THE LEVEL OF CURCUMINOIDS IN THAI MEDICAL HERBS

KANATEE WATTANAVORANUN

MASTER OF SCIENCE
IN
ANTI-AGING AND REGENERATIVE SCIENCE

SCHOOL OF ANTI-AGING AND REGENERATIVE MEDICINE
MAE FAH LUANG UNIVERSITY

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Kanatee Wattanavoranun

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ABSTRACT

Curcuminoids, the principal natural yellow pigments comprising curcumin, demethoxycurcumin and bisdemethoxycurcumin. This study is undertaken to find out the level of curcuminoids content in Thai medicinal herbs. Six kind of plants were selected to study. Rhizomes of *Curcuma longa, Zingiber officinale, Kaempferia parviflora, Curcuma aromatic, Zingiber montantum and Curcuma xanthorrhiza* collected from Chonburi Province, Thailand were carried out to dried and powdered for determining curcuminoids content. The powdered samples were analyzed by UV-Visible spectrophotometry methods. It was complied with Thai Herbal Pharmacopeias 1995 Page 44. The methodology and data were verified for selectivity, linearity, accuracy and precision. Verification of UV-Visible spectrophotometric method verified according to the guidelines of International Conference on Harmonization (ICH). From the study, curcuminoids were found in *Curcuam longa* and *Curcuma aromatic*. The average of curcuminoids content in *Curcuma longa* and *Curcuma aromatic* were 8.45% w/w and 0.92% w/w , respectively. This method was found selective for determination of curcuminoids and showed a considerably high level of accuracy (94-104%). The data showed a good linear relationship with a correlation coefficient greater than 0.999 for curcumin standard and indicated that the method

had an excellent intraday precision and intermediate precision. The results of the study might be useful for further development in herbal pharmaceutical industry and drug development.

Keywords: Curcuminoids/*Curcuma longa/Curcuma aromatic*

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CHAPTER 1

INTRODUCTION

1.1 Statement and Significance of the Problem

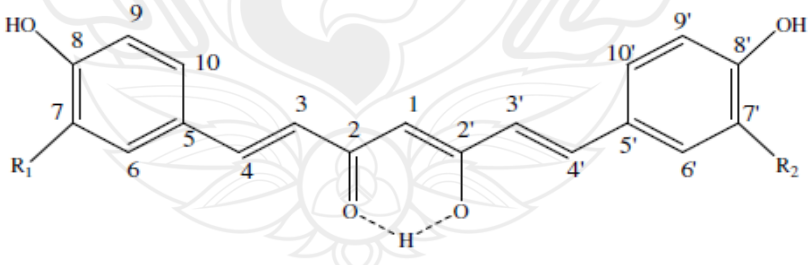
Plants are important sources of chemical compounds for research and development of new drugs. Thailand is a country endowed with a variety of medicinal plants with strong potential for therapeutic applications. Recently, there are a lot of uses of herbal products for the prevention and treatment of diseases in Thailand. The popularity of Thai herb use has been growing along with more herb-related occupational groups and herbal medicine schools that teach how to apply herbs in regular medical treatments. Many research studies on Thai herbs were also conducted. At the same time, herbal medicine has been seen some form of revival, advancing at a greater pace in community acceptance of their therapeutics effects. This field is bringing forward new lead drug discoveries as well as safe and efficacious plant-based medicines. In turn, this leads to growing number of sales of commercialised medicinal herbs and most importantly, growing number of pharmaceutical companies that involve in the research and development of plants as a source for modern medicine.

In general use, herbs are any plants used for flavoring, food, medicine, or perfume. Culinary use typically distinguishes herbs as referring to the leafy green parts of a plant (either fresh or dried), from a "spice", a product from another part of the plant (usually dried), including seeds, berries, bark, roots and fruits. Some plants contain phytochemicals that have effects on the body. There may be some effects when consumed in the small levels that typify culinary "spicing", and some herbs are toxic in larger quantities. Herbs have long been used as the basis of traditional Chinese herbal medicine, with usage dating as far back as the first century CE[3] and far before. In India, the ayurveda medicinal system is based on herbs. Medicinal use of herbs in Western

cultures has its roots in the Hippocratic (Greek) elemental healing system, based on a quaternary elemental healing metaphor. Famous herbalist of the Western tradition include Avicenna (Persian), Galen (Roman), Paracelsus (German Swiss), Culpepper (English) and the botanically inclined Eclectic physicians of 19th century/early 20th century America (John Milton Scudder, Harvey Wickes Felter, John Uri Lloyd). Modern pharmaceuticals had their origins in crude herbal medicines, and to this day, some drugs are still extracted as fractionate/isolate compounds from raw herbs and then purified to meet pharmaceutical standards.

There are diversity of natural resources and forestry in Thailand. Each region represents its own unique, according to the difference in climates and terrain types. This brings about the variety of herbal plants and each specie of herbal plants contain different kind of active compounds and may be different in quantity.

Among the Curcuma species, *C. longa*, *C. aromatic and C. xanthorrhiza* are popular. The main constituent of Curcuma species are curcuminoids and bisabolane-type sesquiterpenes. Curcumin is the most important constituent among natural curcuminoids found in these plants. (Werayut Pothitirat & Wandee Gritsanapan, 2005). Curcuminoids are well known for their antioxidant, anti-inflammatory, antitumour and cytotoxic properties. These polyphenolic compounds are natural yellow pigments comprising curcumin, demethoxycurcumin and bisdemethoxycurcumin (Figure 1.1).



Compound	R_1	R_2
Curcumin	OMe	OMe
Demethoxycurcumin	H	OMe
Bisdemethoxycurcumin	H	H

Figure 1.1 Chemical Structures of Curcuminoids

At present, a variety of analytical methods for quantitative analysis of curcuminoids content were reported. Most of them are spectrophotometric methods. The other methods such as HPLC, HPTLC has been developed for their analysis but in plasma and urine. (Rohman, 2012) However these methods are instrumental, laborious, time-consuming and expensive. The method of choice in this study is UV-Visible spectrophotometry. In Thai Herbal Pharmacopoeia and the Standard of ASEAN Herbal Medicine, it is an official standard method to determine the total level of curcuminoids content in *Curcuma longa.* This study is undertaken to determine level of curcuminoids content in Thai medicinal herbs. The study is concerned in Zingiberaceae species because there are some researches about curcuminoids contents in Zingiber species. There are six kinds of plants in this study as follows *Curcuma longa, Zingiber officinale, Kaempferia parviflora, Curcuma aromatic, Zingiber montanum and Curcuma xanthorrhiza.* (วิภาดา กันทยศ และยิ่งยง ไพสุขศานติวัฒนา, 2554)

1.2 Objective

The objective of this study is to find out the level of curcuminoids content in Thai medicinal herbs.

1.3 Scope of the Study

1.3.1 Six kinds of Plants were selected as *follows Curcuma longa, Zingiber officinale, Kaempferia parviflora, Curcuma aromatic, Zingiber montantum,Curcuma xanthorrhiza.* All plants were collected from reliable source.

1.3.2 The study is based only on type of plants from Chonburi Province without temperature and moisture control in planting area. These factors may affect in the level of curcuminoids contents. The age of plants is 12 months.

1.4 Hypothesis

Some medical plants have been found curcuminoids content more than the amount of curcuminoids founded in Curcuma *longa.*

1.5 Study Design

Analytical research

CHAPTER 2

LITERATURE REVIEW

This chapter provides an extensive review of the literature and research related to principal selection. The chapter will be divided into sections that include (1) Knowledge of curcuminoids (2) Plant under studied (3) Analytical methods for analysis of curcuminoids (4) Recommended selection analytical methods

2.1 Knowledge of Curcuminoids

2.1.1 Physical Properties

Curcumin is the principal curcuminoid of the popular Indian spice turmeric, which is a member of the ginger family (Zingiberaceae). The other two curcuminoids are desmethoxycurcumin and bis-desmethoxycurcumin. The curcuminoids are polyphenols and are responsible for the yellow color of turmeric. Curcumin can exist in at least two tautomeric forms, keto and enol. The enol form is more energetically stable in the solid phase and in solution. Curcumin is brightly yellow colored and may be used as a food coloring. The active ingredient of curcumin is diferuloylmethane, a hydrophobic polyphenol with a characteristic yellow colour. (Akram et al., 2010; ปรีชา บุญจูง และคณะ, 2549)

2.1.2 Chemical properties

In chemical terms it is bis-a, b-unsaturated b-diketone, a linear diarylheptanoid compound, where two oxy-substituted aryl moieties are linked together through a seven carbon chain (Figure 2.1). The aryl rings may be substituted by varying numbers of hydroxy or methoxy groups in a symmetrical or asymmetrical fashion to produce

analogues of curcumin or curcuminoids. Curcumin is the most abundantly occurring natural analogue at 77%, followed by demethoxycurcumin (17 %) in which one methoxy group is absent, then bis-demethoxycurcumin (3 %) in which the methoxy group is absent from both the aryl rings (Figure 2.1)

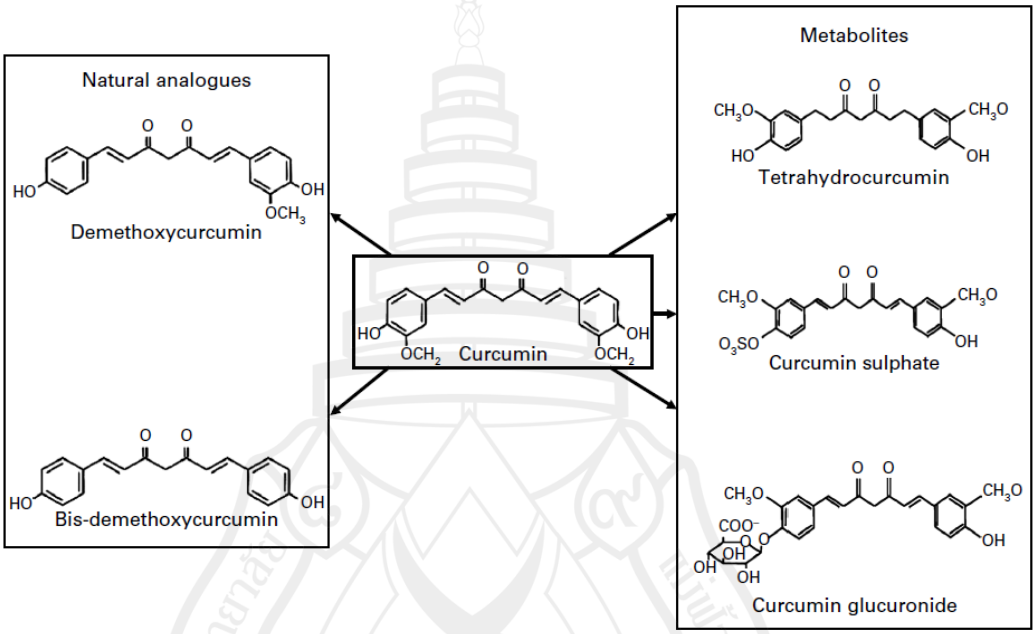


Figure 2.1 Chemical Structure of Curcumin, its Natural Analogues and Principle Metabolites

Curcumin has a molecular weight of 368.37 and a melting point of 183 _C. Commercial grade curcumin contains the curcuminoids desmethoxycurcumin (MW 338; typically 10–20%) and bisdesmethoxycurcumin (MW 308; typically less than 5%. On ultraviolet–visible spectrophotometric investigation, maximum light absorption of curcumin occurs at 420 nm. (Sharma, Gescher & Steward, 2005)

2.1.3 Therapeutic Properties of Curcumin

Curcumin has antioxidant, anti-inflammatory, antiviral and antifungal actions. Studies have shown that curcumin is not toxic to humans. Curcumin exerts anti-

inflammatory activity by inhibition of a number of different molecules that play an important role in inflammation.

2.1.3.1 Anti-inflammation

The volatile oils and curcumin of Curcuma longa exhibit potent anti-inflammatory effects. Oral intake of curcumin in instances of acute inflammation was found to be as effective as cortisone or phenylbutazone, and one-half as effective in cases of chronic inflammation. In rats with Freund's adjuvant-induced arthritis, oral intake of Curcuma longa significantly reduced inflammatory swelling compared to controls. In monkeys, curcumin inhibited neutrophil aggregation associated with inflammation. C. longa's anti-inflammatory properties may be attributed to its ability to inhibit both biosynthesis of inflammatory prostaglandins from arachidonic acid, and neutrophil function during inflammatory states. Curcumin may also be applied topically to counteract inflammation and irritation associated with inflammatory skin conditions and allergies, although care must be used to prevent staining of clothing from the yellow pigment. (Itokawa, Shi, Akiyama, Morris-Natschke & Lee, 2008)

2.1.3.2 Anticarcinogenic Effects

Animal studies involving rats and mice, as well as *in vitro* studies utilizing human cell lines, have demonstrated curcumin ability to inhibit carcinogenesis at three stages: tumor promotion, angiogenesis, and tumor growth. In two studies of colon and prostate cancer, curcumin inhibited cell proliferation and tumor growth. The anticarcinogenic effects of turmeric and curcumin are due to direct antioxidant and free-radical scavenging effects. (Itokawa et al., 2008)

2.1.3.3 Antioxidant Effects

Water- and fat-soluble extracts of turmeric and its curcumin component exhibit strong antioxidant activity, comparable to vitamins C and E. A study of ischemia in the feline heart demonstrated that curcumin pretreatment decreased ischemia-induced changes in the heart. An *in vitro* study measuring the effect of curcumin on endothelial heme oxygenase-1, an inducible stress protein, was conducted utilizing bovine aortic endothelial cells. Incubation (18 hours) with curcumin resulted in enhanced cellular resistance to oxidative damage. (Itokawa et al., 2008)

2.1.3.4 Antimicrobial Effects

Turmeric extract and the essential oil of Curcuma longa inhibit the growth of a variety of bacteria, parasites, and pathogenic fungi.

2.1.3.5 Gastrointestinal Effects

Constituents of *Curcuma longa* exert several protective effects on the gastrointestinal tract. Sodium curcuminate inhibited intestinal spasm and p-tolymethylcarbinol, a turmeric component, increased gastrin, secretin, bicarbonate, and pancreatic enzyme secretion. Turmeric has also been shown to inhibit ulcer formation caused by stress, alcohol, indomethacin, pyloric ligation, and reserpine, significantly increasing gastric wall mucus in rats subjected to these gastrointestinal insults. (Itokawa et al., 2008)

2.1.3.6 Curcumin enhances immunity

Curcumin can also help the body fight off cancer should some cells escape apoptosis. When researchers looked at the lining of the intestine after ingestion of curcumin, they found that CD4+ T-helper and B type immune cells were greater in number. In addition to this localized immune stimulation, curcumin also enhances immunity in general. (Itokawa et al., 2008)

2.2 Plant Under Studied

2.2.1 Curcuma longa (Khamin Chan) (Figure 2.2) (Turmeric, n.d.)

Synonyms Curcuma; Yellow Root; Indian Saffron

Category: Stomachic; carminative ; pharmaceutic aid (colouring agent); astringent Turmeric is the dried rhizome of curcuma longa Linn. (Family Zingiberaceae)

Constituents: Turmeric yields about 7 percent of a yellow volatile oil containing turmerone and zingiberone as major constituents and many other sesquiterpenes and monoterpenes; yellow colouring matter including curcumin or diferuloylmethane, desmethoxycurcumin, and bisdesmethoxycurcumin.

The Thai Herbal Pharmacopoeia (THP) recommended that dried turmeric should contain not less than 6.0% v/w of turmeric oil and 5.0% w/w of total curcuminoids (Prachachon Co, Ltd., 1995).

Dose 3-9 g.: Research on Curcuma has been focused on Alzheimer's, Arthritis, Cancer and Diabetes. In laboratory experiments on rodents, Curcuma can break up the Alzheimer's amyloid-beta polymers, suppress arthritic inflammation, induce apoptosis in some cancer types and improve insulin sensitivity. Human trials of Curcuma for some illnesses are underway.

Figure 2.2 *Curcuma Longa*

2.2.2 *Zingiber officinale* (Ginger) (Figure 2.3)

Ginger (*Zingiber officinale Rosc*.) (Ginger, n.d.; Malhotra & Singh, 2003) is a creeping perennial on a thick tuberous rhizome, which spreads underground. The plant has narrow; lanceolate to linear-lanceolate, 15-30 cm long leaves which die of each year. The odour and taste are characteristic, aromatic and pungent. The pungency of ginger is due to gingerol, an oily liquid consisting of homologous phenols. It is formed in the plant from phenylalanine, malonate and hexonate. In the fresh ginger rhizome, the gingerols were identified as the major active components and gingerol [5-hydroxy-1-(4-hydroxy-3-methoxy phenyl) decan-3-one] is the most abundant constituent in the gingerol series. The powdered rhizome contains 3-6% fatty oil, 9% protein, 60-70% carbohydrates, 3-8% crude fiber, about 8% ash, 9-12% water and 2-3% volatile oil. The volatile oil consists of

mainly mono and sesquiterpenes; camphene, betaphellandrene, curcumene, cineole, geranyl acetate, terphineol, terpenes, borneol, geraniol,limonene, linalool, alpha-zingiberene (30-70%), beta-sesquiphellandrene (15-20%), betabisabolene (10-15%) and alpha-farmesene. In dried ginger powder, shogaol a dehydrated product of gingerol is a predominant pungent constituent up to biosynthesis.

The ginger bears an enormous number of pharmacological activities such as cardio protective activity, anti-inflammatory activity, anti-microbial activity, antioxidant property, anti-proliferative activity, neuro-protective activity and hepatoprotective activities which have been proved. Among those, neuro-protective activity as well as effect of ginger in colon cancer has facilitated the extent of the further research with a positive outcome.

Figure 2.3 *Zingiber officinale*

2.2.3 ***Kaempferia parviflora*** (Figure 2.4)

Kaempferia Parviflora (Thai Ginseng) is a herb that has some historical and medicinal usage for treating metabolic ailments and improving vitality in Thailand and limited to surrounding regions. It is also reported to be an aphrodisiac compound and physical enhancer. (ว่านนางคำ, ม.ป.ป.)

Currently, the research on Thai Ginseng is at a moderate level and starting to get human trials. It appears to be 'healthy' and a good source of a class of Bioflavonoids compounds with methoxy groups added to them, known as methoxyflavones.

That being said, the research on its aphrodisiac effects in mice indicate that low doses are weak to moderate in potency and higher doses fail to exert any aphrodisiac effect. It does not appear to increase Testosterone in otherwise normal rats (although it may in castrated rats), and although it appears to have a variety of mechanisms to be pro-erectile, these have not been tested for potency in a living system. The mechanism of pro-erectility is fairly unique and interesting, but the one study to investigate whether or not it could inhibit PDE5 (one of the mechanisms of Viagra) failed to establish whether it was selective. Selective PDE5 inhibitors are good pro-erectiles without many side-effects, but the non-selectivity (currently not established) may lead to gastrointestinal side effects.

Figure 2.4 *Kaempferia parviflora*

2.2.4 *Curcuma aromatic* (Figure 2.5)

Curcuma aromatica Salisb. (Family: Zingiberaceae) is a species occurring wild throughout India and cultivated chiefly in West Bengal and Travancore. The rhizomes are light yellow (internally orange) and possess a camphoraceous odour. They are sometimes used as a substitute for turmeric (*Curcuma longa*). *C. aromatica* is never used as a spice but only as an aromatic for cosmetic purposes and in indigenous medicine for external applications on skin diseases, bruises and sprains. The colouring matter in the rhizomes of

C. aromatica is predominantly curcumin and demethoxycurcumin, a trace of bis-demethoxycurcumin can be found when high levels of samples are taken for thin layer separation. C. aromatica is used for preventing and treating coronary heart disease, for epilepsy, as anti-allergic and in autoimmune diseases . (Saowaluck Bua-in & Yingyong Paisooksantivatana, 2009;ไพล, ม.ป.ป.)

Figture 2.5 *Curcuma aromatic*

2.2.5 ***Zingiber montantum*** (Figure 2.6)

Cassumunar ginger (*Zingiber montanum* (Koenig) Link ex Dietr.) belongs to the Zingiberaceae family. It is probably native to India and is now widely cultivated in tropical Asia. In Thai traditional medicine, the rhizomes are consumed to relieve asthma, and muscle and joint pain. The main constituent, terpinen-4-ol and (E)-1(3, 4-dimethylphenyl) butadiene (DMPBD) has been found to be effective against a range of pathogenic bacteria and also has anti-inflammatory activity. New, complex curcuminoids, cassumunins A, B, C, have been isolated from rhizomes of Z. montanum and they possess stronger antioxidant activity than that of curcumin.

Figure 2.6 *Zingiber montantum*

2.2.6 *Curcuma xanthorrhiza* (Figure 2.7)

Curcuma xanthorrhiza belongs to Zingiberacae family.It is a native plant of Indonesia. (Mangunwardoyo, Deasywaty & Usia, 2012)

Most people use the rhizome of this plant as they believe it has medicinal effect. The rhizomes of *Curcuma xanthorrhiza* Roxb contain volatile oil, saponin, flavonoid and tannin]. Chemistry analysis showed that the main substances of *Curcuma xanthorrhiza* Roxb are starch (48.18-59.64%), fiber (2.58-4.83%), volatile oil such as, phelandren, camphor, tumerol, sineol, borneol, and xanthorrhizol (1.48-1.63%), and also curcuminoid like, curcumine and desmetoxicurcumine (1.6-2.2%).

Figure 2.7 *Curcuma xanthorrhiza*

2.3 Analytical Methods for Analysis of Curcuminoids

At present, a variety of methods for Quantitative analysis of curcuminoids content were reported. Most of them are based-spectrophotometric techniques, expressed as the total color content of the sample. However, using this technique it is not possible to separate and to quantify the curcuninoids individually. (Uthai Sotanaphun, Thawatchai Phaechamud1 & Pranom Dechwisissakul, 2007). For this reason, chromatographic-based techniques and electrophoresis are among the methods of choice for determination of curcuminoids attributed to their separation capacities. (Rohman, 2012)

2.3.1 **Spectroscopic Techniques** (Rohman, 2012)

2.3.1.1 UV-Vis spectrophotometry

The official standard method for determination of curcuminoids or Curcuma-based products is UV-Vis spectrophotometry which is relied on the direct measurements of sample in certain solvents. In some organic solvents, curcuminoids show the intensive absorption intensity at wavelength of 420 – 430 nm. The quantification of curcuminoid using UV-Vis spectrometry technique was usually expressed as the total curcuminoids content.

2.3.1.2 Infrared spectroscopy

Infrared (IR) spectroscopy, especially in combination with chemometrics technique, has been widely used for determination of analytes of interest in food, agricultural, and pharmaceutical products. The method allows rapid and sensitive, ease in sample preparation, and non destructive technique meaning that the used samples can be used for further analysis.

2.3.2 Flow Injection Analysis (FIA)

FIA system with on-line detections using ultraviolet (UV) at 250 nm and fluorometric (FL) using λex of 397 nm and λem 508 nm is developed for analysis of curcuminoids in *C. longa.*

FIA was conducted at ambient temperature using various organic solvents, either alone or in combination with water as carrier solution delivered at flow rate of 1.0 ml/min.

2.3.3 Chromatographic-based Methods

Chromatography-based methods are emerging analytical technique in chemical analyses which are appropriate for qualitative and quantitative determination of a large number of samples. Due to its advantageous properties, namely low cost in operation, ease in sample preparation, and the availability of several detection systems, thin-layer chromatography (TLC) was regularly used for the identification, separation, quantification or semiquantitative purposes of natural pigments, including curcuminoids . However, high-performance liquid chromatography (HPLC) is a method of choice for curcuminoids attributed to the high precision and accuracy offered and low detection limit achieved. Due to its advantageous properties, namely low cost in operation, ease in sample preparation, and the availability of several detection systems, thin-layer chromatography (TLC) was regularly used for the identification, separation, quantification or semiquantitative purposes of natural pigments, including curcuminoids. However, high-performance liquid chromatography (HPLC) is a method of choice for curcuminoids attributed to the high precision and accuracy offered and low detection limit achieved. Furthermore, in order to improve the separation power, multi-development in TLC and gradient elution in HPLC are the preferred methods for analysis of samples. Capillary electrophoresis was currently developed as an optional technique for the analysis of curcuminoids. (Werayut Pothitirat & Wandee Gritsanapan, 2005)

2.4 Recommended Selection Analytical Methods

Recommended content of curcuminoids for Standard of ASEAN Herbal Medicine and THP is not less than 5% calculated as curcumin. Spectrophotometry is the official technique for the quantitative determination. Other techniques such as HPTLC, HPLC and CE have also been reported for the quantitative analysis of the individual curcuminoids. However these methods are instrumental, laborious, time-consuming and

expensive. UV-Visible spectrophotometry methods was chosen because it is a rapid, simple, selective and precise method for assay curcuminoids content. (Uthai Sotanaphun et al., 2007)

Thus, this study was undertaken to find out the level of curcuminoids content in Thai medicinal herbs by UV-Visible spectrophotometry methods. It is complied with Thai Herbal Pharmacopoeias1995 Page44 (THPI).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials and Instrument

3.1.1 Chemicals

Reference standard of curcumin was purchased from Sigma Chemical (U.S.A.) and supported by Defence Pharmaceutical Factory. All chemicals and reagents were analytical grade.

3.1.2 Plants

3.1.2.1 *Curcuma longa*

3.1.2.2 *Zingiber officinale*

3.1.2.3 *Kaempferia parviflora*

3.1.2.4 *Curcuma aromatic*

3.1.2.5 *Zingiber montantum*

3.1.2.6 *Curcuma xanthorrhiza*

All plants are from Chonburi Province and the period of time which plants were collected is 12 months.

3.1.3 Instrument

UV Spectrophotometer Hitachi U-2800

3.2 Methods

The scope of this study was divided into three parts as the scheme in Figure 3.1. The details are as follows.

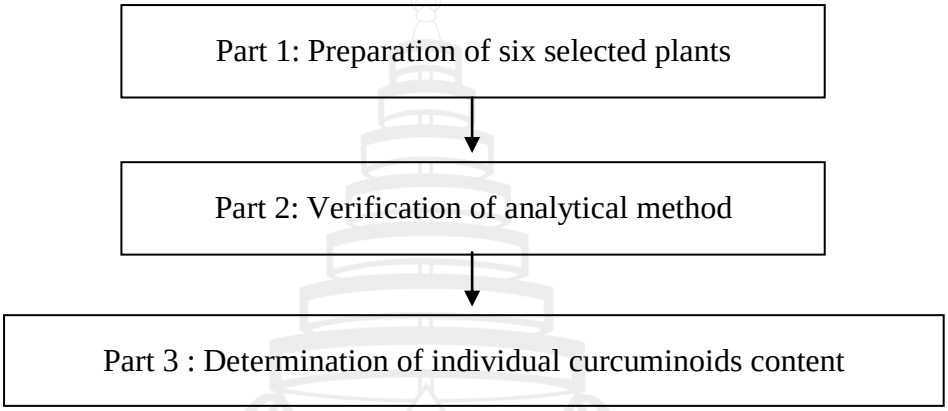


Figure 3.1 Scope of the Study

3.2.1 Part 1 Preparation of Six Selected Plants

Six rhizomes of Plants were selected as follows *Curcuma longa, Zingiber officinale, Kaempferia parviflora, Curcuma aromatic, Zingiber montantum, Curcuma xanthorrhiza.* All plants were collected from reliable source. They were grown in the same farm and harvested at the same period (age;10-12 months). Fresh rhizomes were cleaned,washed with deionised water,sliced and dried at 60°c in a hot air oven for 48 hours. Dried rhizomes were cut in small pieces and powdered by electronic mill. Then, keep them in closed container.

Powder of samples

Figure 3.2 *Kaempferia parviflora (Krachai Dam)*

Figure 3.3 *Curcuma xanthorrhiza*

Figure 3.4 *Zingiber montantum (Prai)*

Figure 3.5 *Curcuma aromatic (Wan Nang Kham)*

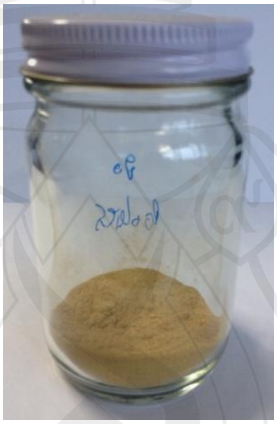


Figure 3.6 *Zingiber officinale (Ginger)*

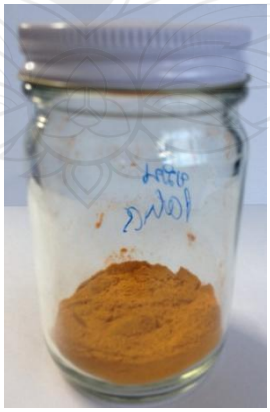


Figure 3.7 *Curcuma longa (Khamin Chan)*

3.2.2 Part 2 Verification of Analytical Method (ICH Harmonised Tripartite Guideline, 1994)

The methodology and data were verified for selectivity, linearity, accuracy and precision. Verification of UV-visible spectrophotometric method verified according to the guidelines of the International Conference on Harmonization (ICH), Verification of Standard Method is defined for the first use of a standard method within the laboratory.

3.2.2.1 Selectivity is a measure of a method's freedom from interferences. (ICH Harmonised Tripartite Guideline, 1994) Six kinds of herb powder were prepared in the same concentration 4 µg/ml, they are all in methanol. For measure the curcuminoids peak the wavelength was set at 420 nm and for scanning curcuminoids in samples we set the wavelength interval at 200-700 nm.

3.2.2.2 Linearity: To validate the UV-visible spectrophotometer method, calibration curve was obtained at 5 different concentrations of curcumin. Least square regression method was used to analyze the linearity and slope. Intercept and correlation coefficient were also determined.

3.2.2.3 Precision: The precision of the developed UV-visible spectrophotometric method was also determined as inter-day, intra-day and repeatability. Inter-day precision was determined by performing experiments in each of sample on three different days of the week whereas, intra-day precision of developed method was determined by performing individual experiments on each sample at the same day. (United nations office on drugs and crime Vienna, 2009) Repeatability was also determined by repeating the analysis of samples for six times.

3.2.2.4 Accuracy: Recovery studies at three different levels to judge the accuracy of developed method of UV-visible. To calculate the %Recovery and relative standard deviation (RSD), experiments were performed in triplicates spectroscopy was assessed. (United nations office on drugs and crime Vienna, 2009)

3.2.3 Part 3 Determination of individual CurcuminoidsC

Analytical method is complied with Thai Herbal Pharmacopoeias 1995 Page44. The official standard method to determine the level of curcuminoids content is UV-Vis spectrophotometry technique.

Procedure of curcuminoids assay (THPI 1995)

Curcuminoids content Not less than 5.0 percent w/w of curcuminoids, calculated as curcumin, when determined by the following method.

Standard curcumin solution Dissolve about 20 mg of curcumin, accurately weighed, in sufficient methanol to produce 50 ml.

Standard curcumin curve Transfer 0.2, 0.4, 0.6, 0.8, 1.0 ml of standard curcumin solution into five 100-ml volumetric flasks, respectively, dilute to volume with methanol, and mix. Measure the absorbances of standard solutions relative to the blank at 420 nm. Plot the readings and draw the curve of best fit.

Sample preparation Transfer about 300 mg of each sample, accurately weighed, into a 10-ml volumetric flask, add tetrahydrofuran to volume and mix. Set aside at room temperature for 24 hours with frequent shaking. Dilute 1.0 ml of the clear supernatant liquid with methanol to produce 25.0 ml. Transfer 1.0 ml of this solution into a 50-ml volumetric flask, dilute to volume with methanol and mix well. Measure the absorbance of the sample solution, and by reference to the Standard curcumin curve, calculate the content of curcuminoids as curcumin in the sample.

Statistical analyses: Microsoft EXCEL® (Microsoft Corporation, USA) was used to compute the Standard regression curve analysis. We also used the same software to calculate the mean, SD, %recovery and RSD.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Verification of Analytical Method

The methodology and data were verified for selectivity,linearity, accuracy and precision.

4.1.1 Selectivity

This parameter is concerned with the extent to which other substances interfere with the identification and, where appropriate, quantification, of the analyte(s) of interest. It is a measure of the ability of the method to identify and quantify the analytes in the presence of other substances, either endogenous or exogenous, in a sample matrix under the stated conditions of the method. (United nations office on drugs and crime Vienna, 2009)

Representative wavelength scannings below show that there are no interferences with the curcuminoids peak in each sample, we have found curcuminoids peak in *Curcuma aromatic* and *Curcuma longa* (Khamin Chan) at the same wavelength as found in curcumin standard 420 nm. Therefore, this method was found selective for determination of curcuminoids.

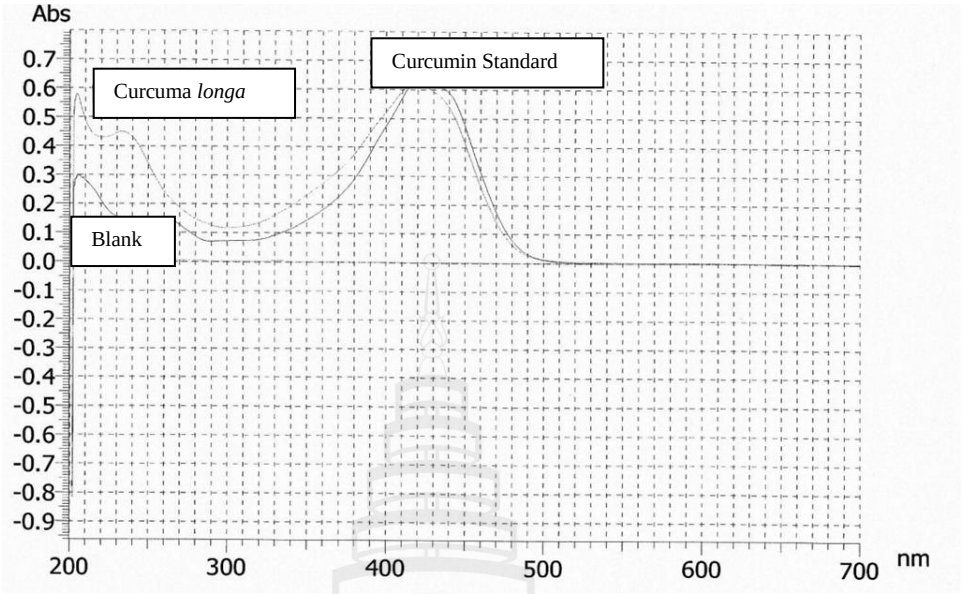


Figure 4.1 Wavelength scanning of Curcumin Standard VS *Curcuma longa* (Khamin Chan)

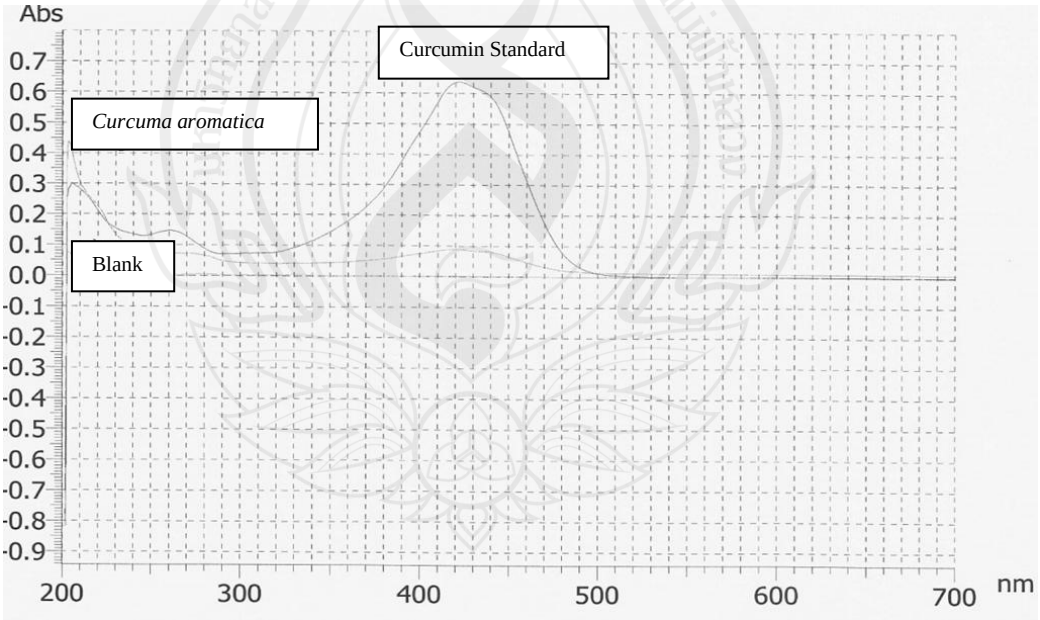


Figure 4.2 Wavelength scanning of Curcumin Standard VS Curcuma *aromatic* (Wan Nang Kham)

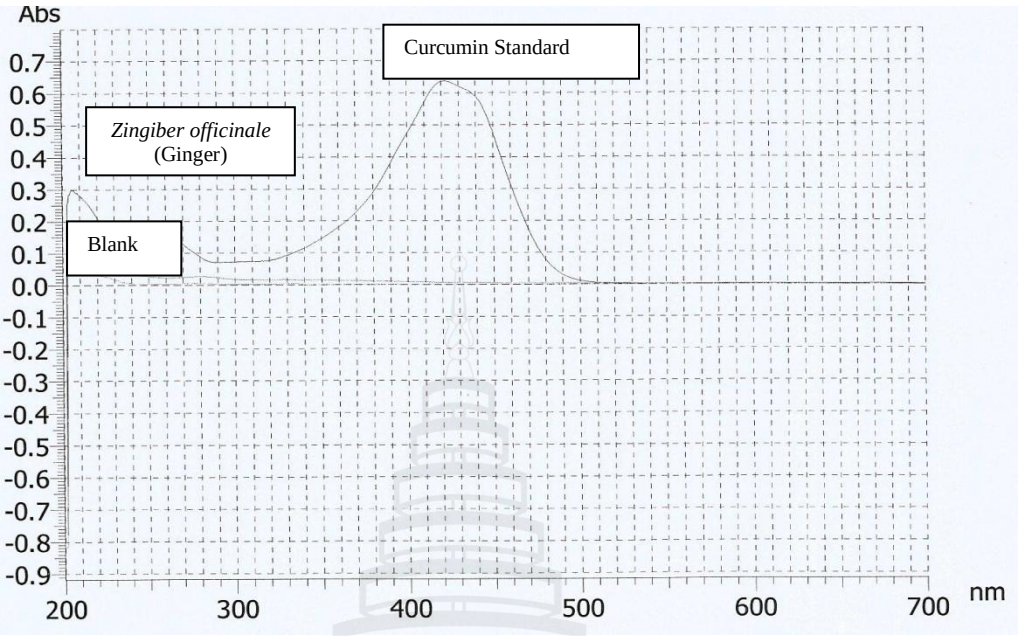


Figure 4.3 Wavelength scanning of Curcumin Standard VS *Zingiber officinale* (Ginger)

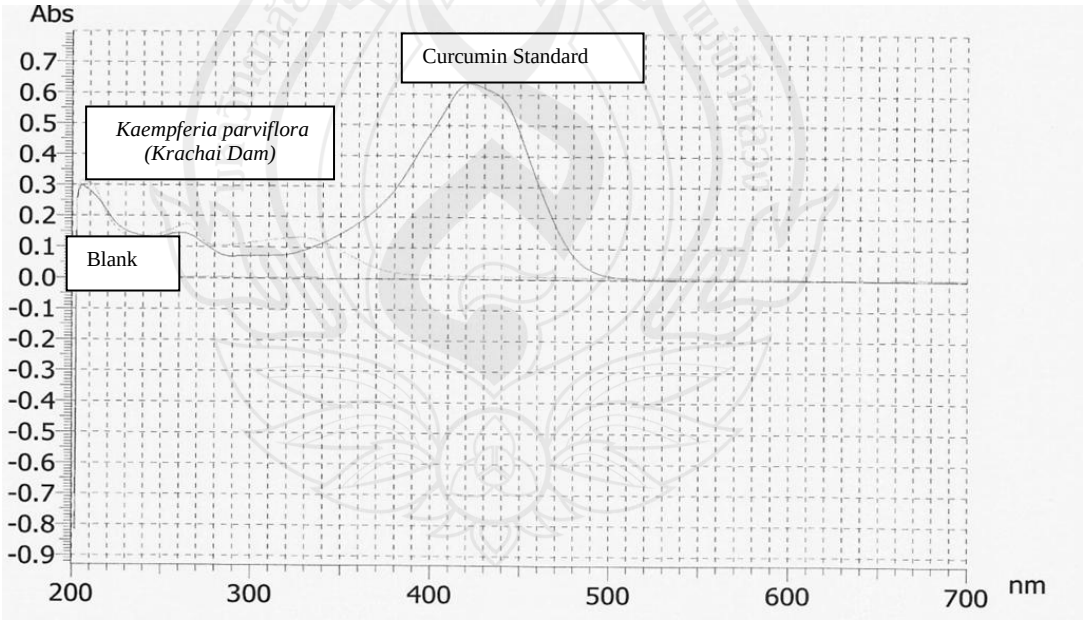


Figure 4.4 Wavelength scanning of Curcumin Standard VS Kaempferia *parviflora (Krachai Dam)*

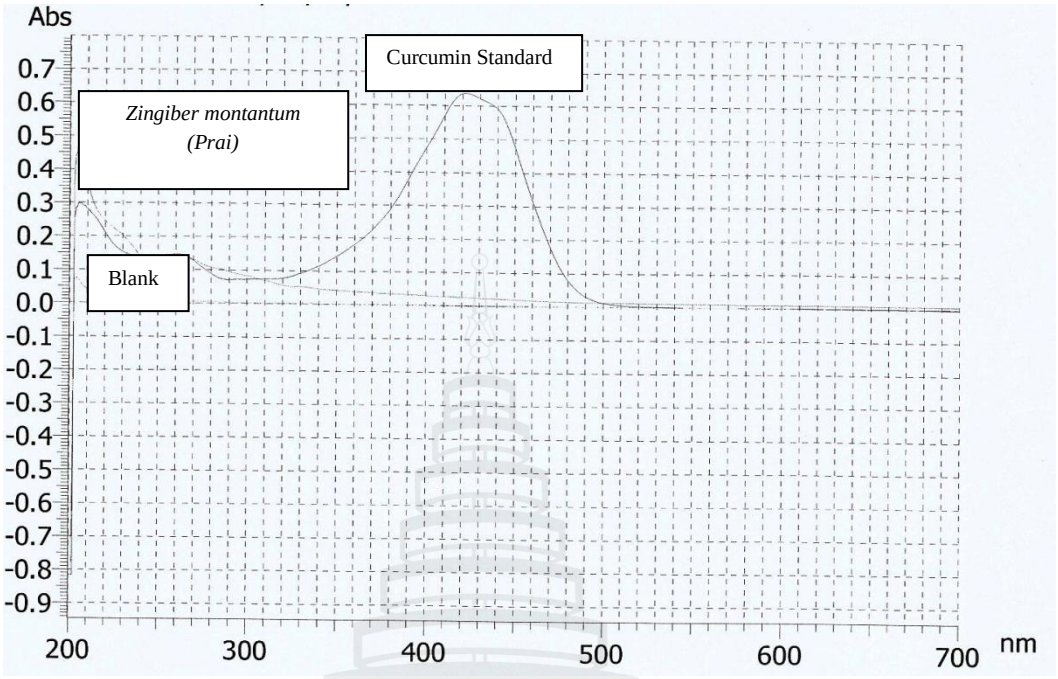


Figure 4.5 Wavelength scanning of Curcumin Standard VS *Zingiber montantum (Prai)*

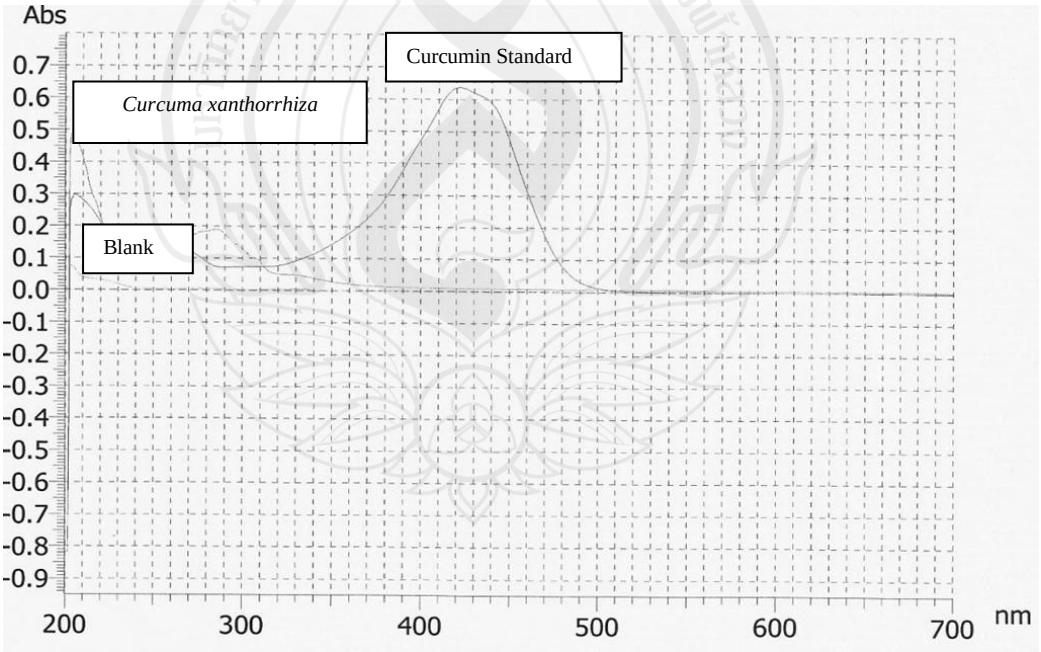


Figure 4.6 Wavelength scanning of Curcumin Standard VS Curcuma *xanthorrhiza*

4.1.2 Linearity

Traditionally, methods are described as linear when there is a directly proportional relationship between the method response and concentration of the analyte in the matrix over the range of analyte concentrations of interest (working range). (United nations office on drugs and crime Vienna, 2009) The working range is predefined by the purpose of the method and may reflect only a part of the full linear range. Acceptance criteria usually involve a Goodness of Fit test. A high correlation coefficient (*r2*) of 0.99 is often used as criterion of linearity.

Five different concentrations of curcumin standard were prepared in methanol from a fresh stock of o.4mg/ml. The data showed a good linear relationship with a correlation coefficient (*r2*) greater than 0.999 for curcumin standard. The linear regression equation of curcuminoids is Y=153.16X+0.0087 and the correlation coefficient (r^2) is 0.9999. (Figure 4.7)

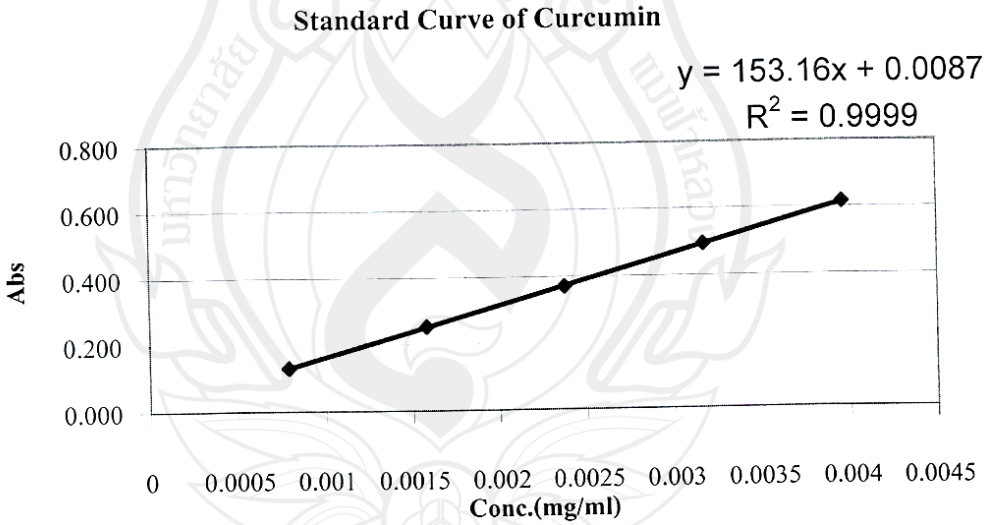


Figure 4.7 Standard Curve of Curcumin

4.1.3 Precision

Precision is a measure of the closeness of the analytical results obtained from a series of replicate measurements of the same measure under the conditions of the method. It reflects the random errors which occur in a method. Two commonly accepted sets of conditions under which precision is measured are repeatable and reproducible conditions. (United nations office on drugs and crime Vienna, 2009)

Intraday precision refers to the use of analytical procedure within a laboratory over a short period of time using the same operator with the same equipment whereas interday precision involves estimation of variations in analysis when a method is used within a laboratory on different days. Repeatability (intraday) was assessed by analyzing the samples six times a day. Intermediate precision (interday) was established by analyzing these samples six times a day for at least three different days.

Precision is usually measured as the coefficient of variation or relative standard deviation of analytical results obtained from independently prepared quality control standards. Acceptable precision at the lower concentrations is 20%. At higher concentrations better precision would be expected. These acceptance criteria may be widened in some instances.

The Standard deviation and %RSD for intra-assay precision and intermediate precision for each sample was showed in the table below. The data indicated that the method had an excellent intraday precision and intermediate precision.

4.1.3.1 Repeatability (Intraday Precision)

Table 4.1 Day 1; Repeatability (Intraday Precision)

Sample	Absorbance (at 420 nm)	Curcuminoids content (%w/w)	Average of Curcuminoids (% w/w)	SD (SD<2)	%RSD
Intraday (n=6)					
Day 1					
Curcuma longa 1	0.286	7.95	8.03	0.06	0.75
Curcuma longa 2	0.292	8.13			
Curcuma longa 3	0.329	8.01			
Curcuma longa 4	0.246	8.04			
Curcuma longa 5	0.319	7.99			
Curcuma longa 6	0.310	8.07			
Curcuma aromatic 1	0.051	1.25	1.31	0.054	4.12
Curcuma aromatic 2	0.051	1.25			
Curcuma aromatic 3	0.055	1.37			
Curcuma aromatic 4	0.055	1.37			
Curcuma aromatic 5	0.053	1.31			
Curcuma aromatic 6	0.056	1.31			

Table 4.2 Day 2; Repeatability (Intraday Precision)

Sample	Absorbance (at 420 nm)	Curcuminoids content (%w/w)	Average of Curcuminoids (% w/w)	SD (SD<2)	%RSD
Intraday (n=6)					
Day 2					
Curcuma longa 1	0.328	8.66	8.71	0.190	2.18
Curcuma longa 2	0.342	9.04			
Curcuma longa 3	0.333	8.80			
Curcuma longa 4	0.328	8.66			
Curcuma longa 5	0.327	8.64			
Curcuma longa 6	0.320	8.47			

Table 4.2 (continue)

Sample	Absorbance (at 420 nm)	Curcuminoids content (%w/w)	Average of Curcuminoids (% w/w)	SD (SD<2)	%RSD
Curcuma aromatic 1	0.056	1.37	1.43	0.055	3.85
Curcuma aromatic 2	0.077	1.49			
Curcuma aromatic 3	0.077	1.49			
Curcuma aromatic 4	0.056	1.37			
Curcuma aromatic 5	0.066	1.40			
Curcuma aromatic 6	0.070	1.44			

Table 4.3 Day 3; Repeatability (Intraday Precision)

Sample	Absorbance (at 420 nm)	Curcuminoids content (%w/w)	Average of Curcuminoids (% w/w)	SD (SD<2)	%RSD
Intraday (n=6)					
Day 3					
Curcuma longa 1	0.340	9.02	9.20	0.170	1.85
Curcuma longa 2	0.346	9.06			
Curcuma longa 3	0.370	9.33			
Curcuma longa 4	0.350	9.10			
Curcuma longa 5	0.374	9.45			
Curcuma longa 6	0.365	9.21			
Curcuma aromatic 1	0.050	1.10	1.08	0.031	2.87
Curcuma aromatic 2	0.051	1.11			
Curcuma aromatic 3	0.049	1.10			
Curcuma aromatic 4	0.049	1.09			
Curcuma aromatic 5	0.047	1.02			
Curcuma aromatic 6	0.048	1.09			

4.1.3.2 Intermediate precision (Interday Precision)

Table 4.4 Intermediate precision (Interday Precision)

Sample	Curcuminoids content (%w/w)			Average of curcuminoids (%w/w)	SD (SD<2)	%RSD
	Day1	Day 2	Day 3			
Curcuma longa	8.03	8.71	9.20	8.65	0.59	6.82
Curcuma aromatic	1.31	1.43	1.08	1.27	0.18	14.17

4.1.4 Accuracy

Accuracy of an analytical method is the closeness of test results to true value. This is a measure of the difference between the expectation of the test result and the accepted reference value due to systematic method and laboratory error. It is usually expressed as a percentage. (United nations office on drugs and crime Vienna, 2009) It is common to estimate accuracy by analysing samples spiked at three different concentrations (low, medium, high) covering the working range. The concentrations of these standards should be different from those used for preparing the calibration curves and they should be prepared from a different stock standard solution. The % recovery for the standard analysis and reference analysis method for all the three concentration level ranged from 94.0% to 104.0% showing that any small change in the drug concentration can be accurately determined with high accuracy. The results obtained from the standard addition and reference analysis method were also found supporting the accuracy of the proposed method. Accuracy data are presented in Table 4.5

Table 4.5 Accuracy of curcumin analysis

Curcumin Sample	Low Conc. (0.000907 mg/ml)		Medium Conc. (0.002721 mg/ml)		High Conc. (0.004534 mg/ml)	
	Measured Value	% Accuracy	Measured Value	% Accuracy	Measured Value	% Accuracy
1	0.00090	99.23	0.00258	94.00	0.00465	102.00
2	0.00095	104.00	0.00270	99.00	0.00459	101.00
3	0.00091	100.00	0.00269	98.00	0.00432	95.00
Mean	0.00092	101.08	0.00266	97.00	0.00452	99.33
SD		2.56		2.65		3.79
%RSD		2.00		2.00		3.00

4.2 Determination of Individual Curcuminoids Content

Analytical method is complied with Thai Herbal Pharmacopoeias 1995 Page44. The official standard method to determine the level of curcuminoids content is UV-Vis spectrophotometry technique. Spectral scan-λmax of curcumin was found to be at 420 nm.

4.2.1 Assays

Wavelength at λ max 420 nm which was used to analyze each curcuminoid content in the samples is the same wavelength recommended by Thai Herbal Pharmacopoeia. Curcumin content in the samples were showed in the table.

Table 4.6 Level of Curcuminoids in sample

Sample	Absorbance at 420 nm	Average of Curcuminoids (%W/W)	Average	SD (SD<2)
Curcuma longa (Khamin Chan) S1	0.320	8.47	8.45	0.028
Curcuma longa (Khamin Chan) S2	0.320	8.43		
Curcuma aromatic (Wan Nang Kham) S1	0.046	0.85	0.92	0.099
Curcuma aromatic (Wan Nang Kham) S2	0.051	0.99		
Zingiber *officinale* (Ginger) S1	0.006	N/A	N/A	N/A
Zingiber *officinale* (Ginger) S2	0.003	N/A	N/A	N/A
Kaempferia *parviflora (Krachai Dam)* S1	0.002	N/A	N/A	N/A
Kaempferia *parviflora (Krachai Dam)* S2	0.008	N/A	N/A	N/A
Zingiber *montantum* (Prai) S1	0.006	N/A	N/A	N/A
Zingiber *montantum* (Prai) S2	0.003	N/A	N/A	N/A
Curcuma *xanthorrhiza* S1	0.016	N/A	N/A	N/A
Curcuma *xanthorrhiza* S2	0.011	N/A	N/A	N/A

CHAPTER 5

CONCLUSION

Six kind of Thai medical herbs were selected to study about curcuminoids level (*Curcuma longa, Zingiber officinale, Kaempferia parviflora, Curcuma aromatic, Zingiber montanum and Curcuma xanthorrhiza).* There are the previous reports studied about curcuminoids in Zingiberaceae species. Various methods were developed for screening curcuminoids. Spectrophotometry is the official technique for the quantitative determination. However, using this technique it is not possible to separate and to quantify the curcuminoids individually. For this reason other techniques such as HPTLC, HPLC, CE and IR have been reported for the quantitative analysis of the individual curcuminoids.

In this study UV-Visible Spectrophotometry method was selected to use for analysis the curcuminiods in samples. This method is recommended as an official method for quantitative analysis of curcuminoids is simple, reliable, accurate, reproducible and specificity. From the result, we can find curcuminoids in two kinds of plants as follow Curcuma *longa* and Curcuma *aromatic* though there are previous study about curcuminoids in Zingiberaceae species. Due to UV-Visible Spectrophotometry method is the official method of THPI for analysis curcumin but It may be not sensitive enough for detect curcuminoids in some plants because of the amount of curcuminoids is very low or some variation in chemical structure. For the further study, a method development is necessary for finding the curcuminoids in that medicinal plants although we can find them in low level but it might be very good bioavailability forms that can provide many pharmacotherapeutic effects for disease prevention and treatment.

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CURRICULUM VITAE

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