PHYTOCHEMICAL AND QUANTIFICATION STUDIES OF SECONDARY METABOLITES IN *CURCUMA XANTHORRHIZA* AND *CURCUMA HEYNEANA*

AINUL INAYAH HAMZAH

UNIVERSITI TEKNOLOGI MALAYSIA

PHYTOCHEMICAL AND QUANTIFICATION STUDIES OF SECONDARY METABOLITES IN *CURCUMA XANTHORRHIZA* AND *CURCUMA HEYNEANA*

A dissertation submitted in partial fulfillment of the
requirements for the award of the degree of
Master of Science

Faculty of Science
Universiti Teknologi Malaysia

NOVEMBER 2017

DEDICATION

I humbly dedicated this thesis to my beloved mother, Hjh. Maimunah bt Hj. Ali, who is the strong and gentle woman, taught me to put full trust on Allah, being passion and hard work.

My late father, Hj. Hamzah bin Hj. Hamdan, the person who instilled the spirit and encouraged me to believe in myself.

My sister, Dr. Zuhra bt Hamzah who has always been my inspiration, supports and motivates me until I arrive at this juncture of my life.

My siblings Hamidah, Alsyukri, Zuhra, Alridza, Alyusra, Alsauffeen and Syafik Effendy for all their love, patience, kindness and support.

I love all of you with all my heart. I hope everyone of you feel happy and proud with what I've done

ACKNOWLEDGEMENT

In the name of Allah, the most Gracious and the most Merciful. Praise to Allah, his majesty and uncountable blessing for the completion of this thesis.

I wish to convey my most sincere appreciation and gratitude to my supervisor, Dr Shajarahtunnur bt Jamil for her unmeasurable professional guidance, encouragement, valuable advices and constructive criticism from beginning of this research until final review of the thesis. Thank you so much Dr. I always be grateful for having the opportunity to study under your supervision. I am also grateful to Dr Norazah bt Basar for her comments, suggestions and guidance which contributed a lot towards the improvement of this thesis, especially knowledge in HPLC.

I am also indebted to the staffs of Universiti Teknologi Malaysia, Mr Amin, Mr Azmi, Mr Subri and Mr Zoulkifli for their help in handling several instruments for data collection in this research and for sharing their knowledge in the data analysis.

My thanks extended to lab friends, Muhammad Kamal Ja'far, Nurshahirah Mohd Rozi, Nur Syamimi Shafie, Siti Awanis Abdullah, Siti Nur Atiqah Md Othman, Zafneza Arriffin, and Zairunnisa Zainudin. Thank you for your cooperation and knowledge sharing which help me a lot in acquiring the skills and knowledge in Natural Products research and also for motivational support that strengthened me mentally and emotionally.

Finally, great appreciation goes to my family for being by my side through thick and thin, encouraging, motivating and supporting me until the completion of this thesis.

Once again, thank you very much.

ABSTRACT

Phytochemical and quantification studies had been carried out on the secondary metabolites in *Curcuma xanthorrhiza* and *C. heyneana*. Phytochemical screening of the methanol extracts of both species revealed the presence of flavonoids, steroids, saponins, phenolics, tannins, terpenoids, proteins and cardiac glycosides. Extraction of the dried rhizomes of both species was conducted by cold maceration method using *n*-hexane, dichloromethane and ethyl acetate to yield *n*-hexane, dichloromethane and ethyl acetate extracts. Isolation of chemical constituents from all crude extracts was performed using silica gel vacuum liquid chromatography and column chromatography. Purification of the *n*-hexane, dichloromethane and ethyl acetate extracts of *C. xanthorrhiza* yielded ar-curcumene, germacrone, xanthorrhizol and curcumin while purification of the *n*-hexane, dichloromethane and ethyl acetate of *C. heyneana* obtained ar-turmerone, β-sitosterol, xanthorrhizol and curcumin. Structure of all pure compounds were characterised using ^{1}H NMR, ^{13}C NMR, DEPT, COSY, HMQC, HMBC, FTIR and also by comparison with literature data. Antioxidant activity of the methanol extracts of *C. xanthorrhiza* and *C. heyneana* together with the pure compounds were conducted using 2,2-diphenyl-1-picrylhydrazyl scavenging, ferric reducing antioxidant potential and 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) assays. The methanol extract of *C. xanthorrhiza*, curcumin and xanthorrhizol displayed strong antioxidant activity in 2,2-diphenyl-1-picrylhydrazyl assay with SC_{50} values of 66.15, 71.88 and 79.99 μg/mL, respectively. The methanol extract of *C. heyneana*, ar-turmerone, germacrone and β-sitosterol showed moderate antioxidant activity in 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) assay with SC_{50} values of 113.71 143.70, 142.95 and 133.13 μg/mL, respectively. Ar-curcumene showed weak antioxidant activity in ferric reducing antioxidant potential assay with SC_{50} value of 160.62 μg/mL. Curcumin, germacrone and xanthorrhizol were selected as the chemical markers for the quantification studies of both *Curcuma* extracts. HPLC analysis gave good precision and accuracy, as well as sufficient limit of detection and quantitation. Curcumin was found to be the major compound in *C. heyneana* with concentration of 16.90 μg/mL while in *C. xanthorrhiza*, the concentration of curcumin was only 0.65 μg/mL. Xanthorrhizol was major compound in methanol extract of *C. xanthorrhiza* but minor compound in methanol extract of *C. heyneana* with the concentrations of 44.12 and 0.82 μg/mL. Germacrone was found in concentration of 4.43 and 8.05 μg/mL in methanol extracts of *C. xanthorrhiza* and *C. heyneana*. Identification of compounds by validation method proved that curcumin, germacrone and xanthorrhizol were present in the methanol extracts of both species. HPLC chromatogram displayed curcumin absorption band at 380 nm with retention time of 4.24 min, while germacrone and xanthorrhizol absorption bands were observed at 270 nm with retention time of 13.32 min and 16.54 min, respectively.

ABSTRAK

Kajian fitokimia dan kuantitatif telah dilakukan ke atas metabolit sekunder dalam *C. xanthorrhiza* dan *C. heyneana.* Penyaringan fitokimia ke atas ekstrak metanol kedua-dua spesies menunjukkan kehadiran flavonoid, steroid, saponin, fenolik, tannin, terpenoid, protein, dan kardiak glikosida. Pengekstrakan sampel kering rizom kedua-dua spesies dilakukan melalui kaedah rendaman menggunakan *n*-heksana, diklorometana dan etil asetat untuk menghasilkan ekstrak *n*-heksana, diklorometana dan etil asetat. Pengasingan sebatian kimia daripada kesemua ekstrak mentah dilakukan menggunakan silika gel kromatografi cecair vakum dan kromatografi turus. Penulenan ekstrak *n*-heksana, diklorometana dan etil asetat *C. xanthorrhiza* menghasilkan ar-kurkumena, germakron, xanthorrhizol dan kurkumin. Manakala penulenan ekstrak *n*-heksana, diklorometana dan etil asetat *C. heyneana* memperoleh ar-turmeron, β-sitosterol, xanthorrhizol dan kurkumin. Struktur kesemua sebatian tulen dicirikan menggunakan ^{1}H NMR, ^{13}C NMR, DEPT, COSY, HMQC, HMBC, FTIR dan juga perbandingan dengan data literatur. Aktiviti antioksidan ekstrak metanol *C. xanthorrhiza* dan *C. heyneana* bersama-sama dengan sebatian tulen dijalankan menggunakan cerakin 2,2-difenil-1-pikrilhidrazil, potensi penurunan antioksidan ferik dan asid 2,2-azino-bis(3-etilbenzotiazoline-6-sulfonik). Ekstrak metanol *C. xanthorrhiza*, kurkumin dan xanthorrhizol menunjukkan aktiviti antioksidan yang kuat dalam cerakin 2,2-difenil-1-pikrilhidrazil dengan nilai SC_{50} 66.15, 71.88 dan 79.99 μg/mL, setiap satu. Ekstrak metanol *C. heyneana*, ar-turmeron, germakron dan β-sitosterol menunjukkan aktiviti antioksidan sederhana dalam cerakin asid 2,2-azino-bis(3-etilbenzotiazoline-6-sulfonik) dengan nilai SC_{50} setiap satu 113.71, 143.70, 142.95 dan 133.13 μg/mL. Ar-kurkumena menunjukkan aktiviti antioksidan yang lemah dalam cerakin potensi penurunan antioksidan ferik dengan nilai SC_{50} 160.62 μg/mL. Kurkumin, germakron dan xanthorrhizol dipilih sebagai penanda kimia dalam kajian kuantifikasi kedua-dua ekstrak *Curcuma*. Analisis HPLC memberikan ketepatan dan kebolehpulihan pengujian yang baik, serta had pengesanan dan kuantifikasi yang mencukupi. Kurkumin adalah sebatian utama dalam *C. heyneana* dengan kepekatan 16.90 μg/mL manakala dalam *C. xanthorrhiza* kepekatan kurkumin hanya 0.65 μg/mL. Xanthorrhizol adalah sebatian utama dalam ekstrak metanol *C. xanthorrhiza* dan sebatian minor dalam ekstrak metanol *C. heyneana* dengan kepekatan 44.12 dan 0.82 μg/mL. Germakron ditemui dengan kepekatan 4.43 dan 8.05 μg/mL dalam ekstrak metanol *C. xanthorrhiza* dan *C. heyneana*. Pengenalpastian sebatian melalui kaedah pengesahan membuktikan kurkumin, germakron dan xanthorrhizol hadir dalam ekstrak metanol kedua-dua spesies. Kromatogram HPLC memaparkan jalur penyerapan kurkumin pada 380 nm dengan masa pengekalan 4.24 min, manakala germakron dan xanthorrhizol dalam metanol menunjukkan jalur penyerapan pada 270 nm dengan masa pengekalan masing-masing 13.32 min dan 16.54 min.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SCHEMES

LIST OF ABBREVATIONS

δ	-	Chemical shift
λ	-	Wavelength
σ	-	Standard Deviation
μ	-	Mean
br d	-	Broad doublet
d	-	Doublet
dd	-	Doublet of doublets
m	-	Multiplet
n	-	Number of replicates
R^2	-	Coefficient of determination
s	-	Singlet
sext	-	Sextet
t	-	Triplet
μg	-	Microgram
μg/mL	-	Microgram per millilitre
μL	-	Microlitre
μM	-	Micromolar
μm	-	Micrometre
cm	-	Centimetre
cm^{-1}	-	Per centimetre
g	-	Gram
Hz	-	Hertz
mg	-	Milligram
min	-	Minute
mL	-	Millilitre
mL/min	-	Millilitre per minute

mm	-	Millimetre
mM	-	Millimolar
mAU	-	Absorbance unit
nm	-	Nanometre
ppm	-	Part per million
ºC	-	Degree celcius
ν_{max}	-	Maximum frequency
%	-	Percent
^{13}C	-	Carbon 13
^{1}H	-	Proton
AA	-	Ascorbic acid
A_{blank}	-	Absorbance value of the control sample
ABTS	-	2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
ACN	-	Acetonitrile
A_{sample}	-	Absorbance value of the tested sample
ATR	-	Attenuated Total Reflectance
CC	-	Column Chromatography
CH_2Cl_2	-	Dichloromethane
CHD	-	*C. heyneana* dichloromethane extract
CHE	-	*C. heyneana* ethyl acetate extract
CHH	-	*C. heyneana n*-hexane extract
CHM	-	*C. heyneana* methanol extract
COSY	-	Homonuclear Correlation Spectroscopy
CXD	-	*C. xanthorrhiza* dichloromethane extract
CXE	-	*C. xanthorrhiza* ethyl acetate extract
CXH	-	*C. xanthorrhiza n*-hexane extract
CXM	-	*C. xanthorrhiza* methanol extract
DAD	-	Diode Array Detector
DEPT	-	Distortionless Enhancement by Polarization Transfer
DPPH	-	2,2-diphenyl-1-picrylhydrazyl
Et_2O	-	Diethyl ether

EtOAc	-	Ethyl acetate
Fe^{3+}-TPTZ	-	Ferric tripyridyltriazine
$FeSO_4.7H_2O$	-	Iron (II) sulphate heptahydrate
FRAP	-	Ferric Reducing Antioxidant Potential
HMBC	-	Heteronuclear Multiple Bond Coherence
HMQC	-	Heteronuclear Multiple Quantum Coherence
HPLC	-	High Performance Liquid Chromatography
IC_{50}	-	Inhibition concentration at 50%
IR	-	Infrared
LOD	-	Limit of detection
LOQ	-	Limit of quantification
MeOH	-	Methanol
MS	-	Mass Spectrometry
NMR	-	Nuclear Magnetic Resonance
R	-	Recovery
R_f	-	Retention factor
RSD	-	Relative Standard Deviation
SC_{50}	-	Percentage of scavenging at 50%
TFA	-	Trifluoroacetic acid
TLC	-	Thin Layer Chromatography
UV	-	Ultraviolet
v/v	-	Volume over Volume
VLC	-	Vacuum Liquid Chromatography

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Natural products chemistry is the studies of various products made up of living material, animals or plants. Globalization era has developed new era on the uses of natural products in commercial market for various purposes such as herbal medicines and cosmetics. Herbal medicines are herbal products that comprises of active ingredient of plant parts such as rhizomes or other plants' materials or mixture. Statistic showed that 80% of the world population, relies on plant based medicines or products which have biomedical benefits and contribute towards improving human health.

Zingiberaceae is the flowering ginger plants widely grow in tropic and subtropic regions, especially in Southeast Asia, including Malaysia, Indonesia and Brunei [1-2]. Peninsular Malaysia is one of the Zingiberaceae richest region with more than 18 genera and 160 species [3-4]. Among the genera that could be found in Malaysia are *Alpinia*, *Achasma*, *Amomum*, *Boesenbergia*, *Costus*, *Curcuma*, *Elettaria*, *Etlingera*, *Globba, Hedychium, Kaempferia*, *Nicolaia* and *Zingiber* [3].

One of the well-known genus in Zingiberaceae family is *Curcuma*, comprises approximately 40 species including *Curcuma aeruginosa, C. angustifolia, C. aromatica, C. domestica, C. longa, C. mangga, C. phaeocaulis, C. viridiflora, C. xanthorrhiza* and *C. zedoaria* [3-4]. Among these species *C. xanthorrhiza* (temu

lawak) and *C. heyneana* (temu giring) are the most commonly being investigated in Malaysia. *Curcuma* is a wild plant species which growth in Southeast Asia with height between 0.5 to 3 ft. It has oblong leaves with solid green or dark red blotch along the centre of the leaves. This genus also has pink to burgundy red or purest white flowers that blooming during summer. It has yellowish orange rhizomes with gingery or lemony aromatic scent. The rhizomes were traditionally used for stomachic and carminative [5].

Phytochemical studies on these species have revealed that these plants are rich of sesquiterpenoids and curcuminoids [3-6]. Hundreds of sesquiterpenoids and curcuminoids had been reported and most of them possessed biological activities such as anti-aging, anti-carcinogenic, anti-coagulation, antimicrobial, antioxidant, anti-inflammatory, anti-mutagenic, estrogenic, hepatoprotective, hypocholesterolic, hypolipidaemic, immunomodulatory and antichronic diseases [6]. Thus, both species were commonly used in Malaysia as one of the active ingredients in the traditional health supplement for the treatment of health problems. Table 1.1 shows the traditional uses of *C. xanthorrhiza* and *C. heyneana* as remedy.

Table 1.1: Traditional uses of *C. xanthorrhiza* and *C. heyneana* as Remedy

Species	Functions
C. xanthorrhiza	1. Indigestion and rheumatism treatment 2. Remedy for the inflammation in postpartum uterine bleeding 3. Stimulating menstruation by stimulating blood flow in pelvic area and uterus 4. Stimulating the flow of bile from liver and cure hepatitis [7] 5. Treating chronic diseases like bacterial infections, hypertension and heart disorders [8-9]
C.heyneana	1. Eliminate the intestinal worms [10] 2. Remedy for abdominal pain, constipation, swelling, arthritis, hepatitis, eye pain, diarrhoea, irregular menstruation, fever and slimy phlegm [10-11] 3. Boost appetite 4. Improve stamina and cure skin diseases [12]

1.2 Problem Statement

Nowadays, plant derived products has been widely utilized throughout consumer in Malaysia. These products can be found in commercial markets as cosmetics, nutraceutical and medicinal products. However, dangerous situation might expose to consumer when the commercialized products were lacking in quantification due to the limited standard chemical markers. Therefore, this study is conducted not only to identify the chemical constituents presence in these plants and determine their potential bioactivities but also to provide the scientific data on the quantification of the chemical markers in the crude extracts rhizomes of *C. xanthorrhiza* and *C. heyneana*.

1.3 Significance of Study

This research focused on quantification of the pure compounds isolated from the rhizomes of *C. xanthorrhiza* and *C. heyneana*. Malaysia is lagging behind in terms of herbal industry even though being home of various species of herbal and medicinal plants. Therefore, it is interesting to conduct scientific studies on local plant species to identify the active chemical markers and provide significant information especially on the identification of the chemical markers of *C. xanthorrhiza* and *C. heyneana*. This study would provide important database and information for development and production of safe and quality herbal products.

1.4 Objectives of Study

Due to the reasons as mentioned above, this research was conducted with several objectives stated as below:

1. To extract the rhizomes of *C. xanthorriza* and *C. heyneana* using cold maceration method.
2. To conduct phytochemical screening on the methanol extract of both species.

3. To fractionate and purify the fractions from both species using chromatographic methods and to characterize the isolated compounds from both species using spectroscopic methods.
4. To evaluate antioxidant activity of methanol extracts and pure compounds from both species using DPPH, FRAP and ABTS
5. To quantify the chemical markers of both species using HPLC.

1.5 Scope of Study

This study focused on the quantification of compounds from the rhizomes of *C. xanthorrhiza* and *C. heyneana*. The dried rhizomes of *C. xanthorrhiza* and *C. heyneana* were extracted using maceration technique. The crude extracts of the dried rhizomes contain various chemical constituents and the major compounds were isolated and purified by Vacuum Liquid Chromatography (VLC) using silica gel 60 (Merck, 230-400 mesh) and column chromatography (CC) using silica gel 60 (Merck, 70-230 mesh). Each fraction was monitored by Thin Layer Chromatography (TLC) using Merck silica gel plate (60 F_{254}, 0.20 mm thickness). Ultra violet light was used to visualize the spots on the TLC plate. The structure of the pure compounds was characterized by 1D and 2D Nuclear Magnetic Resonance Spectroscopy (NMR) and Infrared Spectroscopy (IR). Quantification of the chemical compounds of both *Curcuma* species was conducted using High Performance Liquid Chromatography (HPLC).

The methanol extracts and pure compounds of *C. xanthorrhiza* and *C. heyneana* were tested using antioxidant assays which are 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay, ferric reducing antioxidant power (FRAP) assay and 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) free radical scavenging assay.

REFERENCES

1. Schumann, K. (1904). Zingiberaceae. *Das Pflanzenreich*, *46*(*4*).
2. Pongboonrod, S. (1979). Medicinal Plants of Thailand. 448.
3. Holttum, R. E. (1950). The Zingiberaceae of the Malay Peninsula. *Gard. Bull., Singapore*, *13*(1), 1-249.
4. Ibrahim, H., Khalid, N., and Hussin, K. (2007). Cultivated Gingers of Penisular Malaysia: Utilization, Profiles and Micropagation. *Gardens' Bulletin Singapore, 59*(1, 2)*,* 71-88.
5. Skornickova, J., and Sabu, M. (2005). The Identity and Distribution of *Curcuma xanthorrhiza* Roxb. (Zingeberacae). *Garden Bulletin Singapore,* 57, 199-210.
6. Ravindran, P. N., Babu, K. N., and Sivaraman, K. (2007). Turmeric The Genus *Curcuma*. *CRC Press*, *45*.
7. Lin, S. C., Lin, C. C., Lin, Y. H., Supriyatna, S., Teng, C. W., Am, J., and Chin, M. (1995), *23*, 243.
8. Yasni, S., Imaizumi, K., Sin, K., Sugano, M., and Nonaka, G. (1994). *Food Chem Toxic, 32*, 273.
9. Diastuti, H., Syah, Y. M., Juliawaty, L. D., and Singgih, M. (2014). Antibacterial *Curcuma xanthorrhiza* Extract and Fractions. *Journal of Mathematical and Fundamental Sciences*, *46*(3), 224-234.
10. Nawangningrum, D., Widodo, S., Suparta, I. M., and Holil, M. (2004). Penyakit dan Pengobatan Ramuan Tradisional, *8*(2), 43-45.
11. Dharma, A. (1985). Tanaman Obat Tradsional Indonesia. 265-266.
12. Atun, S., Arianingrum, R., Aznam, N., and Nurestri, S. (2010). Phytochemical Study on Some *Curcuma* Species from Indonesia. 25.
13. Halim, M. R., Tan, M. S., Ismail, S., and Mahmud, R. (2012). Standardization and Phytochemical Studies of *Curcuma xanthorrhiza* Roxb. *International Journal of Pharmacy and Pharmaceutical Science, 4*(3), 606-610.

14. Mangunwardoyo, W. (2012). Antimicrobial and Identification of Active Compounds *Curcuma xanthorrhiza* Roxb. *International Journal of Basic and Applied Sciences Ijbas-Ijens, 12*(1).

15. Anjusha, S., and Gangaprasad, A. (2014). Phyochemical and Antibacterial Analysis of Two Important *Curcuma aromatica* Salisb. and *Curcuma xanthorrhiza* Roxb. (Zingeberacae) . *Journal of Pharmacognosy and Phytochemistry, 3*(3), 50-53.

16. Lee, J., Jung, Y., Shin, J., Kim, H., Moon, B., Ryu, D., & Hwang, G. (2014). Secondary Metanolite Profiling of Curcuma Species Grown at Different Locations using GC/TOF and UPLC/Q/TOF MS. *Molecules, 19*(7), 9535-9551.

17. Haining, L., and Gaimie, S. (2012). Naturally Occuring Diarylheptanoids - A Supplementary Version. *Rec. Nat. Prod, 6*(4), 321-333.

18. Uehara, S., Yasuda, I., Akiyama, K., Morita, H., Takeya, K., and Itokawa, H. (1987). Diarylheptanoids from the Rhizomes of *Curcuma xanthorrhiza* and *Alpina officinarum*. *Chemical and Pharmaceutical Bulletin, 35*(8), 3298-3304.

19. Pothitirat, W., and Gritsanapan, W. (2005). Qualitative Analysis of Curcumin, Demethoxycurcumin and Bisdemethoxycurcumin in the Crude Curcuminoid Extract from *Curcuma longa* in Thailand by TLC-densitometry. *Journal of Pharmaceutical Sciences, 32*(1-2), 23-30.

20. Payton, F., Sandusky, P., and Alworth, W. (2007). NMR Study of the Solution Structure of Curcumin. *Journal of Natural Products, 70*(2), 143-146.

21. Jitoe, A., Masuda, T., Tengah, I., Suprapta, D., Gara, I., and Nakatani, N. (1992). Antioxidant Activity of Tropical Ginger Extracts and Analysis of the Contained Curcuminoids. *Journal Agricultural Food Chemistry, 40*, 1337-1340.

22. Morikawa, T., Matsuda, H., Ninomiya, K., and Yoshikawa, M. (2002). Potent Protective Effect of Sesquiterpenes and Curcumin from *Curcuma Zedoaria* Rhizome on Liver Injury Induced by D-Galatosamin/Lipopolysaccharide or Tumor Necrosis Factor-alpha. *Biol. Pharm. Bull., 25*(5), 627-631.

23. Masuda, T., Isobe, J., Jitoe, A., and Nakatani, N. (1992). Antioxidative Curcuminoids from Rhizomes of *Curcuma xanthorrhiza*.

24. Keeratinijakal, V., and Konkiatpaiboon, S. (2017). Distribution of Phytoestrogenic Diarylheptanoids and Sesquiterpenoids Components in *Curcuma comosa* Rhizomes and its Related Species. *Revista Brasileira de Farmacognosia, 27*(3), 290-296.

25. Claeson, P., Panthong, A., Tuchinda, P., Reutrakul, V., Kanjanapothi, D., Taylor, W., and Santisuk, T. (1993). Three Non-Phenolic Diarylheptanoids with Anti-Inflammatory Activity from *Curcuma xanthorrhiza*. *Planta Medica, 59*(5), 451-454.

26. Claeson, P., Pongprayoon, U., Sematong, T., Tuchinada, P., Reutrakul, V., Sootornsaratune, P., and Taylor, W. (1996). Non-Phenolic Linear Diarylheptanoids from *Curcuma xanthorrhiza*: A Novel Type of Topical Anti-Inflammatory Agents: Structure-Activity Relationship. *Planta Med., 62*(3), 236-240.

27. Claeson, P., Panthong, A., Tuchinda, P., Reutrakul, V., Kanjanapothi, D., Taylor, W., and Santisuk, T. (2007). Three Non-Phenolic Diarylheptanoids with Anti-Inflammatory Activity from *Curcuma xanthorrhiza*. *Planta Medica, 59*(5), 451-454.

28. Kiuchi, F., Goto, Y., Sugimoto, N., Akao, N., Kondo, K., and Tsuda, Y. (1993). Nematocidal Activity of Turmeric: Synergistic Action of Curcuminoids. *Chem. Pharm. Bull., 41*, 1640-1643.

29. Nahar, L., and Sarker, S. (2007). Phytochemistry of the Genus *Curcuma* in Turmeric. *Press: Boca Raton*, 71-106.

30. Chokchaisiri, R., Pimkaew, P., Piyachaturawat, P., Chalermglin, R., and Suksamrarn, A. (2014). Cytotoxic Sesquiterpenoids and Diarylheptanoids from the Rhizomes of *Curcuma elata* Roxb. *Records of Natural Products, 8*(1), 46.

31. Breitmaier, E. (2006). Terpenes: Importance, General Structure and Biosynthesis. . *Terpenes: Flavors, Fragrances, Pharmaca, Pheromones*, 1-9.

32. Sirat, H., Jamil, S., and Hussain, J. (1998). Essential Oil of *Curcuma aeruginosa* Roxb. from Malaysia. *Journal of Essential Oil Research, 10*(4), 453-458.

33. Srivastava, A., and Shah, N. (2011). Constituents of the Rhizome Essential Oil of *Curcuma amada* Roxb. from India.

34. Jarikasem, S., Thubthimthed, S., Chawananoraseth, K., and Suntorntanasat, T. (2003). Essential Oil from Three Curcuma Species Collected in Thailand. *Bioprospecting and Ethnopharmacology, 1*(675), 37-40.

35. Oguntimein, B., Weyerstahl, P., and Marshall, H. (1990). Essential Oil of *Curcuma longa* L. Leaves. *Flav. Frag. J., 5*, 89-90.

36. Leela, N., Tava, A., Shafi, P., John, S., and Chempakam, B. (2002). Chemical Composition of Essential Oils of Turmeric (*Curcuma longa* L.). *Acta Pharm*(52), 137-141.

37. Awasthi, P., and Dixit, S. (2009). Chemical Composition of *Curcuma longa* Leaves and Rhizomes Oil from the Plains of Northen India. *Journal of Young Pharmacists, 1*(4), 312.

38. Jantan, I., Ahmad, A., Ali, N., Ahmad, A., and Ibrahim, H. (1999). Chemical Composition of the Rhizome Oils of Four Curcuma Species from Malaysia. *Journal of Essential Oil Research, 11*(6), 719-723.

39. Mccaron, M., Mills, A., Whittaker, D., Sunny, T., and Verghese, J. (1995). Comparison of the Monoterpenes Derived from Green Leaves and Fresh Rhizomes of *Curcuma longa* L. from India. *Flavour and Fragrance Journal, 10*(6), 355-357.

40. Zhu, J., Lowernedza, A., Hong, M., Jie, S., Wang, Z., Yingmao, D., and Brantner, A. (2013). Chemical Composition and Antimicrobial Activity of Three Essential Oils from *Curcuma wenyujin*. *Natural Product Communications, 8*(4), 523-526.

41. Halim, A., and Rohaimi, M. (2014). Validation of GC-MS Method for Standardization of *Curcuma xanthorrhiza* Extracts using Biochemical Markers, Ar-curcumene and Xanthorrhizol. *Doctoral Dissertation*. *Universiti Sains Malaysia*. 87-95.

42. Jantan, I., Saputri, F., Qaisar, M., and Buang, F. (2012). Correlation between Chemical Composition of *Curcuma domestica* and *Curcuma xanthorrhiza* and their Antioxidant Effect on Human Low-Density Lipoprotein Oxidation. *Evidence-based Complementary and Alternative Medicine*. 1-10.

43. Uehara, S., Yasuda, I., Takeya, K., and Itokawa, H. (1992). Terpenoids and Curcuminoids of the Rhizomes of *Curcuma xanthorrhiza* Roxb . *Journal of the Pharmaceutical Society of Japan, 112*(11), 817-823.

44. Feng, J., Xu, M., Huang, X., Liu, H., Lai, M., and Wei, M. (2013). GC/MS Analysis of Essential Oil from *Curcuma aromatica* Rhizomes of Different Growth Periods. *Journal of Chinesee Medicinal Materials, 36*(12), 1926-1929.

45. Diastuti, H., Syah, Y., Juliawaty, L., and Singgih, M. (2014). Antibacterial Activity of Germacrane Type Sesquiterpenes from *Curcuma heyneana* Rhizomes. *Indonesian Journal of Chemistry, 14*(1), 32-36.

46. Park, J., Mohamed, M., Jung, Y., Shrestha, S., Lee, T., Lee, C., Baek, N. (2014). Germacrane Sesquiterpened Isolated from the Rhizomes of *Curcuma xanthorrhiza* Roxb. Inhibit UVB-Induced Upregulation of MMP-1,-2 and -3 Expression in Human Keratonocytes. *Archieves of Pharmacal Research*. 93-99

47. Sukari, A., Wah, T., Saad, S., Rashid, N., Rahmani, M., Lajis, N., and Hin, T. (2010). Bioactive Sesquiterpenes from *Curcuma ochrorhiza* and *Curcuma heyneana*. *Natural Product Research, 24*(9), 838-845.

48. Zhang, C., Fan, P., Li, M., and Lou, H. (2014). Two New Sesquiterpenoids from the Rhizomes of *Curcuma xanthorrhiza*. *Helvetica Chimica Acta, 97*(9), 1295-1300.

49. Saifudin, A., Tanaka, K., Kadota, S., and Tezuka, Y. (2013). Sesquiterpenes from the Rhizomes of *Curcuma heyneana*. *Jounal of Natural Products, 76*(2), 223-229.

50. Li, W., Feng, J., Xiao, Y., Wang, Y., Xue, X., and Liang, X. (2009). Three Novel Terpenoids from the Rhizomes of *Curcuma longa*. *Journal of Asian Natural Products Research, 11*(6), 569-575.

51. Garg, S., Naquvi, A., Bansal, R., Bahl, J., and Kumar, S. (2005). Chemical Composition of the Essential Oil from the Leaves of *Curcuma zedoaria* Rosc. of Indian Origin. *Journal of Essential Oil Research, 17*(1), 29-31.

52. Zwaving , J., and Bos, R. (1992). The Essential Oil of Five *Curcuma* Species. *Flavors, Fragrances, Journal, 7*, 19-22.

53. Firman, K., Kinoshita, T., Itai, A., and Sankawa, U. (1988). Terpenoids from *Curcuma heyneana*. *Phytochemistry, 27*(12), 3887-3891.

54. Sirat, H., and Lee, L. (2009). Chemical Components of the Rhizome Oil of *Curcuma heyneana* Val. *Malaysian Journal of Science, 28*(3), 323-328.

55. Ahmad, S., Ali, M., Ansari, S., and Ahmed, F. (2011). Phytoconstituents from the Rhizomes of *Curcuma aromatica* Salisb. *Journal of Saudi Chemical Society, 15*(3), 287-290.

56. Singh, S., Kumar, J., Saikia, D., Shanker, K., Thakur, J., Negi, A., and Banerjee, S. (2010). A Bioactive Labdane Diterpenoid from *Cucuma amada* and its Semi synthethic Analogues as Antitubercular Agents. *European Journal of Medicinak Chemistry, 45*(9), 4379-4382.

57. Abas, F., Lajis, N., Shaari, K., Israf, D., Stanslas, J., Yusuf, U., and Raof, S. (2005). A Labdane. Diterpene Glucoside from the Rhizomes of *Curcuma mangga*. *Journal Natural Product, 68*(7), 1090-1093.

58. Afzal, A., Oriqat, G., Khan, M., Jose, J., and Afzal, M. (2013). Chemistry and Biochemistry of Terpenoids from *Curcuma* Related Species. *Journal of Biologically Active Products from Nature, 3*(1), 1-55.

59. Nurcholis, W., Ambarsari, L., Sari, N. L., and Darusman, L. K. (2012). Curcuminoid Contents, Antioxidant and Anti-Inflammatory Activities of *Curcuma xanthorrhiza* Roxb. and *Curcuma domestica* Val. Promising Lines from Sukabumi of Indonesia. *Prosiding Seminar Nasional Kimia Unesa*, 284-292.

60. Nisar, T., Iqbal, M., Raza, A., Safdar, M., Iftikhar, F., and Waheed, M. (2015). Estimation of Total Phenolic and Free Radical Scavenging of Turmeric (*Curcuma longa*). *American-Eurasian J. Agric. & Environ. Sci, 15*(7), 1272-1277.

61. Donipati, P., and Sreeramulu, S. (2015). In Vitro Bio Evaluation and Correlation of Antioxidant Activity of Different Extracts of *Curcuma amada*. *Journal of Pharmaceutical, Chemical and Biological Science, 3*(3), 373-377.

62. Hutapea, J., Djumidi, and Sutjipto. (2001). Indonesia Medicinal Plants Inventory: *Curcuma xanthorrhiza* Roxb. *1*.

63. Reddy, A., and Lokesh, B. (1996). Effect of Curcumin and Eugenol on Iron-Induced Hepatic Toxicity in Rats. *Toxicology, 107*(1), 39-45.

64. Naksuriya, O., Okonogi, S., Schiffelers, R., and Hennink, W. (2014). Curcumin Nanoformulations: A Review of Pharmaceutical Properties and Preclinical Studies and Clinical Data Related to Cancer Treatment. *Biomaterials, 35*, 3365-3383.

65. Taher, Z., and Sarmidi, R. (2015). Optimization Processing Parameters for *Curcuma xanthorrhiza* Oleoresin Yield and its Antioxidant Activity. *International Journal of Biotechnology for Wellness Industries, 4*, 97-102.

66. Lukiati, B., Aulanni'am, and Darmanto, W. (2012). The Effects of *Curcuma heyneana* Ethanolic Extract on the Superocide Dismutase Activity and Histological Pancreas of Type 1 Diabetes Mellitus Rats. *International Journal of Basic and Applied Sciences, 12*(2).

67. Jalip, I., Suprihatin, Wiryanti, I., and Sinaga, E. (2013). Antioxidant Activity and Total Flavonoids Content of *Curcuma* Rhizome Extract. *Proceeding International Conference*, 93-99.

68. Asouri, M., Atae, R., Ahmadi, A., Amini, A., and Moshaei, R. (2013). Antioxidant and Free Radical Scavenging Activities of Curcumin. *Asian Journal of Chemistry, 25*(13), 7593-7595.

69. Hamdi, O., Ye, L., Kamarudin, M., Hazni, H., Paydar, M., Looi, C., and Awang, K. (2015). Neuroprotective and Antioxidant Constituents from *Curcuma zedoaria* Rhizomes. *Records of Natural Products, 9*(3), 349.

70. Gupta, R., Sharma, A., Dobhal, M., Sharma, M., and Gupta, R. (2011). Antidiabetic and Antioxidant Potential of Beta-sitosterol in Streptozotocin-induced Experimental Hyperglycemia. *Journal of Diabetes, 3*(1), 29-37.

71. Jain, V., Prasad, V., Pal, R., and Singh, S. (2007). Standardization and Stability Studies of Neuroprotective Lipid Soluble Fraction Obtained from *Curcuma longa*. *Journal of Pharmaceutical and Biomedical Analysis, 44*(5), 1079-1086.

72. Paramasivam, M., Aktar, M., Poi, R., Banerjee, H., and Bandyopadhyay, A. (2008). Occurence of Curcuminoids in *Curcuma longa*: A Quality Standardization by HPTLC. *Bangladesh Journal of Pharmacognosy, 3*(2), 55-58.

73. Li, S., Yuan, W., Deng, G., Wang, P., Yang, P., & Aggarwal, B. (2011). Chemical Composition and Product Quality Control of Turmeric (*Curcuma longa* L.). *Pharmaceutical Crops, 2*, 28-54.

74. Yang, F., Li, S., Chen, Y., Lao, S., Wang, Y., Dong, T., and Tsim, K. (2005). Identificationa and Quantification of Eleven Sesquiterpenes in Three Species of *Curcuma* Rhizomes by Pressurized Liquid Extraction and Gas Chromatography-Mass Spectroscopy. *Journal of Pharmaceutical and Biomedical Analysis, 39*(3), 552-558.

75. Hong, S., Lee, G., Rahman, S., Hamdi, O., Awang, K., Nugroho , N., and Malek, S. (2014). Essential Oil Content of the Rhizomes of *Curcuma purpurascens* (Temu tis) and its Antiproliferative Effect on Selected Human Carcinoma Cell Lines. *The Scientific World Journal*. 1-7.

76. Devi, L., Rana, V., Devi, S., Verdeguer, M., and Blazquez, M. (2012). Chemical Composition and Antimicrobial Activity of the Essential Oil of *Curcuma leucorhiza* Roxb. *Journal of Essential Oil Research, 24*(6), 533-538.

77. Erpina, E., Rafi, M., Daruman, L., Vitasari, A., Putra, B., and Rohaeti, E. (2017). Simaltaneous Quantification of Curcuminoids and Xanthorrhizol in *Curcuma xanthorrhiza* by High Performance Liquid Chromatography. *Journal of Liquid Chromatography and Related Technologies, 18*(14).

78. Rohman, A., Sudjadi, Devi, Ramadhani, D., and Nugroho, A. (2015). Analysis of Curcumin in *Curcuma longa* and *Curcuma xanthorrhiza* using FTIR Spectroscopy and Chemometrics. *Research Journal of Medicinal Plant, 9*(4), 179-186.

79. Du, Z., Zheng, S., Chen, G., and Lv, D. (2011). A Short Synthesis of Bisabolane Sesquiterpenes. *Molecules, 16*(9), 8053-8061.

80. Yan, J., Chen, G., Tong, S., Feng, Y., Sheng, L., and Lou, J. (2005). Preparative Isolation and Purification of Germacrone and Curdione from the Essential Oil of the Rhizomes of *Curcuma wenyujin* by High-Speed Counter-Current Chromatography. *Journal of Chromatography, 1070*(1), 207-221.

81. Verma, S. (2014). Development of a Rapid Separation Process for Curcumin from *Curcuma longa* L. Rhizomes and its Quantification by HPLC-PDA. *World Journal of Pharmacy and Pharmaceutical Sciences, 3*(7), 752-761.

82. Lee, H., Shin, W., Song, C., Cho, K., and Ahn, Y. (2001). Insectidal Activities of Ar-turmerone Identified in *Curcuma longa* Rhizome against Nilaparvata lugens (Homopetra: Delphacidae) and Plutella xylostella (Lepidoptera: Yponomeutidae). *Journal Asia-Pacific Etnomol, 4*(2), 181-185.

83. Chaturvedula, V., and Prakesh, I. (2012). Isolation of Stigmasterol and Beta-sitosterol from Dichloromethane Extract of *Rubus suavissomus*. *International Pharmaceutical Journal, 9*(1), 239-242.

84. Pyrzynska, K., & Pekal, A. (2013). Application of Free Radical Diphenylpicrylhydrazyl (DPPH) to Estimate Antioxidant Capacity of Food Samples. *Anal. Methods*(5), 428.

85. Ja'afar, M., Jamil, S., & Basar, N. (2017). Antioxidant Activity of Leaf Extracts of *Globimetula braunii* (Engler) *van tiegh Parasitizing* on Piliothonningii and *Parkia biglobosa*. *Journalteknologi, 79*(5), 43-47.

86. Thaipong, K., Boonprakob, U., Crosby, K., Zevallos, L., & Byrne, D. (2006). Comparison of ABTS, DPPH, FRAP and ORAC Assyas for Estimating Antioxidant Activity from Guava Fruit Extracts. *Journal of Food Composition and Analysis, 19*(6), 669-675.

87. Shabir, G. (2004). A Practical Approach to Validation of HPLC Methods under Current Good Manufacturing Practices. *Jornal of Validation Technology, 10*, 210-218.

88. Shabir, G. (2005). Step-by-step Analytical Methods Validation and Protocol in the Quality System Compliance Industry . *Journal of Validation Technology, 10*, 314-325.

89. Anguilar, M., Osorio, N., Bernal, I., Navarrete, A., & Bye, R. (2007). Development and Validation of a Liquid Chromatography Method for Quantification of Xanthorrhizol in Roots of *Iostephane heterophylla* (Cav). *Journal of AOAC International, 90*(4), 892-896.

90. Gugulothu, D., and Patravale, V. (2012). A New Stability-Indicating HPLC Method for Simultaneous Determination of Curcumin and Celecoxib at Single Wavelength: An Application to Nanoparticle Formulation. *Pharmaceut Anal Acta, 4*(4), 2135-2153.

91. Bakalyar, S., and Henry, R. (1976). Variables Affecting Precision and Accuracy in High-Performance Liquid-Chromatography. *Journal of Chromatography*(126), 327-345.

92. Sharma, K., Agrawal, S., & Gupta, M. (2012). Development and Validation of UV-Spectrophotometric Method for the Estimation of Curcumin in Bulk Drug and Pharmaceutical Dosage Forms. *International Journal of Drug Development and Research*.

93. Lu, D. Y., Cao, Y., Li, L., Zhu, Z. Y., Dong, X., Zhang, H., and Lou, Z. Y. (2011). Comparative Analysis of Essential Oils found in Rhizomes *Curcuma* and Radix *Curcuma* by High Performance Liquid Chromatography. *Journal of Pharmaceutical Analysis*, *1*(3), 203-207.

94. Iqbal, E., Salim, K., and Lim, L. (2015). Phytochemical Screening, Total Phenolics and Antioxidant Activities of Bark and Leaf Extracts of *Goniothalamus velutinus* (Airy Shaw) from Brunei Darulssalam. *Journal of King Saud University Science, 27*(3), 224-232.

95. Sofowora, A. (1993). Medicinal Plants and Traditional Medicinal in Africa. *2*, 134-156.

96. Firdouse, S., and Alam, P. (2011). Phytochemical Investigation of Extract of *Amorphophallus campanulatus* tubers. *International journal of Phytomedicine, 3*(1), 32.

97. Yadav, M., Chatterji, S., Gupta, S., and Watal, G. (2014). Preliminary Phytochemical Screening of Six Medicinal Plants Used in Traditional Medicine. *International Journal of Pharmacy and Pharmaceutical Science, 6*(5), 539-542.

98. Najihah, M., Mawardi, R., Ee, G., Mohd, A., Maizatuakmal, Y., Muhammad, A., and Go, G. (2012). Antioxidant, Antimicrobial and Tyrosinase Inhibitiry Activities of Xanthones Isolated from *Atorcapus obtusus* F. M. Jarret. *Molecules, 17*, 6071-6082.

99. Fu, R., Zhang, Y., Guo, Y., and Chen, F. (2014). Determination of Phenolic Content and Antioxidant Activities of Extracts of *Jatropha curcas* L. Seed Shell, a By-Product a New Source of Natural Antioxidant. *Industrial Crops and Products, 58*, 265-270.

100. Zou, Y., Chang, S., Gu, Y., and Qian, S. (2011). Antioxidant Activity and Phenolic Composition of *Lentlis* (*Lens Culinaris var Morton*) Extracts and its Fractions. *Journal of Agricultural Food Chemistry, 59*(6), 2268-2276.

101. Channarong. (2012). Total Reducing Antioxidant Capacity of Thai Herbal Aromatic Powder (*Ya Hom*) Measured by FRAP Assay. *Thai Pharmaceutical and Health Science Journal, 7*(3), 111-114.

102. Shahwar. (2012). Ferric Reducing Antioxidant Power of Essential Oils Extracted from *Wucalptus* and *Curcuma* Species. *Asian Pacific Journal of Tropical Biomedicine*, 1633-1636.