EVALUATION OF TOXIC HEAVY METALS CONTENT IN TRADITIONAL MEDICINE FROM EAST COAST REGION, MALAYSIA

BY

REEM SAADI KHALID

A thesis submitted in fulfilment of the requirement for the degree of Master of Pharmaceutical Chemistry

Kulliyyah of Pharmacy

International Islamic University
Malaysia

JUNE 2013

ABSTRACT

Traditional medicine (TM) is the oldest form of health care style known to humanity; it has been used in different cultures throughout the history. According to the World Health Organization (WHO) reports, more than 70% of the world population use TM. The broad use of TM is often attributed to the accessibility, affordability and availability of such products to the majority of the world's population. Asians are well-known for their reliance on TM. Malaysia has a long tradition and rich legacy from three main cultural groups (Malay, Chinese and Indian) of using TM, a large section of the population in this country is depending on TM for their healthcare needs. The huge demand for TM in Malaysia has significantly increased the Malaysian TM market from US$ 385million in 2000 to US$ 1.29 billion in 2005. Due to the global wide diffusion of TM the safety, efficacy and quality control of such products became significant concern from various health institutes. Presence of toxic substances such as heavy metals is often reported in TM products. This study has been initiated with a prime focus of detecting the amount of heavy metals namely arsenic (As), cadmium (Cd), lead (Pb), nickel (Ni), zinc (Zn) and iron (Fe) in locally available traditional medicines both registered and unregistered medicinal products in various dosage forms from the East Coast region of Malaysia. The determination of Zn and Fe were conducted using Flame Atomic Absorption Spectrometer (FAAS), while Pb, Cd and Ni analysis were conducted using Graphite Furnace Atomic Absorption Spectrometer (GFAAS) and As detection was performed with Hydride Generation Atomic Absorption Spectrometer (HGAAS).TM samples were collected from three states of Malaysia namely Pahang, Terengganu and Kelantan. Total of sixty TM samples from various dosage forms such as capsule (50%), pill (25%), powder (21.6%) and tablet (3.4%) were analysed to determine the content of heavy metal using AAS. Three different acid digestion methods were compared to optimize the best sample preparation technique for analyzing TM samples. They were nitric –perchloric acid digestion (Method-A) nitric acid digestion (Method-B) and hydrochloric –nitric acid (Method-C) digestion respectively. It was found that Method-C showed the highest recovery compared to the other two methods (Method-A and Method-B) and the difference was found statistically significant ($p < 0.05$). Method validation was performed using QC standard sample, spiked TM samples and standard reference material (SRM). It was found that the limit of detection (LOD) for As, Cd, Pb, Ni, Zn and Fe were 0.11ppb, 0.1ppb, 1.17 ppb, 2.01 ppb, 0.01 ppm and 0.09 ppm respectively. Limit of quantification (LOQ) were 1.1 ppb for As, 1ppb for Cd, 11.7 ppb for Pb, 20.1 ppb for Ni, 0.1 ppm for Zn and 0.9 ppm for Fe. The recovery percentages for QC samples were ranged from 95.12- 102.4 which reflects the accuracy of the method. While the relative standard deviation (RSD) that represents the precision of the method for QC samples were in the range of 3.23-0.2. For spiked TM samples the recovery range and the RSD range were 95-105 and 0.11-5.0 respectively. All the validation results were within the specification limit of American Organization of Analytical Chemistry (AOAC) guideline. The accuracy of the method was further checked by the analysis of SRM. The recovery percentages of all metals were in the range of 94.5-108. Among the sixty TM samples it was found that As, Cd, Pb, Ni, Zn and Fe were present in 43%, 81%, 90%, 100%, 100% and 93% of the total samples with a concentrations range of 0.214-1.325, 0.1-1.23, 1.2-19.3, 2.01-36.3, 13.2-391 and 103.3- 1484.7 µg/g respectively. The results further revealed the fact that 36 % of samples contain Cd higher than the permissible limit and 10% of the samples were found having Pb above the permissible limit set by NPCB.

خلاصة البحث

الطب التقليدي يعتبر من اقدم أشكال الرعاية الصحية التي عرفتها البشرية و الذي تم استخدامه من قبل شعوب مختلفة على مدى العصور التاريخية. وفقا لتقارير منظمة الصحة العالمية فان اكثر من 70% من سكان العالم يستخدمون الطب التقليدي. ان الانتشار الواسع لهذا النوع من الادوية غالبا ما تعود اسبابه الى توفرها وكذلك القدرة على تحمل تكاليفها من قبل غالبية سكان العالم. الاسيويين عرفوا باعتمادهم الكبير على الطب التقليدي. ماليزيا لها تراث غني و تقليد عريق متوارث من ثلاث مجموعات ثقافية رئيسية (الملايو و الصينية و الهندية) في استعمال الطب التقليدي حيث ان شريحة واسعة من السكان يعتمدون عليه في تلبية احتياجات الرعاية الصحية لديهم. ان الطلب الكبير على هذه الادوية ادى الى زيادة ملحوظة في حجم السوق الماليزي لهذه المنتجات و الذي ارتفع من 385 مليون دولار امريكي في العام 2000و صولا الى 1.2بليون دولار امريكي في العام 2005. نظرا للانتشار العالمي الواسع لهذه الادوية فقد اصبحت سلامة و فعالية وجودة هذه المنتجات يشكل اهتماما كبيرا من قبل مختلف المؤسسات الصحية. وجود المواد السامة كالمعادن الثقيلة في مثل هذه المنتجات غالبا ما يرد ذكره. شرعت هذه الدراسة بهدف رئيسي وهو الكشف عن كمية المعادن الثقيلة و هي الزرنيخ و الكادميوم والرصاص و النيكل و الزنك والحديد في الادوية و المنتجات الطبية التقليدية المسجلة و غير المسجلة و المتاحة في الاسواق المحلية لمناطق الساحل الشرقي لماليزيا. و اجري تحليل العينات للكشف عن المعادن الثقيلة باستخدام مطياف الامتصاص الذري حيث تم الكشف عن كل من الزنك و الحديد بواسطة تقنية لهب مطياف الامتصاص الذري بينما تم الكشف عن الكادميوم و الرصاص و النيكل بواسطة فرن الكرافيت مطياف الامتصاص الذري اما الزرنيخ فقد تم تحليليه بطريقة تكوين الهيدريد مطياف الامتصاص الذري. جمعت عينات الادوية التقليدية من ثلاثة ولايات و هي تحديدا بهانج و ترنجانو و كلانتان. المجموع الكلي للعينات الدوائية كان ستين عينة في اشكال دوائية مختلفة الكبسول كان يشكل 50% و25% الحبوب الدائرية الشكل و 21.6% المسحوق و 3.4% فقط للاقراص. في هذه الدراسة تمت مقارنة ثلاثة طرق مختلفة لتحضير العينات بواسطة التحلل الحامضي وذلك بهدف ايجاد الطريقة الافضل و التي تم اختيارها على اساس اعطائها التركيز الاعلى للعنصر. و تحديدا كانت هذه الطرق هي الاولى عبارة عن التحلل بواسطة حامض النتريك-البيركلوريك و الثانية التحلل بطريقة حامض النتريك فقط والثالثة هي التحلل بمزيج من حامض الهيدروكلوريك و النتريك بنسبة 1:3.اثبت النتائج ان الطريقة الثالثة كانت الافضل حيث اعطت التراكيز الاعلى للعناصر كافة و قد وجدت الفروقات واضحة بدلالة النتائج الاحصائية. تم التحقق من صحة ودقة الطريقة التحليلية و ذلك باستخدام العينات القياسية و العينات الدوائية وكذلك المواد النموذجية القياسية. و قد وجد ان حد الكشف الادنى لكل من الزرنيخ و الكادميوم و الرصاص والنيكل 0.11, 0.1, 1.17, 2.01 جزء في البليون على التوالي بينما كان لكل من الزنك والحديد 0.09.0.01جزء في المليون على التوالي. اما الحد الادنى من القياس الكمي فقد وجد انه 1.17, 1.0 , 11.7 , 20.1 جزء في البليون لكل من الزرنيخ,الكادميوم,الرصاص و النيكل و0.9.0.1 جزء في المليون لكل من الزنك و الحديد على التوالي.التحقق من صحة النتائج تم عن طريق ايجاد نسب التراكيزالتي تم الحصول عليها للعينات القياسية المحضرة مع التراكيز الاصلية و التي تراوحت ما بين 95.12-102.4 %.اما التحقق من دقة النتائج فقد استدل عليه من قيم الانحراف المعياري النسبي و الذي تراوح ما بين 0.2-3.2 %. النتائج التي تم الحصول عليها عكست صحة و دقة الطريقة التحليلية. هذا و قد تم استخدام العينات الدوائية كخطوة اضافية للتاكد من صحة و دقة الطريقة الحليلية حيث تم اضافة تراكيزمعينة من كل معدن الى عينات مختلفة و من ثم تم تحليلها لاجل معرفة مدى صحة و دقة النتائج و ذلك بمقارنتها بالتراكيز الاصلية للعينات وقد تراوحت نسب التراكيز التي تم الحصول عليها الى التراكيز الاصلية ما بين 95- 105 % .اما قيم الانحراف المعياري النسبي فقد تراوح بنسبة 0.11-5.0%. الخطوة الثالثة للمزيد من التحقق من دقة الطريقة التحليلية اجريت بواسطة تحليل المواد النموذجية القياسية و كانت نسب التراكيز التي تم الحصول عليها لجميع المعادن تتراوح ما بين 94.5-108. جميع نتائج التحقق من صحة و دقةالطريقة التحليلية كانت في حدود مواصفات المنظمة الأمريكية للكيمياء التحليلية.اظهرت النتائج انه ما بين المجموع الكلي للعينات الدوائية و الذي بلغ الستين عينة ان الزرنيخ والكادميوم والرصاص و النيكل و الزنك والحديد موجودة بنسبة 43%,81%,90%.100%,100%,93% من العينات و بمعدل تراكيز تتراوح 0.214-1.325, 0.1-1.2,1.2-19.3, 2.01-36.3, 13.2-391, 103.3-1484.7 ميكروغرام / غرام على التوالي.كما اظهرت النتائج ايضا ان 36% و 10% من العينات كانت تحتوي على عنصر الكادميوم و الرصاص على التوالي بتراكيز اعلى من الحد المسموح به و المقرر من قبل المكتب الوطني للرقابة الدوائية في ماليزيا.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion; it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Pharmaceutical Chemistry.

…….....……..
A.B.M Helal Uddin
Supervisor

I certify that I have read this study and that in my opinion; it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Pharmaceutical Chemistry.

……..…….....…….
Qamar Uddin Ahmed
Examiner

This thesis was submitted to the Department of Pharmaceutical Chemistry and is accepted as a fulfilment of the requirement for the degree of Master of Pharmaceutical Chemistry.

……..…….....….
Nik Mohd Idris Bin Nik Yusoff
Head of Department
Pharmaceutical Chemistry

This thesis was submitted to the Kulliyyah of Phamacy and is accepted as a fulfilment of the requirement for the degree of Master of Pharmaceutical Chemistry.

……..…….....…….
Mohamed bin Awang
Dean, Kulliyyah of Pharmacy

DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

Reem Saadi Khalid

Signature……. Date..……

INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA

DECLARATION OF COPYRIGHT AND AFFIRMATION OF FAIR USE OF UNPUBLISHED RESEARCH

EVALUATION OF TOXIC HEAVY METALS CONTENT IN TRADITIONAL MEDICINE FROM EAST COAST REGION, MALAYSIA

Affirmed by Reem Saadi Khalid

....
Signature Date

This is for you my beloved Isra, for being always there for your mother. I hope it will be an inspiration for you to continue on with your scientific career and never give up.

ACKNOWLEDGEMENTS

In the name of Allah, the Most Gracious and the Most Merciful may His peace and blessings be upon our beloved prophet Muhammad (SAW).Alhamdulillah, Thanks to Allah (S.W.T), for His willing to give me the opportunity to complete this research work.

Although only my name appears on the cover of this dissertation, a great many people have contributed to its production. I owe my gratitude to all those people who have made this dissertation possible.

I would like to express my appreciation to the Dean of Kulliyyah of Pharmacy Dr, Mohammed B. Awang for his guidance and support. Special appreciation goes to my supervisor, Dr A.B.M Helal Uddin for his patient guidance, constructive comments, suggestions and his valuable input throughout the thesis work that effectively contributed to the success of this research. My sincere thanks to brother Mohammad Alaama and to brother Abdul Rahman for their valuable help and support. I also like to express my gratitude to brother Khairul Affif for support.

My acknowledgement also goes to the entire staff of Pharmaceutical Chemistry Department, especially to brother Adisham, sister Zihan, sister Azora and brother Razif for their cooperation and support.

I sincerely acknowledged the constant love and support of all members of my family. I can't express my gratitude to my Mother in words, for her unconditional kind and support. Many thanks go to Ir. Ahmmad Farouk Al -Abdaly for his support and valuable contribution to the research. A lot of thanks to my beloved daughter Isra, she helped me through several ideas and suggestions from a student perspective and she was extremely supportive throughout the study time and always. Uncountable thanks to my dear brother Ahmmad Saadi and my beloved sister Zeena Saadi for their concerns, care, well wishes and all kinds of support.

I would like to express my regards to Perkin Elmer Sdn. Bhd. and their staff especially Lillian for their cooperation. I also wish to acknowledge Dr. Noor Lide bt Abu Kassim for her assistance in our sampling subject.

Same acknowledgement goes to you all dear friends, whose name were not mentioned, this is just due to a space alert however feelings of appreciations have always been with you.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATION

AAS	Atomic Absorption Spectrometer
AHP	American Herbal Pharmacopeia
ANOVA	Analysis of Variance
APHA	American Public Health Association
As	Arsenic
BLL	Blood Lead Level
CAM	Complementary and Alternative Medicines
Cd	Cadmium
CPM	Chinese Proprietary Medicine
Cr	Chromium
Cu	Copper
DCA	Drug Control Authority
EDL	Electrodeless Discharge Lamp
FAAS	Flame Atomic Absorption Spectrometer
FDA	Food and Drug Administration
Fe	Iron
G	Gram
GAP	Good Agricultural Practices
GFAAS	Graphite Furnace Atomic Absorption Spectrometer
GMP	Good Manufacturing Practices
GSP	Good Storage Practice
GTDP	Good Trade and Distribution Practice
HCL	Hollow-Cathode Lamp
HCl	Hydrochloric Acid
$HClO_4$	Perchloric Acid
HDL	High Density Lipoprotein
Hg	Mercury
HGAAS	Hydride Generation Atomic Absorption
HNO_3	Nitric Acid
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectroscopy
ICP-MS	Inductively coupled plasma-mass spectroscopy
ICP-OES	Inductively Coupled Plasma Optical Emission Spectroscopy
INAA	Instrumental Neutron Activation Analysis
IARC	International Agency for Research on Cancer

Kg	Kilogram
KI	Potassium Iodide
L	Liter
LDL	Low Density Lipoprotein
LOD	Limit of Detection
LOQ	Limit of Quantification
$Mg(NO_3)_2$	Magnesium Nitrate
Mg	Magnesium
Mg	Milligram
µg	Microgram
Mn	Manganese
$NaBH_4$	Sodium-Borohydride
NaOH	Sodium Hydroxide
Ng	Nanogram
$NH_4H_2PO_4$	Ammonium Dihydrogen Phosphate
NIST	National Institute of Standards and Technology
NPCB	National Pharmaceutical Control Bureau
Pb	Lead
PL	Permissible Limit
Ppb	Part Per Billion
ppm	Part Per Million
ppt	Part Per Trillion
RSD	Relative Standard Deviation
SD	Standard Deviation
SPSS	Statistical Package for the Social Sciences Computer Software
SRM	Standard Reference Material
TCM	Traditional Chinese Medicine
TGA	Therapeutic Goods Administration
THGA	Transversely-Heated Graphite Atomizer
TM	Traditional Medicine
WHO	World Health Organization
XRF	X-Ray Fluorescence
Zn	Zinc

CHAPTER ONE

INTRODUCTION

1.1 TRADITIONAL MEDICINE

Traditional medicine (TM) is the oldest form of heath care style known to humanity. It can be defined as an integrated summary of knowledge, experience and proficiency to cure various illnesses as well as maintaining well-being using certain therapeutic techniques. The effectiveness of these techniques had been proven through centuries and thus it has been handed over from generation to generation since ages. The complementary and alternative medicine (CAM) is an extensive range of health care practices that are not part of a country's own tradition or not scheduled into its popular health care structure (Chan, 2003). The term CAM typically refers to TM system used in Europe and/or North America (and Australia). When referring in a general sense to all of these regions, the comprehensive TM/CAM is used. There are variety of TM systems originated from different parts around the world such as Asian, African, Arab, Native American, South American and many other cultures (WHO, 2002).

In a broader sense TM refers to a system of treating illness and it consists of mainly two mode of therapies namely medicinal and non-medicinal therapy. Medicinal therapy mode of TM is mainly deals with the treatment of illness or abnormal function of certain body parts through medication. This has similarities with the modern conventional medication. Among the medicinal therapy herbal medicines are one of the most popular and widely used in different TM system. Chinese medicine, Ayurveda, Unani, Naturopathy and Homeopathy types of TM system has adopted herbal as one of their major mode of medicinal therapy for treating different

illness of human. The other non-medicinal techniques are comparatively less common. The acupuncture is commonly used in Chinese medicine and sometimes in Osteopathy. While the manual therapies and spiritual therapies are used in Chinese medicine, Ayurveda, Unani and Naturopathy and the exercises are applied in Chinese medicine, Ayurveda and Naturopathy in a form of Qigong, Yoga and relaxation respectively (WHO, 2002).

Different plants/herbs have been used since ages for therapeutic purposes and they were taken either in a raw form or prepared with some methods such as crushing, drying and mixing with water or milk. In recent years the development of the pharmaceutical industries has influenced the preparation of TM /herbal products. Currently TM is provided in different dosage forms which are similar to pharmaceutical or conventional products. It is manufactured into finished products such as capsules, tablets, pills and powders (Koh & Woo, 2000). It is also dispensed in other forms such as raw, paste and liquid forms for the ease of consumption and retail (Said, Khalil, Fulder & Azaizeh, 2002).

1.1.1 History of Traditional Medicine

The use of traditional medicine has started beyond recorded history. Since the beginning of humanity, the struggle against diseases has been part of everyday life. Plant materials had a very important role in the treatment of various sicknesses. Primitive people treated illness by using plants, animal parts and minerals that were not part of their usual diet. Usually people learned how to distinguish between plants with beneficial effects from those which are inactive by trial and error. Many ancient nations had recognized the importance of herbal remedies with a concept of every

patient should be treated with plants of his/her own land, due to the belief that it brings more cures (Saganuwan, 2010).

One of the earliest written records detailing the use of herbs as a treatment for various illnesses was found in the Egyptian papyrus (Bensky, Gamble & Kaptchuk, 1993). Willow is listed in herbal remedies of ancient Egypt in the Ebers papyrus. The willow tree *Salix Alba* has been used for thousands of years to relieve joint pain and to treat certain heart conditions (Aboelsoud, 2010). In the ancient Mesopotamians Babylonian medical texts prescribed many plant products usually specific parts of the plant such as the leaves, blossoms, seeds and roots to be prepared in various ways either by being crushed, cooked, dried and mixed with an appropriate carrier such as water or milk (Biggs, 2006). The most common use of herbal medicine was used to treat gastrointestinal, dermatological and respiratory ailments.

The Arabic world was the center of scientific and medical knowledge. The present use of Arab botanical remedies has historical roots in pre-historic Arabic medicine. Arab herbalists and physicians in the middle ages accepted the ancient medicinal practices of Mesopotamia, Greece, Rome, Persia and India. Texts from Greece and Rome were translated to Arabic and studied by Islamic scholars. Islamic physicians began to use the regulation of diet and prescription of medicinal herbs for healing their patients. Around the 8th and 11th centuries, Arab physicians advanced their existing knowledge about herbs and their potential medical efficacy. Plant extracts were prepared and taken orally (Saad, Azaizeh, & Said, 2005). Their best input to modern medicine was the discovery of the immune system. Arabs in Baghdad were the first in history to distinct medicine from pharmacological science. The first specialized pharmacy was found in the capital of the Arabic world -Baghdad. In Andalusia (Spain), Arab herbalist and physicians led by Ibn AlBitar introduced about

350 new plant species as medicinal herbs. Historically, Asians are well known for their reliance on traditional medicines (Chinese and Indian Ayurvedic formulation). India was known as a rich place of natural resources and knowledge. People from various parts of the world particularly from Asia used to go to the ancient schools of India to learn health, sciences mainly 'Ayurveda', which has been practiced for over 5000 years, based on the theory that both human and universe are made from the same elements, and the human's body should be treated as one part thus, the entire body should be treated while curing any kind of sickness (Chopra & Doiphode, 2002; Gogtay, Bhatt, Dalvi & Kshirsagar, 2002).

Traditional Chinese medicine had originated in ancient China and dates back over 4000 years ago. It has evolved over thousands of years. TCM practitioners mainly use herbs and sometimes they include some minerals and animal products (Hong, 2004). The pharmacological reference book used by TCM practitioners (materia medica) contains hundreds of medicinal plant substances formulated based on the concept of combining different plants/herbs to increase the therapeutic effect of the medicine. Some of these Chinese traditional herbs are currently practiced in different parts of the world (Ergil, Kramer, & Ng, 2002).

The principles of Malay traditional medicine was basically adopted from the Arabic and Unani medicine and it has influenced by other practices such as Indonesian, Chinese, Indian and orang asli (indigenous people) traditional medicines (Jamal, 2006). The use of herbal products as medicine is extremely ancient and probably predates to the modern *Homo sapiens*. People from ancient cultures collected information on herbs and well developed it in to pharmacopoeias. The earliest recorded evidence of such efforts are in Indian, Chinese, Egyptian, Greek, Roman and

Syrian texts that date back to about 5000 years (Inamdar, Edalat, Kotwal & Pawar, 2008).

1.1.2 Global Market Overview

In general traditional medicine is considered as an alternative treatment which is widely used in the developing countries. Recently it has become more popular in most developed countries as well (Jayaraj, 2010). During the later part of the twentieth century, there was a vast growth in the popularity of traditional healing modalities, mainly herbal remedies.

The World Health Organization (WHO) estimates that more than 70% of the world population use traditional medicine for some aspect of primary health care (Yee, Chu, Xu, & Choo, 2005). This huge percentage of TM usage is mainly due to their availability, accessibility, and affordability to a wide range of the population in different parts of the world.

In many developing countries like Asia and Latin America, populations continue to use TM as a result of historical circumstances and cultural beliefs. The global market for herbal medicines in 2008 was estimated at over US$ 60 billion annually. The sale of herbal medicine is expected to get higher at 6.4% an average annual growth rate (WHO, 2009).

Asians are well known for their high dependency on traditional medicine. In China in 2005 the total sales revenue from traditional Chinese medicines was 14 billion USD and increased by 23.81% compared to one year before. Traditional medicine is used to treat roughly 200 million patients per year and costs about 40% out of the total health care account. In some other Asian countries like Japan, a study conducted in Tokyo, in 1990 showed that 91% of the survey population believed of

the effectiveness of the oriental medicine for chronic diseases, 49% of them had used herbal medicines (WHO, 2009).

In India there are about 25,000 plant-based formulations used in traditional medicine preparations which are recognized in the Indian communities. There are 1.5 million practitioners of traditional medicine and 7800 medicinal drug manufacturing units consuming about 2000 tons of herbs annually (Verma & Singh, 2008).

In the western parts of the world, the United States of America and some European countries, despite all the marvelous advancements in conventional medicine in these countries, the use of traditional herbal medicine is rapidly increasing. This is because TM provides an alternative option mainly for the purpose of maintaining good health and correcting the imbalances caused by modern diets and life styles (Inamdar et al., 2008).

In Europe, according to the 1991 estimation, the herbal medicine market in the European countries was about US$6 billion, about 50% of the total amount goes to Germany, followed by other European countries such as France at US$1.6 billion and Italy at US$0.6 billion while the remaining US$0.8 billion is dispersed among different European countries like the United Kingdom, Spain and the Netherlands. This amount has been increased to US$10 billion in 1996 (Sharma, Shanker, Tyagi, Singh & Rao, 2008).

In the United States of America, the annual costs on traditional medicines was expected about US$2.7 billion in 1997 and the percentage of the population that used complementary and alternative medicine went up to 59% in particular those with chronic conditions such as diabetes and lung disease. Studies in Canada indicate that the use of alternative medicine is up to 42% among children with chronic diseases (Southwood, Malleson, Roberts-Thomson, & Mahy, 1990). In Denmark a study

showed that 53% of 622 patients had used a complementary or alternative medicine at least once (Madsen et al., 2003). In Australia, a recent study shows that 38% of a sample of 580 children and teenagers had used a complimentary medicine (Crawford, Cincotta, Lim & Powell, 2006).

In South Africa 75% of people living with HIV/AIDS use traditional medicine (Chaudhary, Gadhvi, & Chaudhary, 2010). In Ethiopia up to 80% of the population uses traditional medicine and TM is usually sold in open markets (Kassaye, Amberbir, Getachew & Mussema, 2007).

For some of the Latin American countries, it had been reported that 71% of the population in Chile and 40% of the population in Colombia use TM (WHO, 2002).

Current studies show that in the eastern part of the Mediterranean there are about 250 types of herbs used to cure different diseases and they are sold or traded in this region (Said, et al., 2002).

In most of the South Asian countries, traditional herbal medicine is widely used. As it is referred in Malaysia at which there is a rich legacy from three major ethnic groups (Malay, Chinese and Indian) of using TM, a large segment of the population in this country relies on TM mainly herbal products for their healthcare needs. Huge demand of TM has been increasing the total Malaysian market size from US$ 385 million in the year 2000 to US$ 1.29 billion in 2005 (Aziz & Tey, 2009). Malaysia is a country blessed with the rich forests and plenty of assorted medicinal plants. Malaysia's rainforest carry more than 12,000 plant species out of which 2,000 species have been reported to have medicinal value. According to the Drug Control Authority (DCA) of the Ministry of Health, in 2004, there were 219 registered pharmaceutical companies comprising 139 traditional medicine and 80 modern medicine companies (Ang &Lee, 2005).