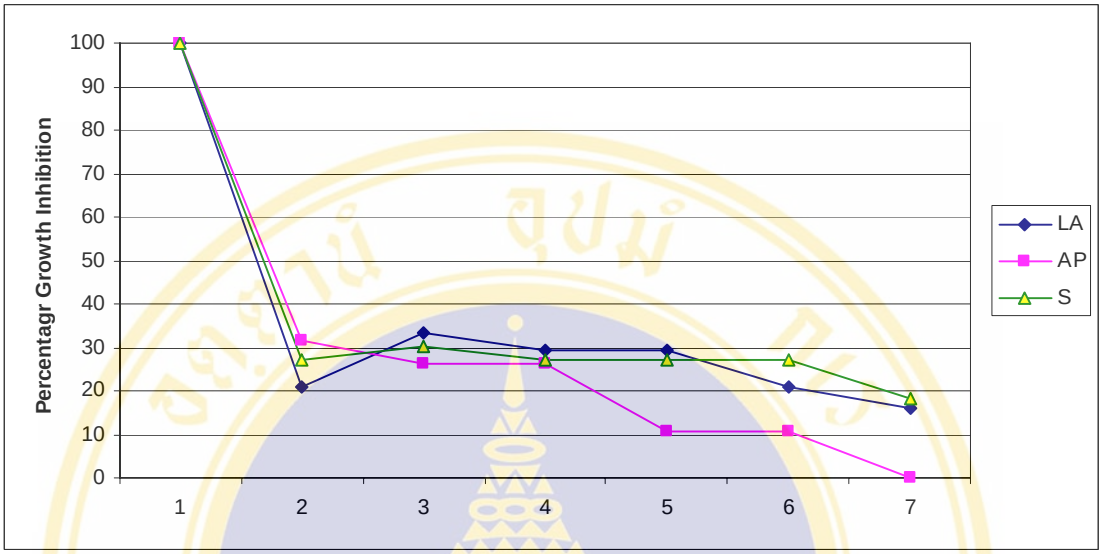


Figure 4.1 Percentage growth inhibition of two strains of *Entamoeba histolytica* trophozoites (AP and S) and one strain of *Entamoeba moshkovskii* trophozoites (LA) after 24 hours exposure to *Andrographis paniculata Nees.* (whole plant).

Herbal aqueous extract of *A. paniculata Nees.* (whole plant) has growth effect on two strains of *E. histolytica* trophozoites (AP and S) and one strain of *E. moshkovskii* trophozoites (LA) and their effects were decreased by increasing dilutions of aqueous extract of *A. paniculata Nees.* (whole plant) (**Figure 4.1**).

Growth of *E. moshkovskii* trophozoites LA strain and *E. histolytica* trophozoites S strain were inhibited by aqueous extract of *A. paniculata Nees.* (whole plant) at the same concentration but more lesser effective to *E. histolytica* trophozoites AP strain (**Figure 4.1**).

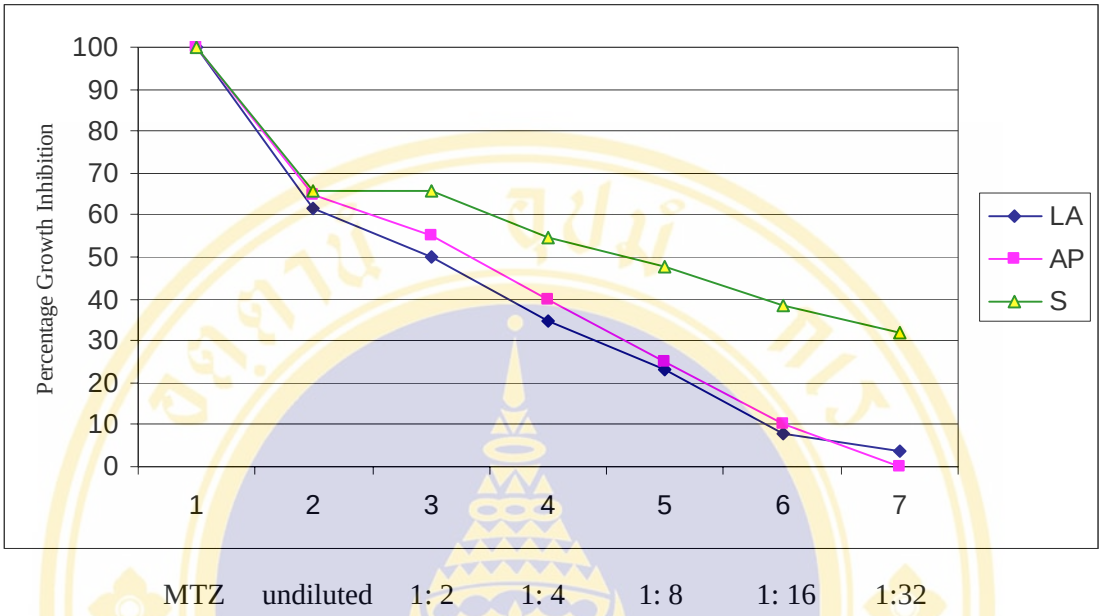


Figure 4.2 Percentage growth inhibition of two strains of *Entamoeba histolytica* trophozoites (AP and S) and one strain of *Entamoeba moshkovskii* trophozoites (LA) after 24 hours exposure to *Euphorbia hirta L.* (whole plant)

Herbal aqueous extract of *E. hirta L.* (whole plant) has growth effect on two strains of *E. histolytica* trophozoites (AP and S) and one strain of *E. moshkovskii* trophozoites (LA) and their effects were decreased by increasing dilution of aqueous extract of *E. hirta L.* (whole plant) (**Figure 4.2**).

Growth of *E. moshkovskii* trophozoites LA strain and *E. histolytica* trophozoites AP strain were inhibited by aqueous extract of *Euphorbia hirta L.* (whole plant) at the same concentration but lesser effective to *E. histolytica* trophozoites S strain (**Figure 4.2**).

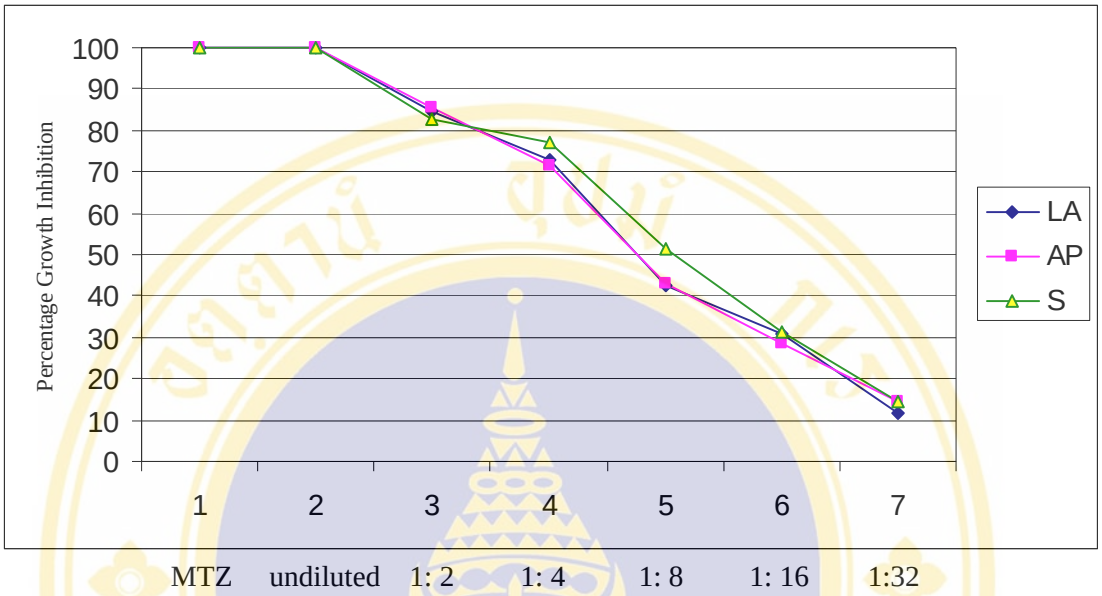


Figure 4.3 Percentage growth inhibition of two strains of *Entamoeba histolytica* trophozoites (AP and S) and one strain of *Entamoeba moshkovskii* trophozoites (LA) after 24 hours exposure to *Lonicera japonica Thunb.* (flowers and leaves)

Undiluted herbal aqueous extract of *L. japonica Thunb.* (flowers and leaves) can kill two strains of *E. histolytica* trophozoites (AP and S) and one strain of *E. moshkovskii* trophozoites (LA) and their effects were decreased by increasing dilutions of aqueous extract of *L. japonica Thunb.* (flowers and leaves) (**Figure 4.3**).

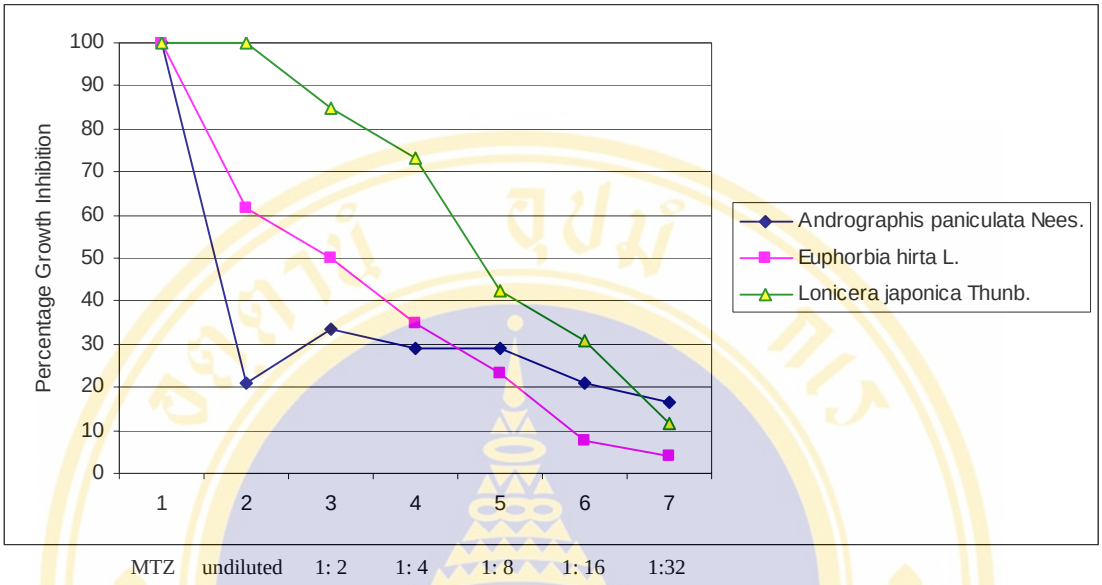


Figure 4.4 Percentage growth inhibition of *Entamoeba moshkovskii* trophozoites LA strain after 24 hours exposure to three medicinal herbs

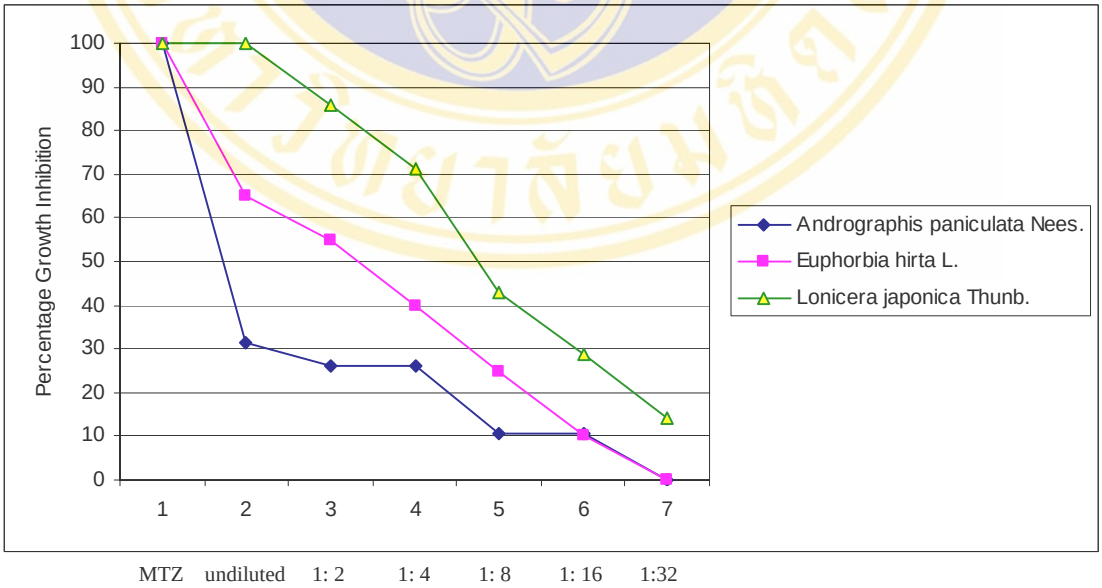


Figure 4.5 Percentage growth inhibition of *Entamoeba histolytica* trophozoites AP strain after 24 hours exposure to three medicinal herbs

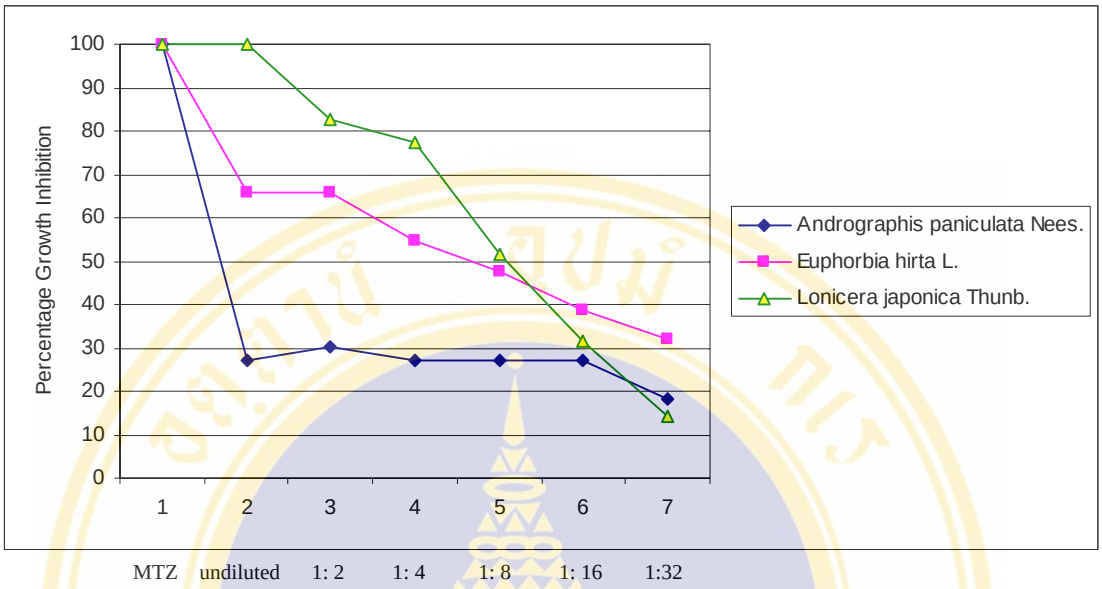


Figure 4.6 Percentage growth inhibition of *Entamoeba histolytica* trophozoites S strain after 24 hours exposure to three medicinal herbs

The growth of two strains of *E. histolytica* trophozoites (AP and S) and one strain of *E. moshkovskii* trophozoites (LA) were highestly inhibited by undiluted herbal aqueous extract of *L. japonica Thunb.* and lower inhibited by higher dilutions of *E. hirta L.* and *A. paniculata Nee*s. aqueous extracts (**Figures 4.4, 4.5** and **4.6**).

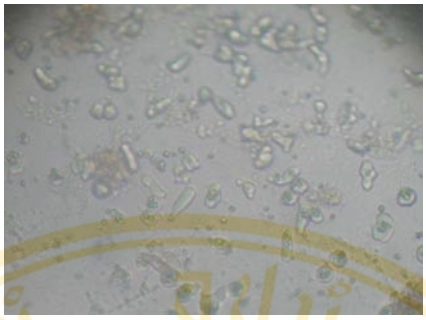


Figure 4.7 Trophozoites of *Entamoeba moshkovskii* LA strain in LE medium

Figure 4.8 Trophozoites of *Entamoeba histolytica* AP strain in LE medium

Figure 4.9 Trophozoites of *Entamoeba histolytica* S strain in LE medium

The growth of trophozoites of *E. moshkovskii* LA strain and *E. histolytica* AP and S strains in LE medium are shown in **Figures 4.7, 4.8** and **4.9**, respectively. They were healthy and active.

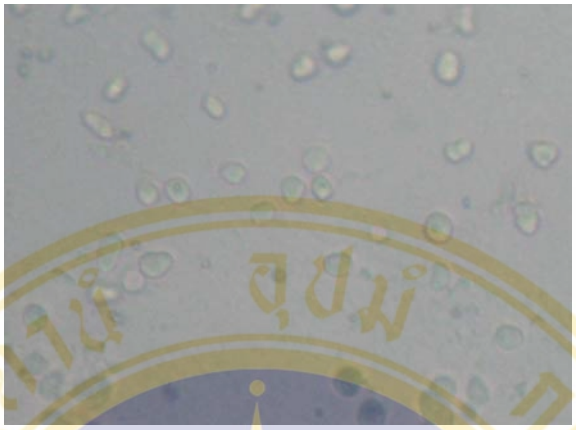


Figure 4.10 Trophozoites of *Entamoeba histolytica* S strain in LE medium After exposure to ice

Trophozoites of *E. histolytica* : S strain in LE medium after expose to ice were detached and rounded up as shown in **Figure 4.10**.

Figure 4.11 Trophozoites of *Entamoeba histolytica* S strain in LE medium after 24 hours exposure to MTZ (M.I.C.= 10 micrograms per milliliter)

Loss of movement and discharge of cytoplasmic components were observed in trophozoites of *E. histolytica* S strain in LE medium after 24 hours exposure to MTZ (M.I.C.= 10 micrograms per milliliter) (**Figure 4.11**).

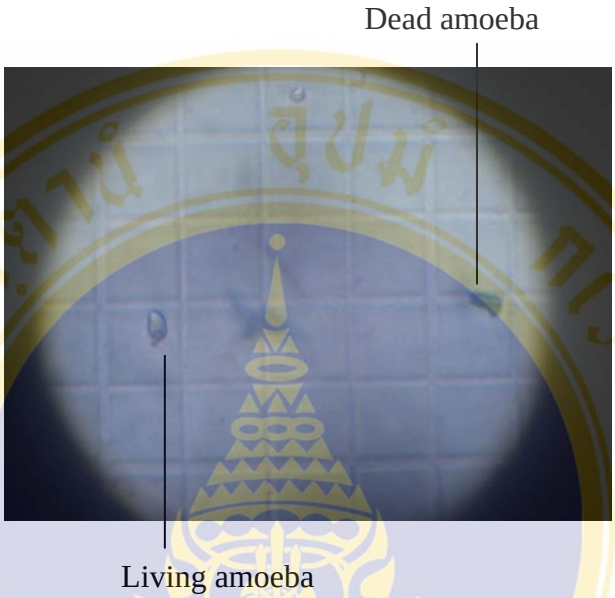


Figure 4.12 Trophozoites of *E. histolytica* AP strain after staining with trypan blue dye

Dead trophozoite of *E. histolytica* : AP strain was non-motile and took on blue stain of trypan blue. The living trophozoite was not pick up the blue stain of trypan blue (**Figure 4.12**).

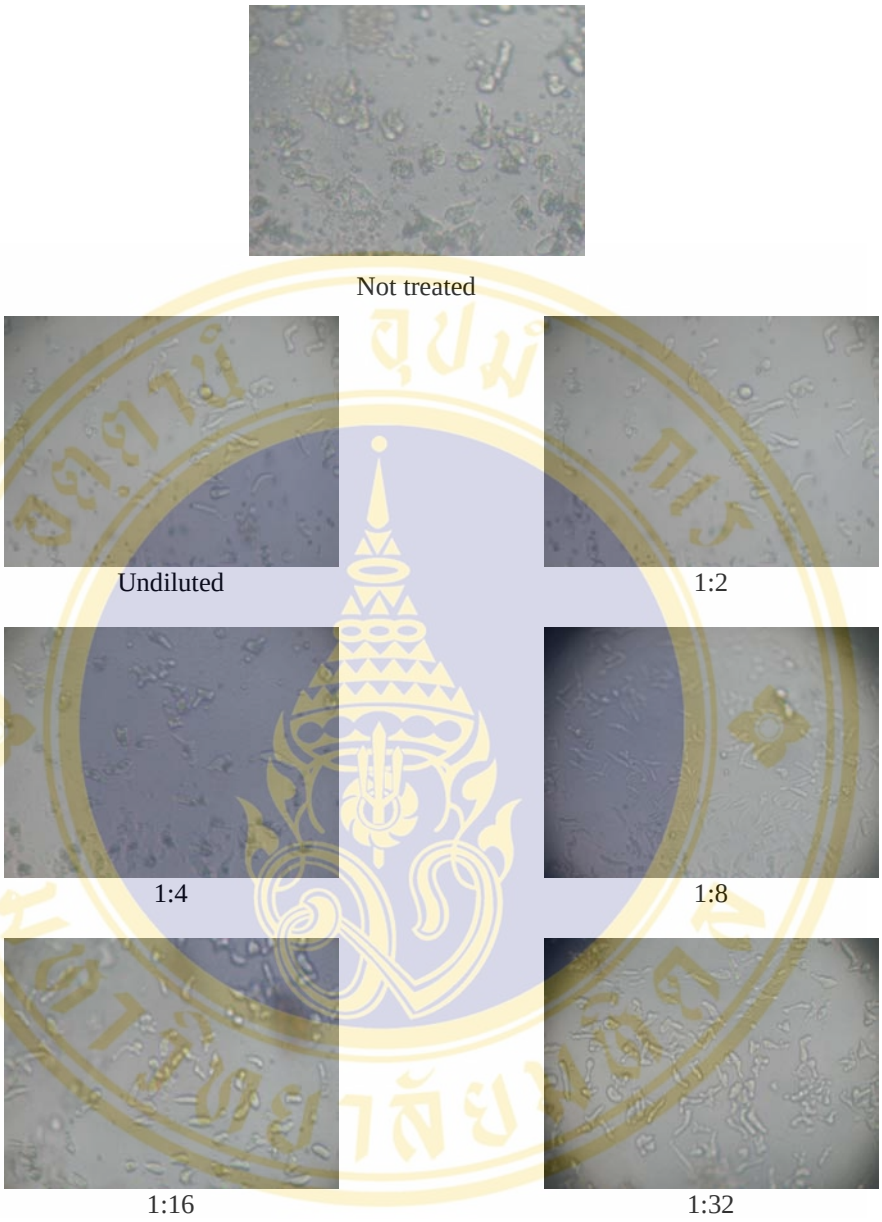


Figure 4.13 Trophozoites of *Entamoeba moshkovskii* LA strain in LE medium after 24 hours exposure to each concentration of *Andrographis paniculate Nees.* (whole plant) aqueous extract

Undiluted aqueous extract of *A. paniculata Nees.* (whole plant) exhibited 20.83 percentage of growth inhibition on throphozoites of *E. moshkovskii* LA strain 31.58 and 27.27 percentage of growth inhibition on trophozoites of *E.histolytica* AP and S strains, respectively (**Figure 4.13, Table 4.2**). When they were more diluted, their effects were decreased substantially.

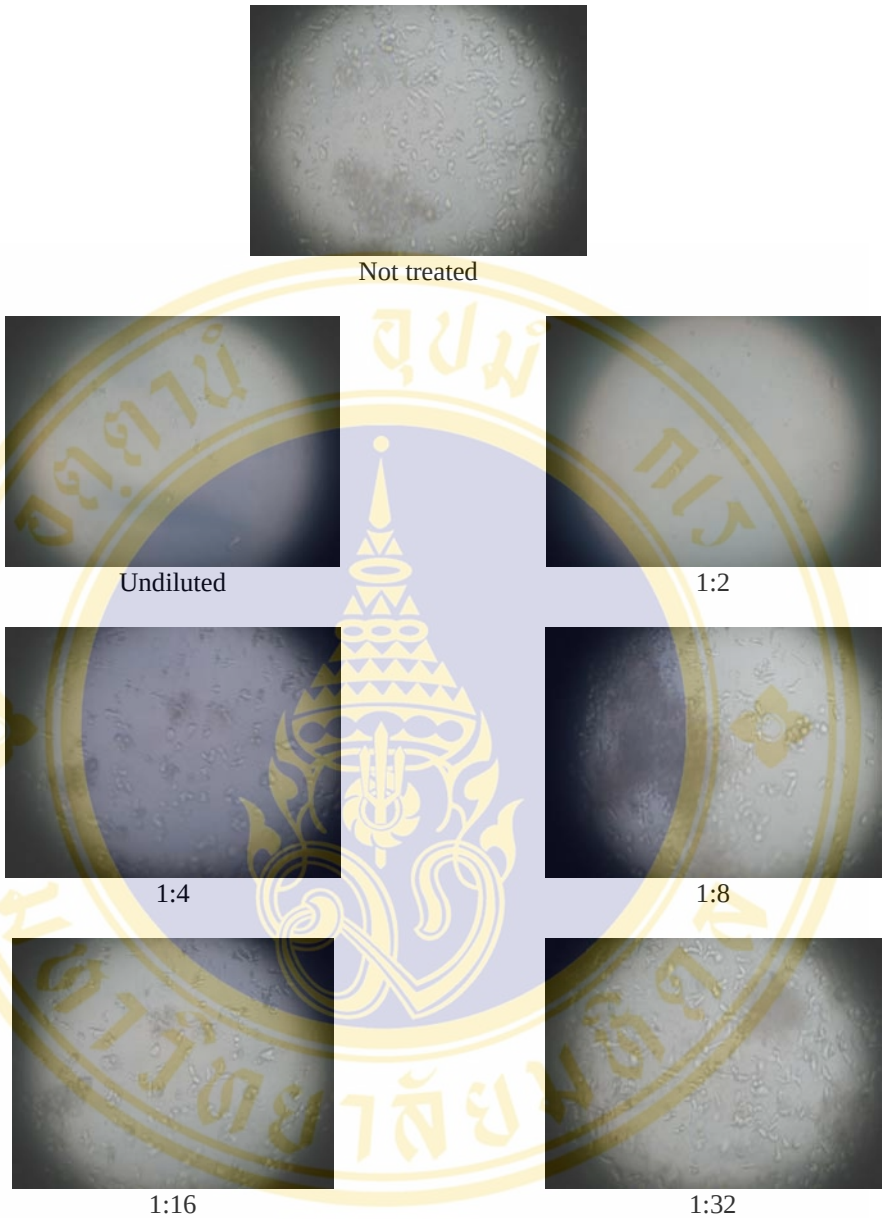


Figure 4.14 Trophozoites of *Entamoeba histolytica* S strain in LE medium after 24 hours exposure to each concentration of *Euphorbia hirta L.* (whole plant) aqueous extract.

Undiluted aqueous extract of *E. hirta L.* (whole plant) exhibited 65.91 percentage of growth inhibition on throphozoites of *E. histolytica* S strain 61.53 and 65 percentage of growth inhibition on trophozoites of *E.histolytica* LA and AP strains, respectively (**Figure 4.14, Table 4.2**). When they were more diluted, their effects were decreased substantially.

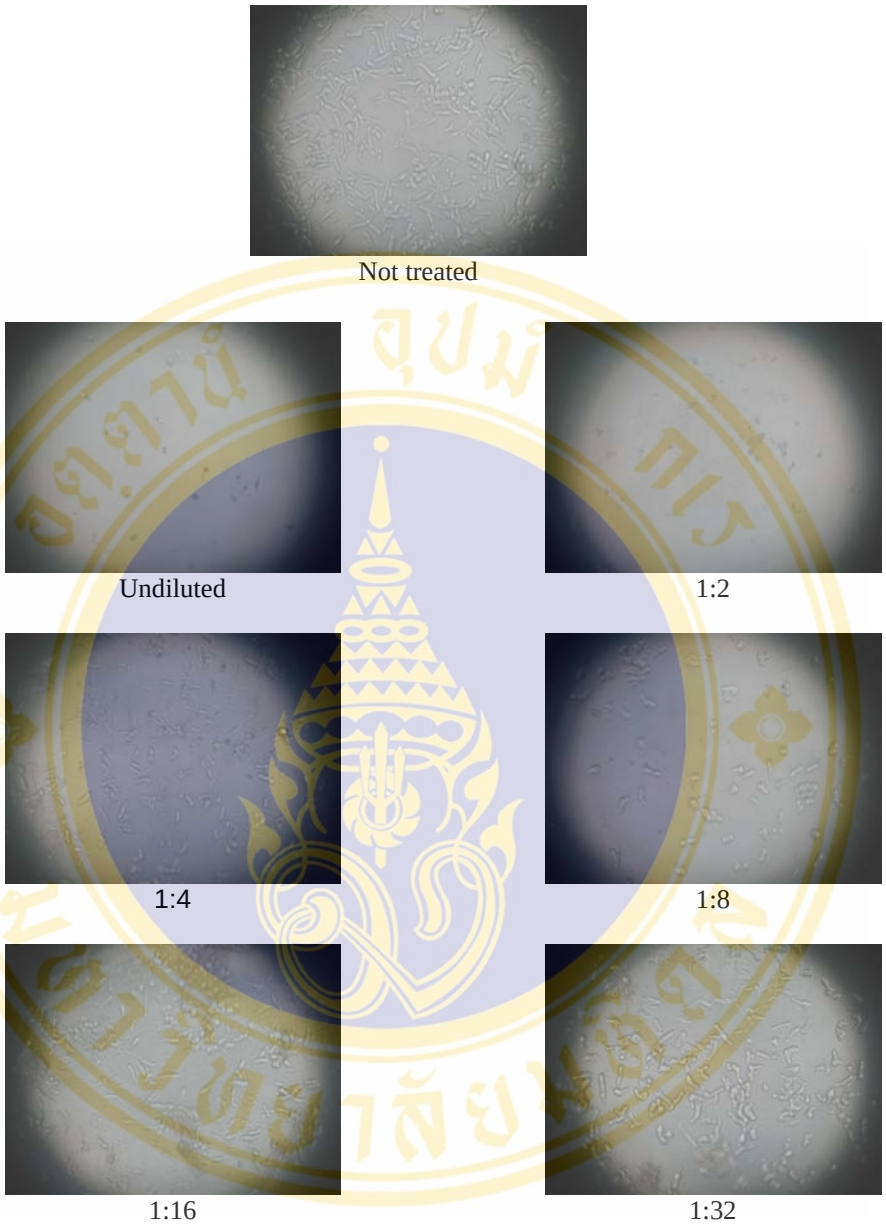


Figure 4.15 Trophozoites of *Entamoeba histolytica* strain AP in LE medium after 24 hours exposure to each concentration of *Lonicera japonica Thunb.* (flowers and leaves) aqueous extract

Undiluted aqueous extract of *L. japonica Thunb.* (flowers and leaves) killed trophozoites of two strains of *E. histolytica* trophozoites (AP and S) and one strain of *E. moshkovskii* trophozoites more than those of more diluted herb (LA) (**Figure 4.15**).

L. japonica Thunb. (flowers and leaves) gave the best amoebicidal effects among the three herbs used in this study.

CHAPTER V
DISCUSSION

This study aimed to assess the in vitro susceptibility of xenic cultures of *E. histolytica* and *E. moshkovskii* to aqueous extracts of *A. paniculata Nees*. (whole plant), *E. hirta L.* (whole plant) and *L. japonica Thunb*. (flowers and leaves). The main factors effective to the growth inhibition of *E. histolytica* were dependent on kinds and concentration of aqueous extract of herb solution was mixed with culture media.

In vitro methods decribed for testing of unknown drugs against *E. histolytica* grown in diphasic media, egg slant made this system unreliable because it was known that there was absorbtion of drug at the interface. This was not the case in axexic culture susceptibility test.

This study was carried out using *E. histolytica* grown with bacterial associates and can be regarded as giving completely picture of what occurs under natural conditions of the small intestines. The role of bacteria associated with amoebae is to maintain anaerobic conditions for survival and growth of amobae. It is difficult to assess accurately whether the drug has direct reaction on the amoebae or indirectly inhibit the bacterial associates which are important for their growth. The amoebicidal effect will be varied by the culture used for testing the drug sensitivity, by pH and redox potential before and after inoculation, by quantity and type of starch used, by the temperature and length of inoculation, etc. The factor of in-vitro culture which should be monitored is the pH value. Culture media used for growing *E. histolytica* in association with bacteria are usually buffered to about pH 7.0 to 7.2 (72).

Andrographis paniculata Nees. is famous used in Thailand as treatment of dysentsry symptom. The chemical constituent is terpenoid that has antidiarrheal activity, antibacterial and antihepatotoxic activity ,etc. At present there is no report of effect of the *A. paniculata Nees.* to *E. histolytica* in both vitro and vivo studies.

In this study, aqueous extracts of *A. paniculata Nees.* (whole plant) had low effect on growth inhibition on two strains of *E. histolytica* trophozoites (AP and S) and

one strain of *E. moshkovskii* trophozoites (LA), that undiluted of aqueous extract of *A. paniculata Nees*. (whole plant) showed 20.83 – 31.58 percentage of growth inhibition of three strains of *Entamoeba* trophozoites.

Chemical constituents of *A. paniculata Nees.* are andrographolide and neoandrographolide which sparingly to dissolve in water, therefore, the action of aqueous extract of *A. paniculata Nees.* (whole plant) were not excert in full action, if use other extraction method to purify each chemical constituent, the study will give more reliable results.

E. hirta L. is a kind of weed flora. It is useful remedy in hayfever. It also has a specific action in destroying the *E. histolytica* that causes intestinal amoebic infection (73). The effect of methanol extract against *E. histolytica* in xenic culture, showed 93.19 percentage mortality, but the study did not report specific chemical content for its action. At present there is no report of the effect of *E. hirta L.* extract to *E. histolytica* both in vitro and in vivo studies.

In this study, the aqueous extract of *E. hirta L.* (whole plant) showed medium effect to inhibit growth of two strains of *E. histolytica* trophozoites (AP and S) and one strain of *E. moshkovskii* trophozoites (LA), compared to undiluted aqueous extract of *E. hirta L.* (whole plant) which were 65.91 – 61.93 percentage of growth inhibition of three strains of *Entamoeba* trophozoites.

The results showed that the aqueous extract of *E. hirta L.* (whole plant) could not destroy *E. histolytica* in which the chemical contents of *E. hirta L.* were less dissolved in water but highly dissolved in methanol. The different extraction solution used may invole different killing and inhibition effect on *Entamoeba* trophozoites.

Lonicera japonica Thunb. is a kind of decorative plant and have fragrance. It is very famous in China for detoxicity but in Thailand, is used for both detoxicity and treatment of diarrheoa. There is no report concerning the effect of *L. japonica Thunb*. on *Entamoeba* trophozoites both in vitro and in vivo studies.

In conclusion, The undilute aqueous extract of *L. japonica Thunb.*(flowers and leavse) showed 100 percentage of growth inhibition of two strains of *E. histolytica* trophozoites (AP and S) and one strain of *E. moshkovskii* trophozoites (LA), that it destroyed *E. histolytica.* It would be great to study the chemical

constituents of *L. japonica Thunb*. to see what is the potent ingredient usable for treatment of intestinal amoebiasis in the future.

The traditional medicine has a crucial role played in the development of novel amoebicides. Several hundreds of herbs and herbal extracts are used in developing countries, such as Thailand, China and India, without regarding on therapeutic, phytochemical and toxicological evaluation. It is expedient to direct research efforts towards the development of novel remedies from traditional drugs. This is particularly essential because of the problems encountered by modern chemotherapy such as drug resistance, toxicity and high costs.

CHAPTER VI
CONCLUSION

A crude drug formulation composed of water extracts of three medicinal herbs *(Andrographis paniculata Nees.* (whole plant), *Euphorbia hirta L*. (whole plant), *Lonicera japonica Thunb.* (flowers and leave). The *Lonicera japonica Thunb.* (flowers and leave) showed the best antiamoebic activity in vitro. The drug formulation also had varying degree of inhibition on xenic *Entamoeba histolytica*. Based on these observations, a detail therapeutic, toxicological and phytochemical evaluation of the crude drug formulation is suggested.

REFERENCES

1. Stanley SL Jr, Reed SL. Microbes and microbial toxin:paradigms for microbial mucousal Interaction. VI. *Entamoeba histolytica*:parasite-host interactions. Am J Physiol Gastrointest Liver Physiol 2001;280:G1049-G1054.
2. Petri WA Jr. Recent advances in amebiasis. Crit Rev Clin Lab Sci 1996;33:1-37.
3. World Health Organisation. Amoebiasis. WHO Wkly Epidemiol Rec 1977;72: 97-100.
4. Towson SM, Borehman PFI, Upcroft P, Upcroft JA. Resistance to the nitroheterocyclic drugs. Acta Trop 1994;56:173-194.
5. Bhopale KK, Pradhan KH, Masani KB, Kaul CL. Additive effect of diloxanide fuorate and metronidazole (EtamizoleR) in mouse caecal amoebiasis. Indian J Exp Biol 1995;33:73-74.
6. Tierney LM, Mcphee JS, Papadaksis MA. Current medical diagnosis and treatment 38th ed., Stadford, Conn. 1999:100.
7. Goodmann A, Rall TW, Nies AS, Taylor P. The pharmacological bases of therapeutics. 8th ed. New York. Pergamon Press, Inc. 1991:1002.
8. Bharti N. Synthetic and naturally occurring biologically active compounds. Ph.D. Thesis, Chemistry. Jamia Millia Islamia A Central University India 2002.
9. Youvraj RS, Kaimal P, Bhatt RM. The antiamoebic effect of a crude drug formulation of herbal extract against *Entamoeba histolytica* in vitro and in vivo. J Ethnopharmacol 1995;45:43-52.
10. Cruz-Vega DE, Aguilar A, Castro-Garza J, Gonzalez-Garze T. Antiamebic activity of aqueous extract from five medicinal plants from Northeastern Mexico. Arch Med Res 2000;31:S17-S18.
11. Ghoshal S, Krishma Prasad BN, Laksmi V. Antiamoebic activity of *Piper longum* fruits against *Entamoeba histolytica* in vitro and in vivo. J Ethnopharmacol 1996;50:166-170.
12. DiMiceli L. Distinguishing between pathogenic and non pathogenic species of *Entamoeba*. Lab Med 2004;35:613-616.

13. Centers for Disease Control and Prevention. Amoebiasis infection 1999;www.cdc . gov/ncidop/amoebias.htm.
14. Boeck WC, Drbohlav J. The cultivation of *Endamoeba histolytica*. Am J Hyg 1925;5:371-407.
15. Dobell C, Laidlaw PP. On the cultivation of *Entamoeba histolytica* and some other entozoic amoebae. Parasitology 1926;18:283-318.
16. Diamond LS. Lumen dwelling protozoa: *Entamoeba,* trichomonads and *Giardia,* p. 67-109. *In* J. B. Jensen (ed.), In vitro cultivation of protozoan parasites. CRC Press, Boca Raton, Fla. 1983.
17. Balamuth W. Improved egg yolk medium for cultivation of *Entamoeba histolytica* and other intestinal protozoa. Am J Clin Pathol 1946;16:380-384.
18. Jones WR. The experimental infection of rats with *Entamoeba histolytica*; with a method for evaluating the anti-amoebic properties of new compounds. Ann Trop Med Parasitol 1946;40:130-140.
19. Diamond LS. A new liquid medium for xenic cultivation of *Entamoeba histolytica* and other lumen dwelling protozoa. J Parasitol 1982;68:958-959.
20. Robinson GL. The laboratory diagnosis of human parasitic amoebae. Trans R Soc Trop Med Hyg 1968;62:285-294.
21. Cleveland LR, Sanders EP. The production of bacteria-free amoebic abscesses in the liver of cats and observations on the amoebae in various media with and without bacteria. Science 1930;77:149-151.
22. Reeves RE, Meleney HE, and Frye WW. A modified Shaffer-Frye technique for the cultivation of *Entamoeba histolytica* and some observations on its carbohydrate requirements. Am J Hyg 1957;66:56-62.
23. Phillips BP. Cultivation of *Endamoeba histolytica* with *Trypanosoma cruzi*. Science 1950;111:8-9.
24. Diamond LS. Improved method for the monoxenic cultivation of *Entamoeba histolytica* Schaudinn, 1903 and *E. histolytica*-like amebae with trypanosomatids. J Parasitol 1968;54:715-719.
25. Diamond LS. Techniques of axenic cultivation of *Entamoeba histolytica* Schaudinn, 1903 and *E. histolytica*-like amebae. J Parasitol 1968;54:1047-1056.

26. Diamond LS. Axenic cultivation of *Entamoeba histolytica.* Science 1961;134:336-337.

27. Diamond LS, Harlow DR, Cunnick CC. A new medium for the axenic cultivation of *Entamoeba histolytica* and other *Entamoeba*. Trans R Soc Trop Med Hyg 1978;72:431-432.

28. Gllin FD, Diamond LS. Clonal growth of *Entamoeba histolytica* and other species of *Entamoeba* in agar. J Protozool 1978;25:539.

29. Thammapalerd N, Tharavanij S, Wattanagoon Y. Axenic cultivation of *Entamoeba histolytica* from liver abscess and its zymodeme.Southeast Asian J Trop Med Public Health 1993;24:480-483.

30. Diamond LS, Clark CG, Cunnick CC. YI-S, a casein-free medium for axenic cultivation of *Entamoeba histolytica*, related *Entamoeba*, *Giardia intestinalis* and *Trichomonas vaginalis*. J Eukaryot Microbiol 1995; 42:277-278.

31. Said-Fernandez S, Vargas-Villarreal J, Castro-Garza J, Mata-Cardenas BD, Navarro-Marmolejo L, Lozano-Garza G, Martinez-Rodriguez H. PEHPS medium: an alternative for axenic cultivation of *Entamoeba histolytica* and *E. invadens*. Trans R Soc Trop Med Hyg 1988;82:249-253.

32. Gillin FD, Diamond LS. Clonal growth of *Entamoeba histolytica* and other species of *Entamoeba* in agar. J Protozool 1978; 25:539-543.

33. Mueller DE, Petri WA Jr. Clonal growth in Petri dishes of Entamoeba histolytica. Trans R Soc Trop Med Hyg 1995;89:123.

34. Farri TA. A simple technique for preparing clone cultures of Entamoeba histolytica. Trans R Soc Trop Med Hyg 1978;72:205-206.

35. Diamond LS, Mattern CFT, Bartgis IL, Daniel WA, Keister DB. Viruses of *Entamoeba histolytica.* VI. A study of host range, In B. Sepúlveda and Diamond LS (ed.), Proceedings of the International Conference on Amebiasis. Instituto Mexicano de Seguro Social, Mexico City, Mexico 1976; p334-345.

36. Diamond LS, Clark CG. A redescription of *Entamoeba histolytica* Schaudinn, 1903 (Emended Walker, 1911) separating it from *Entamoeba dispar* Brumpt, 1925. J Eukaryot Microbiol 1993; 40:340-344.

37. Clark CG. Axenic cultivation of *Entamoeba dispar* Brumpt 1925. *Entamoeba insolita* Geiman and Wichterman 1937 and *Entamoeba ranarum* Grassi 1879. J Eukaryot Microbiol 1995;42:590-593.
38. Kobayashi S, Imai E, Tachibana H, Fujiwara T, Takeuchi T. *Entamoeba dispar*: cultivation with sterilized *Crithidia fasciculata*. J Eukaryot Microbiol 1998;45:3S-8S.
39. ประเสริฐ พรหมณี และคณะ. สมาคมแพทย์แผนโบราณ วัดมหาธาตุ ตำราเภสัช กรรมไทย แผนโบราณ. กรุงเทพฯ: โรงพิมพ์จุฬาลงกรณราชวิทยาลัย วัดมหาธาตุ:2531.
40. โรงเรียนแพทย์แผนโบราณ วัดพระเชตุพนวิมลมังคลารามราชวรมหาวิหาร ตำราประมวลหลักเภสัช. กรุงเทพฯ : โรงพิมพ์สีไทย :2538.
41. Nadkarni AK, Nadkarni KM. Indian Material Medica. Bombay Popular Prakashan 1976;1:101-103.
42. Bensky D, Gamble A, Kaptchuk T. Chinese herbal medicine material medica, rev ed. Seattle : Eastland Press 1993:95.
43. www.vitaminherbuniversity.com.
44. Hu CQ, Zhou BN. Isolation and structure of two new diterpenoid glucosides from *Andrographis paniculata Nees.* Yao Xue Xue Bao 1982;17:435-440.
45. Bone K. The story of *Andrographis paniculata*, a new “immune system” herb. Nutrition and Healing 1998;22:3 ,4, 8, 9 (Review).
46. Leelarasamee A, Trakulsomboom S, Sittisomwong N. Undetectable anti-bacterial activity of *Andrographis paniculata* (Burma) Wall.ex ness. J Med Assoc Thai 1990;73:299-304.
47. Thanagkul B, Chaichantipayut C. Double-blind study of *Andrographis paniculata Nees.* and tetracycline in acute diarrhea and bacillary dysentery. Ramathibodi Med J 1985;8:57-61.
48. Chatuvedi GN, Tomar GS, Tiwari SK, Singh KP. Clinical studies on Kalmegh (*Andrographis paniculata*) in infective hepatitis. J Int Inst Ayurveda 1983;2:208-211.
49. www.ars-grin.gov.
50. Caceras DD, Hancke JL, Burgos RA, Sandberg F, Wikman GK. Prevention of common colds with dried extract. A pilot double blin trial. Phytomedicine 1997;4:101-104.

51. Thamlikitkul V, Dechtiwongse T, Theerapong S, Chantrakul C, Boonroj P, Punkrut W, Eupalakorn W, Boontaeng N, Taechaija S, Petchroen S, et al. Efficacy of *Andrographolide paniculata, Nees* for pharyngotonsillitis in adults. J Med Assoc Thai 1991;74:437-442.
52. Caceras DD, Hancke JL, Burgos RA, Sandberg F, Wikman GK. Use of visual analogue scale measurement (VAS) to asses the effectives of standardized *Andrographis paniculata* extract SHA-10 in reducing the symptoms of common cold. A randomized double blind-placebo study. Phytomedicine 1999;6:217-223.
53. www.altcancer.com.
54. www.cybermango.net.
55. www.ibbilo.org.
56. www.herbs 2000.com.
57. www.innvista.com.
58. www.earthnotes.tripod.com.
59. Galvez J, Zarzuelo A, Crespo ME, Loente MD, Ocete MA, Jimenez J. Antidiarrhoeic activity of *Euphorbia hirta* extract and isolation of an active flavonoid constituent. Planta Med 1993;59:333-336.
60. Australian Naturopathic Network.www.ann.com.
61. Dhar ML, Dhar MM, Dhawan BN, Mehrotra BN, Ray C. Screening of Indian plants for biological activity:part I. Indian Exp Biol 1968;6:232-247.
62. Rao CVK, Ganapaty S. Investigation on *Euphorbia pilulifera L.*. Fitoterapia 1983;54:265-267.
63. Ester TS. *Euphorbia hirta* extracts as immuno-stimulants. Patent:Ger often-4 102, 054 1992:5pp.
64. Lanhers MC, Fleurentin J, Dorfman P, Mortier F, Plet JM. Analgesic, antipyretic and anti-inflammatory properties of *Euphorbia hirta*. Planta Med 1991;57:225-231.
65. Hiermann A, Bucar F. Influence of some traditional medicinal plants of Senegal on prostaglandin biosynthesis. J Ethnopharmacol 1994;42:111-116.
66. ดวงรัตน์ เชี่ยวชาญวิทย์. น้ำนมราชสีห์. ชีวจิต 2547;6:41.
67. www.health.enotes.com.

68. หนังสือพิมพ์ คม ชัด ลึก : 27 มิถุนายน 2548.
69. www.asiancanaerherb.info.
70. Lekkla A. A Thesis of Master of Science (Public Health), Mahidol University : Growth curve inhibition of *Entamoeba histolytica* after exposure to the extract of *Psidium guajava Linn., Garcinia mangostana Linn.* And *Punica granatum Linn*. 1996.
71. Sawangjaroen N, Phongpaichit S, Subhadhirasakul S, Visutthi M, Srisuwan N, Thammapalerd N. The anti-amoebic activity of some medicinal plants used by AIDS patients in southern Thailand. Parasitol Res 2006;98:588-592.
72. Neal RA. The in vitro cultivation of *Entamoeba histolytica*, in Taylor AER (ed) Problems of in vitro culture. Blackwell Scientific Publications, Oxford :pp. 9-26.
73. Moundipa PF, Kamini G, Flore M, Bilong CFB, Bruchhaus I. In vitro amoebicidal activity of some medicinal plants of the Bamun region (Cameroon). Afr J Trad CAM 2005;2:113-121.

APPENDIX

APPENDIX A

Chemical reagents

Chemical reagents for the preparation of modified Boeck and Drbohlav's diphasic medium

- Fresh hen eggs
- Sodium chloride
- Calcium chloride
- Potassium chloride
- Potassium phosphate, monobasic
- Magnesium chloride
- Sodium phosphate, dibasic
- Sodium bicarbonate
- Distilled water
- Sterile rice starch

APPENDIX B

Modified Boeck and Drbohlav's Diphasic medium

The preparation is as follow :

Two fresh hen's eggs are cleaned with 70 % alcohol and broke carefully in a beaker and emulsify them with 25 ml of Ringer's solution. Filter the emulsion through several thickness of sterilized surgical gauze. Dispense 1 ml amounts in to sterile screw cap test tubes. Slant the tubes at an angle of 30° and coagulate in the hot air oven for 20 minutes at 120 °C. Allow the slant to cool and overlay each slant with approximately 3 ml of sterile Ringer's solution. To demonstrate the sterility, incubate the slant at 37 °C for 24 hours. If the overlay fluid is still clear, then the medium can be stored in the refrigerator for future use. Prior to use, warm this medium at room temperature and add about one loopful of sterile rice power (about 10 – 20 mg) and antibiotic (Pennicillin G Sodium and Streptomycin) 2 drops to each sterile overlayed slant.

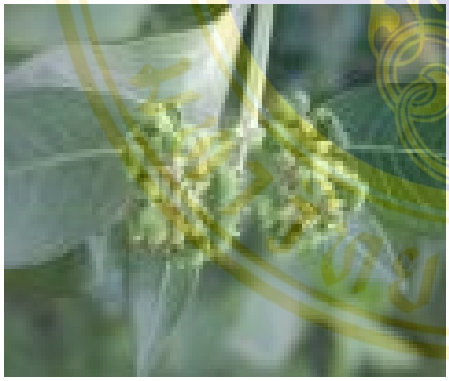
APPENDIX C

Glass ware, plastic ware and instrument

- Test tube with screw caps
- Syringe 1 ml, 5 ml
- Needle No. 18, 2 inches
- Magnetizer
- Pasture pipette
- Pipettes 1 ml, 5 ml and 10 ml
- Volumetric flasks 500 ml and 1,000 ml
- Glass beakers 40 ml, 80 ml, 200 ml, 1,000 ml and 2,000 ml
- Graduated cylinders 1,000 ml
- Bottle with screw caps 100 ml
- Haemocytometer / counting chamber with cover glass
- Millipore filter set
- Whatman membrane filter
- Hot air oven
- Incubator
- Centrifuge
- Inverted microscope
- Refrigerator

APPENDIX D

ANDROGRAPHIS PANICULATA NEES.



EUPHORBIA HIRTA L.

LONICERA JAPONICA THUNB.

BIOGRAPHY

NAME	Miss Jinda Kungvanrattanasin
DATE OF BIRTH	22 October 1974
PLACE OF BIRTH	Bangkok, Thailand
INSTITUTION ATTENTED	Mahidol University, 1993-1996 Bachelor of Nurse Mahidol University, 2000-2006 Master of Science (Public Health) Major Infectious Disease
POSITION AND OFFICE	2 Siriraj Hospital, Bangkok, Thailand Position: Nurse Tel. 0-2419-7939 E-mail:mookjinda@yahoo.com
HOME ADDRESS	3/39 Soi Praram 2 Soi 3, Praram 2 Road, Bangmod, Bangkok 10160