ESSENTIAL OILS, PHYTOCHEMICALS AND BIOACTIVITY STUDIES OF *Curcuma aeruginosa* Aff. AND *Kaempferia rotunda* Linn.

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To my beloved mother and father

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ABSTRACT

The essential oils, phytochemicals and bioactivities of *Curcuma aeruginosa* Aff., known as temu hitam and *Kaempferia rotunda* Linn. or kunyit putih have been studied. Hydrodistillation of the fresh rhizomes of *C. aeruginosa* and *K. rotunda* gave 77.1% and 89.3% oils respectively. These oils were analyzed by GC and GC-MS. The chemical compositions were identified by comparison of the mass spectral data of Wiley Library and Kovats Indices with literature values. A total of 42 components were identified from *C. aeruginosa* with epicurzerenone (19.47%) as the major constituent followed by 1,8-cineole (15.89%), *trans*-β-farnesene (9.75%), β-elemene (6.61%) and camphor (5.84%). *K. rotunda* essential oil was found to have 33 components with high concentration of benzyl benzoate (31.48%), bornyl acetate (5.56%), camphor (5.45%) and camphene (5.04%). Extractions by soxhlet apparatus were carried out on the dried samples to get the crude extracts. Fractionation and purification on the crude extracts of both species resulted in the isolation of sesquiterpenoids, cyclohexane oxide, esters and fatty acid. The structures of the isolated compounds were identified by spectroscopic techniques including IR and NMR (1D and 2D) spectroscopies and mass spectrometry. Seven sesquiterpenoids were isolated from *C. aeruginosa* and identified as curzerenone, furanodienone, germacrone, dehydrocurdione, curcumenone, curcumenol and curzeone. Four compounds were successfully isolated from *K. rotunda* and characterized as benzyl benzoate, crotepoxide, lignoceric acid and stigmasterol. The crude extracts, essential oils and several pure phytochemicals were screened for antibacterial and antityrosinase activities. Disc diffusion method followed by minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) against Gram positive and Gram negative bacteria were used for antibacterial assay. All the essential oil, crude extracts and compounds of *C. aeruginosa* and *K. rotunda* exhibited weak to inactive activity against the entire tested microorganisms. Modified dopachrome method with L-DOPA as the substrate was chosen to screen the antityrosinase activity. Among all the tested samples, the essential oil of *C. aeruginosa* possessed the highest activity with 15.62% inhibition against the mushroom tyrosinase. This inhibition value was far lower compared to the standard kojic acid which showed 81.81% inhibition.

ABSTRAK

Kajian ke atas minyak pati, fitokimia dan bioaktiviti *C. aeruginosa* Aff., yang dikenal sebagai cekur hitam dan *K. rotunda* Linn. atau kunyit putih telah dijalankan. Penyulingan hidro terhadap rizom segar *C. aeruginosa* dan *K. rotunda* masing-masing telah memberikan 77.1% dan 89.3% minyak pati. Minyak ini dianalisis menggunakan GC dan GC-MS. Komposisi bahan kimia telah dikenalpasti melalui perbandingan data spektrum jisim daripada perpustakaan Wiley dan nilai Indeks Kovats daripada literatur. Sebanyak 42 komponen telah dikenalpasti daripada *C. aeruginosa* dengan epikurzerenon (19.47%) sebagai komponen utama diikuti oleh 1,8-sineol (15.89%), *trans*-β-farnesena (9.75%), β-elemena (6.61%) dan kamfor (5.84%). Minyak pati *K. rotunda* terdiri daripada 33 komponen dengan komponen utamanya benzil benzoat (31.48%), bornil asetat (5.56%), kamfor (5.45%) dan kamfena (5.04%). Pengestrakan soxhlet telah dijalankan ke atas rizom kering untuk mendapatkan ekstrak mentah. Pemeringkatan dan penulenan ke atas ekstrak mentah bagi kedua-dua spesies telah mengasingkan sebatian seskuiterpenoid, sikloheksana oksida, ester dan asid lemak. Struktur semua sebatian telah dikenalpasti menggunakan teknik spektroskopi termasuk spektroskopi IR dan NMR (1D dan 2D) serta spektrometri jisim. Tujuh sebatian seskuiterpenoid telah diasingkan daripada *C. aeruginosa* dan dikenalpasti sebagai kurzerenon, furanodienon, germakron, dehidrokurdion, kurkumenon, kurkumenol dan kurzeon. Empat sebatian telah berjaya diasingkan daripada *K. rotunda* dikenalpasti sebagai benzil benzoat, krotepoksida, asid lignoserik dan stigmasterol. Ekstrak mentah, minyak pati dan beberapa fitokimia tulen telah disaring untuk aktiviti antibakteria dan antitirosinase. Kaedah penyebaran cakera diikuti dengan kepekatan perencatan minimum (MIC) dan kepekatan bakterisida minimum (MBC) terhadap bakteria Gram positif dan Gram negatif telah digunakan untuk saringan antibakteria. Kesemua sampel minyak pati, ekstrak mentah dan fitokimia *C. aeruginosa* dan *K. rotunda* menunjukkan keaktifan yang lemah sehingga tidak aktif terhadap seluruh mikroorganisma yang diuji. Kaedah pengubahsuaian dopakrom dengan L-DOPA sebagai substrat dipilih sebagai kaedah untuk menguji aktiviti antitirosinase. Antara semua sampel yang diuji, minyak pati *C. aeruginosa* memiliki aktiviti tertinggi dengan 15.62% perencatan berbanding tirosinase cendawan. Nilai ini adalah jauh lebih rendah daripada piawai asid kojik dengan 81.81% perencatan.

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LIST OF ABBREVIATIONS

α	Alpha
β	Beta
br	Broad
CC	Column Chromatography
COSY	Correlation Spectroscopy
^{13}C	Carbon-13
$CDCl_3$	Deuterated chloroform
$CHCl_3$	Chloroform
cm^{-1}	Per centimeter
δ	Chemical shift
d	Doublet
dd	Doublet of doublets
DEPT	Distortionless Enhancement by Polarization Transfer
DMSO	Dimethyl sulphoxide
DPPH	2,2-Diphenyl-1-picrylhydrazyl
EIMS	Electron Impact Mass Spectrometry
Et_2O	Diethyl ether
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
^1H	Proton
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Coherence
Hz	Hertz
IR	Infrared
J	Coupling constant
KI	Kovats Indices
L	Liter

m	Multiplet
M^+	Molecular ion
m.p.	Melting point
MBC	Minimum Bactericidal Concentration
MIC	Minimum Inhibitory Concentration
MS	Mass Spectrometry
m/z	Mass to charge ion
$MgSO_4$	Magnesium sulphate
NA	Nutrient agar
NB	Nutrient broth
NMR	Nuclear Magnetic Resonance
ppm	Parts per million
R_f	Retention factor
s	Singlet
SiO_2	Silica gel
t	Triplet
TLC	Thin Layer Chromatography
VLC	Vacuum Liquid Chromatography

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CHAPTER 1

INTRODUCTION

1.1 Background of the Problem

Malaysia is a unique country with high biological diversity. There are diverse of plants in a variety of forest which can be found all over Malaysia such as plants of Zingiberaceae, Leguminoceae and Annonaceae. About 12 to 18% of trees, shrubs and herbs from more than 7,000 species of angiosperms and 600 species of ferns in Malaysia are reported to have medicinal values [1]. As a result of chemical studies from isolation of active compounds from various plants, many biologically active plant-derived compounds were discovered and being used in industry according to their medicinal properties [2].

Zingiberaceae which have been reported to be useful as food, traditional medicine, spice, condiment, dye and flavour is one of the largest monocotyledonous family of the order Zinziberales. Approximately 160 species from 18 genera are found, mostly grow naturally in damp, shaded parts of the lowland or hill slopes, as scattered plants or thickets in Peninsular Malaysia [3]. About 23 genera and 200 species of Zingiberaceae have been recognized in Malaysia. Among the common species found are *Curcuma, Zingiber, Alpinia, Achasma, Amomum, Costus, Boesenbergia, Kaempferia, Languas, Elettaria, Globba, Hedychium,* and *Nicolaia* [4].

One of the genus of Zingiberaceae family is *Curcuma* which consists of about 40 species out of 1500 species in Zingiberaceae. Most of the *Curcuma* species are growing in mountainous area of the world [5]. *Curcuma* is a monocotyledon plant with a fleshy complex rhizome and the roots bearing ellipsoid tubers and usually has more or less erect leaf-blades. *Curcuma* species are distributed in India, Southeast Asia and Northern Australia. *Curcuma* species such as *C. longa*, *C. mangga*, *C. aeruginosa*, *C. xanthorrhiza*, *C. zedoaria*, *C. viridiflora*, *C. phaeocaulis*, *C. aromatica*, *C. domestica*, and *C. angustifolia* are well-known as village plants in Malaysia [6].

Beside *Curcuma* species, *Kaempferia* is also one of the genus in Zingiberaceae family. The name *Kaempferia* is taken from a German botanist name, Engelbert Kaempfer (1651-1716). The possible range of distribution for this genus is in Africa and Asia because *Kaempferia* can adapts to life in seasonal climates and more open places. *Kaempferia* is known as small herbs with tuberous root and short rhizomes. The flowers of the genus appear to consist of four lobes, surrounded by three thin and narrow corolla lobes [6].

Both *Curcuma* and *Kaempferia* have their own medicinal values. Some of the medicinal properties reported were antioxidant, antibacterial, anti-inflammatory and anti-tyrosinase. Many antibacterial products are sold today especially for cleaning and hand washing, while antioxidants are widely used in dietary supplement in order to help maintaining human health [7].

An essential oil is natural oil with a distinctive scent secreted by the glands of certain aromatic plants. It is a very complex natural mixtures which includes two groups of components from distinct biosynthetically origin. The main group consists of terpenes and terpenoids while the second group is composed of aromatics and aliphatic constituents of low molecular weight. Essential oils are extracted from the plants by hydrodistillation, steam distillation, extraction with cold neutral fats or solvents such as alcohol or pressing. They are used in preservation of food, antimicrobial, analgesic, anti-inflammatory, and local anesthetic remedies because of their fragrance, antiseptic and medicinal properties [8].

1.2 *Curcuma* Species: Botany, Distribution and Usage

The genus *Curcuma* is naturally distributed throughout south and Southeast Asia with the highest diversity in India and Thailand. This genus comprises of about 70 species of rhizomatous herbs. The genus *Curcuma* is economically important due to its pharmacological effects. Rhizomes of *Curcuma* are useful in traditional medicine in Asia. Several of the *Curcuma* species are reported to possess anti-tumor and anti-virus activities [9].

Previous study on *Curcuma* species showed that the phytochemicals of this species consisted of monoterpenoids, sesquiterpenoids and diarylheptanoids. *C. xanthorrhiza, C. zedoaria, C. longa, C. domestica* and *C. aromatica* are the species found in Malaysia and Indonesia. These species are widely investigated by researchers in the area of natural products and food chemistry due to their wider application in traditional medicine [6].

C. aeruginosa is a native tropical plant of Southeast Asia, including Malaysia, Myanmar, Cambodia, Vietnam, Indonesia and Thailand. It is a perennial plant with leafy shoots of 45–60 cm high and roots or tubers are oblong. This plant is distinguished by red corolla lobes and ferruginous or greenish-blue rhizome. In Malaysia, it is known locally as *temu hitam* [6]. Fresh rhizomes of this species emit the aroma of ginger-like and are mildly aromatic. The rhizomes have been used for many years in traditional medicine especially for gastrointestinal remedies treatment of diarrhea, colic and for postpartum care, uterine involution, treatment of uterine pain and uterine inflammation [10].

C. zedoaria is considered to be native in North-East of India. It is known as zedoary and grows in tropical and subtropical wet forest regions. This plant has fragrant and bears yellow flowers with red and green bracts also has long leaf shoots which can reach 1 meter in height. The underground stem section is large and tuberous with numerous branches. *C. zedoaria* or locally known as '*temu kuning*' is used as a medicine in India and Malaysia. This *Curcuma* species is used as remedies in the countries such as the provinces of Madras, Bengal and also Indonesia because of digestive, diuretic, emollient and antiscurbotic properties [6].

C. xanthorrhiza which is a member of the Zingiberaceae grows in Southeast Asia. This plant is sometimes referred to as Javanese Turmeric or *temu lawak*. They can grow up to 8 feet tall and the flower is yellow in color. It has an aromatic, pungent odor and a bitter taste. The large leaves stem from the root and the large rhizome of this plant contains herbal qualities. The blend of the juice from the rhizomes of *C. xanthorrhiza* is used to treat indigestion and rheumatism or applied to the body after childbirth [3, 6].

C. domestica is a native plant of South Asia and is cultivated extensively throughout the warmer parts of the world. Its rhizomes are oblong, ovate, pyriform and often short-branched. The rhizome is widely used in powder form as food additive to impart flavor and a yellow color. It is very popular in Asian medicine for the treatment of coryza, hepatic disorders and rheumatism [11].

C. mangga (locally known amongst Malays as ‘*temu pauh*’) cultivated in Java and Malaysia. It is a plant of the ginger family which is closely related to turmeric. The structure of the rhizomes is very similar to ginger but it has a raw mango taste and always used for the preparation of food, supplements and traditional medicine. Its aromatic leaves are useful for flavouring steamed and baked fish while its rhizomes are always used in making pickles in several parts of India. The fresh tips of young rhizomes and shoots are sometimes consumed with rice. The rhizomes are useful to treat stomach, chest pains, fever and also used in postpartum care, specifically to aid healing of wounds [6, 12, 13].

1.3 *Kaempferia* Species: Botany, Distribution and Usage

Kaempferia is one of the important genus in Zingiberaceae family which can be found in the Southeast Asia. Plants of the genus are small and herbaceous with short, fleshy or slender rhizomes and one to a few leaves [14]. The *Kaempferia* species can be found in very damp, shaded areas and usually close to streams or in boggy conditions [15].

Several *Kaempferia* species are used to be cultivated in villages for food, spice and folk medicine. Other than that the rhizomes part of *Kaempferia* species has been proven by many researchers to display health benefit properties. The extracts of rhizomes from some *Kaempferia* species can exhibit anti-inflammatory and HIV-1 protease inhibitory activity [16].

K. rotunda Linn. is a stemless and rhizomatous herb. The leaves are erect, oblong, up to 30 cm long, while the flowers are large, light purple and appear before leaves, from radical spike [6]. The leaves and rhizomes of this species are used to treat sprain and stomachic aches by the locals of Northeast India. The paste of the ground rhizomes was mixed with other herbs and applied to the sprains and covered with a bandage while the leaves paste is used as body lotion. This usage of *K. rotunda* is popular in Assam, Manipur and Mizoram area [17].

K. galanga Linn. is said to be native in India and widely cultivated throughout Southeast Asia. This species is known as *Chandramula* by the Assamese live in Northeast India. It is a common village plant in Malaysia known as *Chekur*. *K. galanga* is used both for medicine and to flavor food [6]. The rhizomes of *K. galanga* L. were externally used, taken orally and sometimes used together with the rhizomes of *Z. montanum* and *Z. officinale* as local medicine. The leaves and rhizomes were useful in treating indigestion, cold, pectoral and abdominal pains, headache, carminative and toothache and menstrual pain. They are also effective for dandruff or scabs on the head, antidiarrheal and against poisoning when there is blood vomiting [18-19].

In China, *K. galanga* L. is used to spice food and important ingredient for medicinal industry. The rhizomes of this plant are used traditionally to treat symptoms ranging from hypertension, pectoral and abdominal pains, headache, toothache, rheumatism, dyspepsia, coughs and inflammatory tumor. It also has been used as fragrance to help restlessness, stress, anxiety and depression. While in Japan, it has been used as one of the main ingredients in a scent bag which is recognized as improving sleep or minimizing stressful situations, due to this species possesses a strong characteristic balsamic odor [20].

K. pandurata (*Syn. B. pandurata*) cultivated in some tropical countries including Myanmar, Indonesia, Malaysia, and Thailand is a perennial herb of the Zingiberaceae family. In Indonesia, Malaysia, and Thailand, this species is a popular folk medicine for the treatment of diseases such as ulcer, dry mouth, stomach discomfort, leucorrhea, and dysentery. The fresh rhizomes are used as a flavoring agent in Thai cuisine due to its characteristic aroma [21].

K. parviflora Wall., which is locally known in Thai as *Kra-chai-dam*, belongs to the family Zingiberaceae. *K. parviflora* species has a black rhizome and have been used for the treatment of colic disorder as well as peptic and duodenal ulcers. It is well-known as ‘Thai ginseng’ in Thailand [22].

1.4 Statement of Problem

The plants of *Curcuma* and *Kaempferia* from Zingiberaceae family have great interest due to their variety in essential oil compositions, phytochemicals and biological properties. The essential oils, phytochemicals and bioactivity studies which include antibacterial, antioxidant and anti-inflammatory of *Curcuma* and *Kaempferia* species are well explored. However, the anti-tyrosinase activity of the essential oils and phytochemicals from these species has not been investigated yet.

1.5 Objectives of Study

The objectives of this research are as follows:

(i) To extract the essential oils from the fresh rhizomes of *C. aeruginosa* and *K. rotunda*.

(ii) To determine the chemical compositions of the essential oils.

(iii) To extract and purify phytochemicals from the dried rhizomes of *C. aeruginosa* and *K. rotunda*.

(iv) To identify the structure of the pure phytochemicals.

(v) To screen the antibacterial, antioxidant and anti-tyrosinase of the essential oils, crude extracts and pure phytochemicals.

1.6 Scope of the Study

The essential oils and phytochemicals from the rhizomes of *C. aeruginosa* and *K. rotunda* will be investigated. The essential oils will be extracted from the fresh rhizomes using hydrodistillation technique and the chemical compositions of the essential oils will be analyzed using GC and GC-MS. The crude extracts will be extracted from the dried rhizomes using soxhlet extractor. The extracts will be fractionated using vacuum liquid chromatography and column chromatography to get the pure isolated phytochemicals which will be elucidated structurally using spectroscopic techniques including 1D and 2D NMR, IR, MS and GC-MS. The essential oils, crudes and selected phytochemicals from both species will be screened for antibacterial using disc diffusion method followed by minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC). The modified dopachrome method with L-DOPA will be performed for anti-tyrosinase activity.

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