

**DETERMINATION OF ANTI-BACTERIAL ACTIVITY USING
HOUTTUYNIA CORDATA THUNB. ETHANOL EXTRACT**

BY

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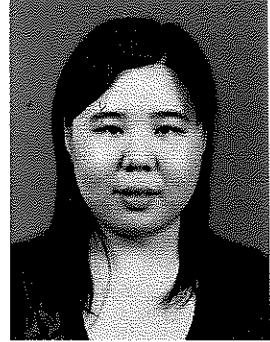
**THESIS SUBMITTED IN FULFILLMENT FOR THE
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DECLARATION

I hereby declare that project report is based on my original work except quotations and citations which have been duly acknowledged. I also declare that it has not been preciously or concurrently submitted for any other degree at Inti International University or other institutions.



A handwritten signature in black ink, consisting of stylized, overlapping loops and lines.

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ABSTRACT

The aim of the study in this project is to assess the anti-bacterial effect of dried *Houttuynia cordata* Thunb. ethanolic extract against *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus*. The extract of medicinal plants were prepared with using conventional method by starting step of pulverized or grounded dried *Houttuynia cordata* Thunb. to coarse powders, then suspended herb in 95% ethanol for 7 days. After that, filtration and evaporation of ethanol, the remaining extracts were oven-dried at 60 °C. For experimental preparation, each extract was re-dissolved in its own solvent to give the desired concentration. Serial dilution of various concentration of *Houttuynia cordata* Thunb. ethanolic extract, 200mg/ml, 100mg/ml, 50 mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml, 3.125mg/ml was prepared. The anti-bacterial activities of *Houttuynia cordata* Thunb. ethanolic extracts were evaluated using the Kirby Bauer Disk Diffusion Method and the inhibitory zones were recorded in millimeters. Control negative, 95% ethanol and control positive, Gentamicin were assessed using Kirby Bauer Disk Diffusion Method. The results of this study showed that various concentration of *Houttuynia cordata* Thunb. ethanolic extracts against *E. coli*, *B. subtilis* and *S. aureus* were showed no anti-bacterial activity. Gentamicin showed zone of inhibition towards *E. coli*, *B. subtilis* and *S. aureus* with zone inhibition of $20.45333 \pm \text{SD } 2.793230$, $19.54333 \pm \text{SD } 0.933293$ and $22.10667 \pm \text{SD } 0.323968$ respectively. Negative result of *Houttuynia cordata* Thunb. ethanolic extract suggest a problem may due to potential factor and limitation.

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LIST OF ABBREVIATIONS

AMR	Anti-microbial Resistance
CA-MRSA	Acquired Methicillin-Resistant <i>Staphylococcus aureus</i>
GPC	Gram-positive cocci
GPO	Government Pharmaceutical Organization
<i>H. cordata</i>	<i>Houttuynia cordata</i> Thunb.
HCP	<i>Houttuynia cordata</i> powder
HEPA	High-Efficiency Particulate Air
HPLC	High Performance Liquid Chromatography
i.e.	That is
IE	Internationale Einheiten
IU	International Unit
MDR	Multidrug-Resistance
MRSA	Methicillin Resistant <i>Staphylococcus aureus</i>
MW	Molecular Weight
ND	Not Detected
SARS	Severe Acute Respiratory Syndrome
SH	Sodium Houttuynfonate
SNH	Sodium New Houttuynfonate
T&CM	Traditional and Complementary Medicines
TCM	Traditional Chinese Medicine
WHO	World Health Organization

LIST OF SYMBOLS

°C	Degree Celcius
°F	Fahrenheit
%	Percentage
<	Less Than
±	Plus Minus
μl	Microliter
μm	Micrometer
cm	Centimeter
g	Gram
g/ml	Gram per Milliliter
kg	Kilograms
mg/ml	Milligram per Milliliter
mg	Milligram
mg/disc	Milligram per Disc
ml	Mililiter
mm	Millimeter
pH	Potential of Hydrogen
SD	Standard Deviation
α	Alpha
β	Beta

CHAPTER 1: INTRODUCTION

Malaysia is a fast developing country with a population about 28 million (Jabatan Perangkaan Malaysia, 2010). Recent research's noted that popular use of Traditional and Complementary Medicines in Malaysia was relatively high as show in Aziz and Tey (Aziz and Tey, 2014). Since ancient time, medical plants have been used in almost all cultures as a source of medicine (Jo, Varghese and Shantaram, 2011). Today, traditional and complementary medicine which use natural anti-microbial (derived from plants) give natural therapeutic aid for various ailments (Jacinta, Farhana and Dayang, 2014). In addition, traditional and complementary medicine has appeared as an important part of modern healthcare despite being practiced before the advent of conventional Western treatments.

Eighty percent of the population in Africa, Asia and Latin America use traditional and complementary medicine every year refer to World Health Organization (WHO) (World Health Organization, Geneva, 2002). There are also nearly half of population from developed countries including United States, Australia, France and Canada use T&CM on a regular basis (Bodeker and Kronenberg, 2002). Studies in Malaysia also proposed that popularity of Traditional and Complementary Medicines use among the elderly is related to its lower cost, lower rate of side effects and better accessibility compared to conventional Western medicines (Silvanathan and Low, 2015).

In fact, general guidelines for methodologies on research and evaluation of traditional medicines of World Health Organize (WHO) has been commented: "Although traditional medicine has been existence and continued to apply over centuries, and it was popularity and widespread used in past decade, but traditional medicines has not yet officially approved in majority of countries. As a result, there does not have enough attention and support in the area such as education, training, research. In order to meet the criteria needed to support its usage worldwide, quantity and quality of the safety and efficacy data on traditional medicine are far from enough. The reasons for an inadequate of research data are due to not only to health

care policies, but also to a lack of appropriated or accepted research methodology for assessing traditional medicine” (WHO, Geneva, 2000).

As WHO’s comment, we need to focus on the lack of study although evidence of the high prevalence of Traditional and Complementary Medicines uses in Malaysia. One of the most important aspects is scholars have to start to see the level of public awareness about safety towards, falsify preparations, herb–drug interactions and side effects of Traditional and Complementary Medicines nowadays (Silvanathan and Low, 2015). Potential of medicinal herbs are investigate and to promote the use of traditional herbal medicine, it is important to intensify the study of medicinal plants that find place in folklore (Nair and Chandra, 2006). In this research, *Houttuynia cordata* Thunb. (*Saururaceae*) is selected to study the anti-bacterial activity of their different concentration of ethanolic extracts against bacterial such as *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*.

According to *Florae Republicae Popularis Sinicae* and *Flora Sichuanica*, *Houttuynia* Thunb. had only one species *Houttuynia cordata* Thunb. (Lu et al., 2006). *Houttuynia cordata* Thunb. is a perennial herbaceous plant and its common names called *Dokudami* (Japanese), *Houttuynia* (English), *Vap Ca* (Vietnamese), *Yu Xing Cao* (鱼腥草 China), *E-Sung-Cho* (Korea) and *Pak Kan Thong* (Thailand). Organism type of *H. cordata* is shrub. *H. cordata* recorded as a folk medicine to cure cough, leucorrhea, nephrotic syndromes, ureteritis, and lung abscesses (Yoon and Shim, 2015). On the one hand, the anti-bacterial activity of *H. cordata* is reported by researcher such as Fu et al., Lu et al. and Yang et al. (Fu et al., 2013; Lu et al., 2006; Yang et al., 2014). There are also survey of the literatures shows *H. cordata* possesses a variety of pharmacological activities including anti-viral (Leardkamolkarn et al., 2011; Choi et al., 2009), immunomodulatory (Sathakarn et al., 2015 ; Lee et al., 2008), anti-SARS (Severe acute respiratory syndrome) activities (Lau et al., 2008), anti-tumor (Zhou et al., 2012;), anti-inflammatory (Lu et al., 2005; Choi et al., 2010; Li et al., 2010; Xu et al., 2015) and anti-oxidative effects (Nuengchamngong et al., 2009), Chemical components of *H.*

cordata including six major types such as volatile oils, flavonoids, alkaloids, fatty acids, sterols and polyphenolic acids (Nuengchamnong et al., 2009).

1.1 Research Objectives

The purpose of this study are:

- I. To determine anti-bacterial activity of *Houttuynia cordata* Thunb. ethanol extract
- II. To identify the different concentration effect of *Houttuynia cordata* Thunb. ethanol extract against Gram- positive *Staphylococcus aureus*, *Bacillus subtilis* and Gram- negative *Escherichia coli*
- III. To measure zone inhibition of Gram- positive *Staphylococcus aureus*, *Bacillus subtilis* and Gram- negative *Escherichia coli*.

1.2 Research Hypothesis

1.2.1 Null Hypothesis

- I. *Houttuynia cordata* Thunb. 200 mg/ml ethanol extracts shows no inhibition against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*.
- II. *Houttuynia cordata* Thunb. 100 mg/ml ethanol extracts shows no inhibition against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*.
- III. *Houttuynia cordata* Thunb. 50 mg/ml ethanol extracts shows no inhibition against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*.
- IV. *Houttuynia cordata* Thunb. 25 mg/ml ethanol extracts shows no inhibition against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*.
- V. *Houttuynia cordata* Thunb. 12.5 mg/ml ethanol extracts shows no inhibition against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*.
- VI. *Houttuynia cordata* Thunb. 6.25 mg/ml ethanol extracts shows no inhibition against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*.