CHEMICAL CONSTITUENTS OF *Curcuma heyneana* AND *Curcuma zedoaria*

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CHEMICAL CONSTITUENTS OF *Curcuma heyneana* AND *Curcuma zedoaria*

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With lots of love to my mother, Norlemar Bt Deris,
the late father, Mohd Yusoff B.Che Deraman
and family members,
for always standing by my side

To my supervisor, Prof. Dr. Hasnah Bt Mohd Sirat
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ABSTRACT

Curcuma zedoaria and C. heyneana are two species belong to the Zingiberaceae family. The essential oils and phytochemical of these two species have been studied. The essential oils of the fresh rhizomes of *C. zedoaria* and *C. heyneana* were extracted by using the hydrodistillation technique and analysed by gas chromatography and gas chromatography–mass spectrometry (GC-MS). Hydrodistillation of *C. zedoaria* gave 71.3% while *C. heyneana* yielded 63.6% of essential oils comprised of monoterpenes, sesquiterpenes, oxygenated monoterpenes, and oxygenated sesquiterpenes. The major component presence in *C. zedoaria* was furanogermenone (42.2%), while in *C. heyneana* were ar-turmerone (13.9%) and germacrone (14.4%). Extraction of dried rhizomes of both *Curcuma* species was done by soxhlet extraction method using chloroform. Fractionation and purification using vacuum liquid chromatography (VLC) and preparative thin layer chromatography (PTLC) successfully isolated ar-turmerone, curcumin and α-turmerone from *C. heyneana* while furanogermenone and isofuranodiene were obtained from *C. zedoaria*. Their structures were elucidated by spectroscopic method such as IR, ^{1}H NMR and ^{13}C NMR spectroscopies. The antioxidant activity of essential oils and crude extracts were carried out using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), and ferric reducing antioxidant power (FRAP) assay. The antioxidant screenings revealed that crude extract of *C. heyneana* had strong antioxidant radical scavenging with DPPH assay (IC_{50} 50.93 µg/mL), ABTS (IC_{50} 90.90 µg/mL) and FRAP (85.70 mM).

ABSTRAK

Curcuma zedoaria dan *C. heyneana* adalah dua spesis yang terdapat dalam kumpulan Zingiberaceae. Kandungan minyak pati dan fitokimia daripada dua jenis spesis tersebut telah dikaji. Minyak pati daripada kedua-dua jenis rizom diekstrak dengan kaedah penyulingan hidro dan seterusnya dianalisis menggunakan kromatografi gas (KG) dan kromatografi gas-spektrometri jisim (KG-SJ). Penyulingan hidro ke atas rizom segar *C. zedoaria* telah memberikan 71.3% manakala *C. heyneana* menghasilkan 63.6% minyak pati, yang diklasifikasikan sebagai monoterpena, sesquiterpena, oksigen monoterpena dan oksigen sesquiterpena. Furanogermenon (42.2%) dikenalpasti sebagai komponen utama dalam *C. zedoaria* manakala ar-turmeron (13.9%) dan germakron (14.4%) ditemui sebagai komponen utama dalam *C. heyneana*. Pengekstrakan kandungan fitokimia daripada rizom kering dilakukan secara kaedah pengekstrakan 'soxhlet' menggunakan kloroform. Pemeringkatan dan penulenan ke atas ekstrak mentah menggunakan kromatografi cecair vakum (KCV) dan kromatografi lapisan nipis penyediaan (KLNP) telah berjaya mengasingkan ar-turmeron, kurkumin dan α-turmeron daripada *C. heyneana* manakala furanogermenon dan isofuranodiena diperolehi daripada *C. zedoaria.* Struktur sebatian tersebut telah dikenalpasti melalui teknik spektroskopi iaitu IM, RMN ^{1}H, dan RMN ^{13}C. Ujian antioksidan ke atas minyak pati dan ekstrak mentah kedua-dua jenis *Curcuma* spesis telah dijalankan menggunakan 2,2-difenil-1-pikrilhidrazil (DFPH), asid 2,2'-azino-bis(3-etilbenzotiozolin-6-sulfonik) (ABTS), dan pengurangan kuasa antioksidan ferik (FRAP). Penyaringan antioksidan menunjukkan ekstrak mentah *Curcuma heyneana* mempunyai aktiviti antioksidan yang kuat dengan ujian DFPH (IC_{50} 50.93 µg/mL), ABTS (IC_{50} 90.90 µg/mL) dan FRAP (85.70 mM).

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LIST OF ABBREVIATIONS

A	-	Alpha
Ar	-	Aromatic
ABTS	-	2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
B	-	Beta
^{13}C	-	Carbon
C.	-	*Curcuma*
Cm		Centimeter
Δ	-	Chemical shifts
$CHCl_3$	-	Chloroform
R^2	-	Coefficient of determination
J	-	Coupling constant
$CDCl_3$	-	Deuterated chloroform
Et_2O	-	Diethyl ether
DEPT	-	Distortionless Ehnancement by Polarization Transfer
DPPH	-	2,2-Diphenyl-1-picrylhydrazyl
D	-	Doublet
Dd	-	Doublet of doublets
EtOAc	-	Ethyl acetate
$FeCl_3.6H_2O$	-	Ferric Chloride Hexahydrate
FRAP	-	Ferric Reducing Antioxidant Power
GC	-	Gas Chromatography
GC-MS	-	Gas Chromatography–Mass Spectrometry
G	-	Gram
Hz	-	Hertz

H	-	Hours
IR	-	Infrared
IC_{50}	-	Inhibition Concentration at 50%
lit.	-	Literature
L	-	Litre
$MgSO_4$	-	Magnesium sulphate
m.p	-	Melting point
mL	-	Millilitre
mM	-	Millimolar
µg/mL	-	Microgram/ millilitre
µL	-	Microliter
Mg	-	Milligram
MHz	-	Megahertz
Min	-	Minute
M	-	Multiplet
Nm	-	Nanometer
NMR	-	Nuclear Magnetic Resonance
Ppm	-	Part per million
cm^{-1}	-	Per centimeter
PTLC	-	Preparative Thin Layer Chromatography
^1H	-	Proton
KBR	-	Potassium bromide
R_f		Retention factor
t_R		Retention time
Ref		Reference
S	-	Singlet
T	-	Triplet
UV	-	Ultraviolet
VLC	-	Vacuum Liquid chromatography

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Natural product and their derivatives have served as therapeutic agent throughout the world and be as important sources in drug discovery [1]. Over 80% of drug substances were derived by natural compounds which come from various sources including terrestrial plants, marine organisms, terrestrial microorganisms, terrestrial vertebrates and invertebrates [1, 2]. Natural products are chemical compounds or substances produced by living organisms and have pharmacological properties which can be used as the basis of treatment of human diseases [3].

Traditional medicines are currently in demand and their popularity is increasing day by day. It is estimated about 80% of the world population still depends on traditional medicine based largely on plants and animal species for their first line of primary health care. It was found 800 plants with medicinal uses have been used in indigenous systems of medicine and about 500 plants are stated in ancient literature [4].

In spite of many successful developments in synthetic chemistry, the potential of higher plants as sources for new drugs is still not exhausted. Of the estimated 250,000 of existing plant species worldwide, only a small percentage has been investigated for the presence of bioactive compounds [5]. Thus, there are more phytochemical studies should be done as the acceptance of people in herbal remedies are increasing.

Malaysia is a unique country, gifted with diverse of plants and recognize as the world's 12 mega biodiversity rich–countries with abundance of natural resources [6]. Ginger, turmeric, black pepper, cinnamon, cloves and nutmeg are recognized as the famous plant species used in traditional medicines.

In Ayurveda medicine, the fresh rhizome of common ginger is prescribed as herbal remedies for asthma and cough by taking fresh juice ginger with some garlic and honey. It has also been used for curing many illness such as indigestion, fever, sinusitis, nausea, allergic reactions, respiratory troubles, headache, backache, toothache, vomiting, and constipation [7]. Some members of the Zingiberaceae family including turmeric (*Curcuma longa*), ginger (*Zingiber officinale*), 'lengkuas' (*Alpinia galanga*) and krachai (*Boesenbergia pandurata*) have been used as condiment for flavouring and herbal remedies to relief from stomach-ache, and diarrhea. There are known to have various antimicrobial properties [8].

Among the species, turmeric is one of the golden spices and most useful herbal in the world. Most of turmeric is used not only in cooking as spice but also for medicinal purposes. About 2500 years ago, most of Asian countries have used turmeric in traditional medicine. This spice also already prescribed in Chinese medicine, Ayurveda in India, and Jamu in Indonesia. Turmeric has been widely cultivated in tropical Asia regions such as India, China and Indonesia since ancient times [9]. Turmeric belongs to ginger family that consists of about 53 genera and over 1200 species [10].

Recently, the developments in studies of pharmacology, chemistry, and phytochemical has increased especially in natural product field. Many genera and species of Zingiberaceae family are traditionally used as condiments and drugs possess variety of important biological properties which are beneficial to mankind. Thus, the chemical constituents of Zingiberaceae plants and biological activities must be continuously studied to prove their pharmaceutical values. Further studies are required for improvement of human health by their bioactive compounds.

1.2 Problem Statement

Several species of *Curcuma* of ginger family are extensively used for many different purposes such as food flavouring, spices, as remedies for stomach ailments, stimulate digestion, and protect the digestive organs, including the intestines, stomach, and liver. There have been many phytochemicals and bioactivity studies conducted on the *Curcuma* species, however there are only few studies reported on the *C. heyneana* [11] and *C. zedoaria* [12]. Previous researches have been done on both *Curcuma* species from different cultivations such as Indonesia and India. The chemical constituents presence might be different due to many factors such as locality and habitat. Thus, further study on the isolation and identification of chemical compounds in rhizomes of *C. heyneana* and *C. zedoaria* are necessary to be carried out to determine the chemical components and the variations. It is also important to screen the bioactivities of the species to evaluate the potential bioactivity of the plants such as antioxidant, anti-inflammatory, and antimicrobial.

1.3 Objectives of Study

The objectives of this research are to investigate the chemical composition of essential oil and the phytochemicals present in two species of *Curcuma* which are *C. heyneana and C. zedoaria*. The first objective is to identify the essential oil constituents from fresh rhizomes. The second objective is to isolate and elucidate the structure of phytochemicals from dried rhizomes. The final part is to evaluate the bioactivities of the essential oils and the crude extracts.

1.4 Significance of Study

Natural products have contributed considerable value to pharmaceutical industry over the past half century. The bioactive phytochemicals such as terpenoids, flavonoids, alkaloids and phenolics give the high value of medicinal plants as the chemical compounds can produce definite physiological action to human body. Thus, this study can contribute to the field of knowledge and provide beneficial information regarding the essential oils and phytochemicals contained in the rhizomes of *C. zedoaria* and *C. heyneana*. In addition, the bioactive oils and extracts of *Curcuma* species can be futher developed in the herbal industries.

1.5 Scope of Study

This study is divided in three parts. The first part is to focus on chemical composition of essential oils from fresh rhizomes of *C. heynaena* and *C. zedoaria*. The essential oil will be extracted by hydrodistillation technique and analysed using GC-MS and Kovats Indices to determine the chemical compositions. The second part is to isolate and identify the phytochemicals of *Curcuma* species. The dried rhizomes will be extracted by soxhlet extraction followed by fractionation using vacuum liquid chromatography (VLC), and purification of the fractions using gravity column chromatography (CC), and preparative thin layer chromatography (Prep-TLC) to obtain the pure compounds. The structure of isolated compound will be characterized using spectroscopic methods including GC-MS, FTIR and NMR. The third part is to evaluate the biological activities of the essential oils and crude extracts from *C. heyneana* and *C. zedoaria* using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), 2,2’azinobis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), and ferric reducing antioxidant power (FRAP) methods.

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