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Studies on the Constituents and Medicinal Properties of Some Zingiberaceous Species

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ABSTRACT Twelve extracts from four Zingiberaceae species (*Curcuma xanthorrhiza, Curcuma heyneana, Kaempferia angustifolia* and *Kaempferia rotunda*) have been screened for antimicrobial assay and cytotoxic screening. *Curcuma xanthorrhiza* showed strong activity in all biological assays. Two cyclohexane diepoxides, crotepoxide and boesenboxide, together with 2'-hydroxy-4,4',6'-trimethoxychalcone were isolated from the rhizomes of *Kaempferia angustifolia*. The structure of these compounds were determined by spectroscopic methods such as infrared (IR), nuclear magnetic resonance (^{1}H, ^{13}C and 2D NMR) and gas chromatography - mass spectrometry (GC-MS) and by comparison with the data reported previously.

ABSTRAK Dua belas ekstrak dari empat spesies (*Curcuma xanthorrhiza, Curcuma heyneana, Kaempferia angustifolia* dan *Kaempferia rotunda*) telah dikaji dengan ujian anti-mikrob dan sitotoksik. *Curcuma xanthorrhiza* menunjukkan aktiviti yang kuat dalam semua ujian biocerakinan. Dua sikloheksana diepoksida, krotepoksida dan boesenboksida, bersama dengan 2'-hidroksi- 4,4',6'-trimetoksikalkon telah dipencil daripada rizom *Kaempferia angustifolia*. Struktur sebatian ini dikenalpasti dengan kaedah spektroskopi seperti inframerah (IR), resonans magnet nukleus (^{1}H, ^{13}C and 2D NMR) dan spektrskopi jisim gas kromatografi (GC-MS) dan dibuat perbandingan dengan data yang telah dilaporkan.

(*Curcuma, Kaempferia,* spectroscopy, antimicrobial and cytotoxic activity)

INTRODUCTION

The Zingiberaceae is one of the largest families of the monocotyledons of Malesia and one of the most important herbaceous components of the ground cover in many types of tropical forests. This family comprises about 52 with about 1500 species in the world. Most of the species can be found in Malesian region, a floristically distinct region that includes Malaysia, Indonesia, Brunei, Singapore, the Philippines and Papua New Guinea, with 25 genera and about 650 species [1].

There are about 25 genera with more than 160 species of Zingiberaceae in Peninsular Malaysia [2]. Many of these are rare, very local in distribution, and consequently highly vulnerable to endangerment. The Zingiberaceae are mainly forest plants found from the lowlands to the highest elevations in Peninsular Malaysia. At least 20 or more ginger species have been cultivated for their use as spices, condiments, flavours, dyes, fresh vegetables (locally known as ulam), medicines, ornamentals and quite recently as cut flowers. One of the earliest uses was spice. The presence of essential oils such as limonene, eugenol, α- pinene and geraniol, in many Zingiberaceae species have made some species important since the time of ancient Greeks. Several species from the genera of *Kaempferia* and *Curcuma* are major ingredients in traditionally prepared tonics called 'Jamu', which are commercially available [3].

Kaempferia is a genus of herbs of over 50 species natives of East Tropical Asia. It is small rhizomatous herbs with usually thick aromatic tuberous roots and short rhizomes. In the indigenous species, the flowers arise in the midst of a few leaves, while in the introduced species

K. rotunda, the inflorescence appears before the leaves. The inflorescence is totally enclosed in the leaf sheaths. The genus is easily recognised, as the flowers appear to consist of four lobes, surrounded by three thin, narrowed corolla lobes.

Kaempferia angustifolia is one of the *Kaempferia* species which is less well known in Malaysia, if to compare with *K. galanga* and *K. rotunda*. *Kaempferia angustifolia* is locally known as *Kunci pepet, Kunci menir* or *Kunci kunot*. *Kaempferia angustifolia* is tuberous and stemless, has small leaves and could be found growing wildly in the forests of West and Centre of Java. It has nice smell and usually used as a medicine to treat cold, stomach-ache and dysentery, while it's rhizome is used for coughs and as a mastigatory.

Kaempferia rotunda is locally known as *Kunir putih, Kunci pepet, Ardong, Temu puteri* and *Kenchur*. In Java, 'Kunir putih' is said to be used for stomach-ache and that the tubercles are used as a cooling medicine. In addition, the rhizomes are serves in cosmetics, while the main tuber is used to treat abdominal illness. The tuber was part of medicine given to stimulate the appetite of lying- in women. The aborigines in the Sulawesi chew the root and spew it on the sick part of the body. In Philippines, the rhizome is used internally in treating gastric complaints, and externally, mixed with oil, as a cicatrising. Ointment of the powder is considered useful in healing wounds. In India, the young leaves are used for poulticing and as body lotion [4].

Curcuma is a genus with about 70 species spread over the tropics. *Curcuma* thrives in a well-watered soil, with plentiful rain, and in a light shade. In open areas and on light soils, bigger and better rhizomes are produced. In Java, they grow spontaneously in hilly regions at 500 – 750 m altitude, in dry grassy fields, and in thick forests. The genus is easily recognised by its inflorescence, which is a spike bearing prominent spiral bracts, which laterally fuse to form pouches. Being rich in volatile oils and having showy flowers, *Curcuma* has been used for spices, medicines, dyes, foods, perfumes, and tonic and as tropical greenhouse ornamentals. Chemical studies have revealed several active components in *Curcuma* such as antioxidant [5], anti-inflammatory and antimicrobial [6] compounds.

Species *Curcuma xanthorrhiza* is normally found at Malaysia. *Curcuma xanthorrhiza* is the well-known traditional medicinal plant used in Malaysia and Indonesia, with the local name "temu lawak" or "temu raya". *C. xanthorrhiza* is cultivated throughout Southeast Asia and occur either wild or cultivated plant. This species is one of the ginger's cultivated in tropical areas and used in traditional medicine and as a spice [7]. The species are also used for treatment of the inflammation in postpartum uterine bleeding (Thailand) and skin treatment (India), besides used as tonic drink in Malaysia and Indonesia, [8].

Curcuma heyneana, which is called 'temu giring' in Javanese, is of wide medicinal value in Indonesia, and is considered to be useful for the treatment of the skin diseases, abrasions and injuries. It is not only found commonly as one of the main ingredients in traditional Indonesian mixed herbal medicines (Jamu), but is also widely used in juice, prepared from fresh rhizomes an anthelminthic against intestinal worms. The drinks are sold widely in the market in Indonesia as an instant powder or liquid as traditional medicine for body cooler. *Curcuma heyneana* has a single green leaf with smooth face and its flower has a yellow crown [9].

Preliminary phytochemical investigation of these species has yielded several compounds including cyclohexane diepoxide derivatives, chalcone and triterpenes. In this paper, we wish to report the bioassay results conducted on these plant species and isolation of chemical constituents of *Kaempferia angustifolia.*

MATERIALS AND METHODS

General

The structure of compounds were determined by spectroscopic methods such as infrared (IR), nuclear magnetic resonance (^{1}H, ^{13}C and 2D NMR) and gas chromatography- mass spectroscopy (GC-MS) and by comparison with the data reported previously.

Plant Material

All plants in this study were collected from Yogyakarta, Indonesia in year 2000. These plants were identified by Dr. Suwijio Pramono of Department of Biological Pharmacy, Faculty of Pharmacy, Gadjah Mada University and the

voucher specimen were deposited at the herbarium of the institution.

Isolation of the Compounds

The finely ground air- dried rhizomes (1.0 kg) of *Kaempferia angustifolia* was extracted successively with petroleum ether, chloroform and methanol for seventy two hours. The extracts were filtered and concentrated down under reduced pressure in a rotary evaporator. Approximately 8.4 g of petroleum ether extract was obtained. The petroleum ether extract was suspended in methanol (50 ml) at room temperature; a solid product (0.16g), which formed was collected and characterised as crotepoxide **1**. Evaporation of the filtrate gave brown oils (8.2 g), which was subjected to column chromatography and eluted with mixtures of n- hexane/ ethyl acetate. This yielded β-sitosterol (0.05 g). Similarly, the chloroform extract (7.5 g) was purified using column chromatography to give boesenboxide **2** (Hex: EA = 7:3 as solvent system) and 2'- hydroxy-4,4',6'-trimethoxychalcone **3** (Hex: EA = 3:2 solvent system).

Crotepoxide **1**. $C_{18}H_{18}O_8$. m.p: 152-154°C (lit. 152- 153°C [10]). IR (cm^{-1}, KBr disc) ν_{max} : 3040, 1765, 1727, 1600, 1373, 1282, 1236, 1121, 722 cm^{-1}. MS m/z (% intensity): $363(M+1)^+$ (0.12), 43(96), 77(69), 97(29), 105(100), 115(25), 163(19), 227(9). ^1H NMR (500MHz, $CDCl_3$) δ: 2.02, 2.11 (2 x OAc), 5.70 (H-2), 4.98 (H-3), 3.10 (H-4), 3.45 (H-5), 3.66 (H-6), 4.55, 4.22 (H-7A,B), 7.45 (*m*-ArH), 7.59 (*p*-ArH), 8.02 (*o*-ArH); ^{13}C NMR (125 MHz, $CDCl_3$) δ : 166.1, 166.4 (2 x OAc), 162.1 (CH_2OCO), 20.1, 20.6 (2 x $COCH_3$), 55.8 (C-1), 65.9 (C-2), 66.8 (C-3), 49.0 (C-4), 44.4 (C-5), 50.2 (C-6), 58.9 (C-7), 123.3 (C-1'), 125.4 (C-2'), 121.0 (C-3'), 131.4 (C-4').

Boesenboxide **2**. $C_{23}H_{20}O_8$ m.p: 171- 172°C (lit. 171- 172°C [10]). IR (cm^{-1}, KBr disc) ν_{max}: 1729, 1607, 1374, 1265, 1115, 713 cm^{-1}; MS m/z (% intensity): 425 $(M+1)^+$ (32), 77(12), 105(42), 136 (42), 154(100). ^1H NMR ($CDCl_3$) δ: 2.07 (OAc), 5.95 (H-2), 5.17 (H-3), 3.19 (H-4), 3.49 (H-5), 3.72 (H-6), 4.63, 4.27 (H-7A,B), 7.45 (*m*-ArH), 7.58 (*p*-ArH), 8.01, 8.05 (*o*-ArH); ^{13}C NMR ($CDCl_3$) δ: 170.0 (OAc), 168.5 (CH_2OCO), 165.4 (OCOPh), 20.6 ($COCH_3$), 59.8 (C-1), 69.3 (C-2), 71.2 (C-3), 53.0 (C-4), 48.2 (C-5), 53.8 (C-6), 62.3 (C-7), 129.0, 129.1 (C-1', C-1"), 129.8 (C-2', C-2"), 128.7 (C-3', C-3"), 133.5 (C-4', C-4").

2'-hydroxy-4,4',6'-trimethoxychalcone **3**. $C_{18}H_{18}O_5$. m.p: 108-110°C (lit. 115°C [10]). IR(cm^{-1}, KBr disc) ν_{max}: 1622, 1514, 1222, 1158, 1032 cm^{-1}. MS m/z (% intensity): 315 $(M+1)^+$(72), 29(5), 43(7), 57(12), 91(7), 107(10), 137(55), 181(30). ^1H NMR ($CDCl_3$) δ: 3.84, 3.86, 3.91 (3 x OMe), 7.56 (H-2, H-6), 6.93 (H-3, H-5), 7.80 (H-α, H-β), 6.11 (H-3'), 5.96 (H-5'), 14.40 (H-OH); ^{13}C NMR ($CDCl_3$) δ: 128.3 (C-1), 130.1 (C-2, C-6), 114.3 (C-3, C-5), 161.4 (C-4), 192.6 (C-β'), 125.1 (C-α), 142.5 (C-β), 106.4 (C-1'), 168.2 (C-2'), 98.8 (C-3'), 166.0 (C-4'), 91.2 (C-5'), 162.4 (C-6'), 55.4 (4-OMe), 55.6 (4'-OMe), 55.8 (6'-OMe).

RESULTS AND DISCUSSION

Compound **1** appeared as colourless crystals of melting point 152-154°C (lit. 152- 153°C [10]), was isolated from petroleum ether extract. IR spectrum showed the presence of aromatic ester at 1727 cm^{-1} while peak at 1373 cm^{-1} revealed acetate. Peak at 1236 cm^{-1} exhibited cyclic epoxy group. The existence of peaks at 1121 cm^{-1} and 722 cm^{-1} were due to aromatic methine and mono-substituted benzene ring. The mass spectrum showed molecular ion peak at *m/z* 362 that corresponded to molecular formula $C_{18}H_{18}O_8$.

^1H NMR spectrum exhibited that two singlet at δ 2.02 and δ 2.11, attributed two methoxy groups. ^{13}C-NMR spectrum gave 18 absorption peaks representing the presence of 18 carbons. The presence of three carbonyl groups were shown by peaks δ 165.6, δ 166.1 and δ 166.4, while the two methoxy group were represented by peaks at δ 22.1 and δ 22.6. Based on these spectral data and comparison with the previous report [11], compound **1** was identified as (1R,2R,4R,5S,6R,7R)-4-benzoyloxymethyl-3,8-dioxatricyclo[5.1.0.0]-octane-5,6-diol- diacetate (Crotepoxide).

Compound **2** was obtained as colourless crystals of melting point 171-172°C (lit. 171-172°C [10]). IR spectrum exhibited the presence of aromatic ester at 1729 cm^{-1}. Peak at 1374 cm^{-1} revealed acetate, whereas peak at 1265 cm^{-1} exhibited cyclic epoxy group. The existence of peaks at 1115 cm^{-1} and 713 cm^{-1} were due to aromatic methine and mono-substituted benzene ring.

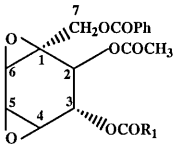
Compound **2** was the derivative of compound **1** with the lack of a methyl group, being replaced by a phenyl group. The mass spectrum showed molecular ion at *m/z* 424, which correspond to molecular formula $C_{23}H_{20}O_8$.

^{1}H NMR spectrum exhibited a singlet at δ 2.07 attributed a methoxyl group. The presence of two pairs of doublet of doublet of doublet at δ 7.45, and δ 8.01 and δ 8.05, represented two pairs of *meta* and *ortho* protons from two different phenyls, respectively. ^{13}C-NMR spectrum gave 23 absorption peaks representing the presence of 23 carbons. Peaks showed the presence of three carbonyl groups δ 170.0, δ 165.4 and δ 165.8, while methoxy group were represented by peak δ 20.6. The HMQC spectrum showed that acetyl was correlated to methyl, while both H-7A and H-7B was assigned to C-7. The HMBC spectrum indicated that OAc was correlated to the carbonyl attached to C-2, while H-7A and H-7B was correlated to C-6, C-1, C-2 and carbonyl that were attached to C-7. Based on these spectral data the compound **2** was identified as boesenboxide.

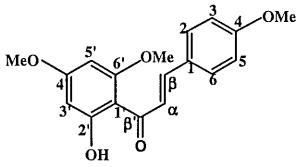
Compound **3** was isolated as yellow rhombic of melting point 108- 110°C (lit. 115°C [10]). IR spectrum exhibited the presence of OH as broad peak at 3450 cm^{-1}, while 1622 cm^{-1} exhibited C=C bonding. A sharp peak at 1514 cm^{-1} revealed methoxy bending, whereas peak at 1347 cm^{-1} exhibited aliphatic ketones. The existence of peaks at 1222 cm^{-1} and 826 cm^{-1} were due to aromatic methine and tri-substituted benzene ring. The mass spectrum showed molecular ion at *m/z* 314, which corresponds to molecular formula $C_{18}H_{18}O_5$.

^{1}H NMR spectrum exhibited three singlets at δ 3.84, δ 3.86 and δ 3.91 attributed to three methoxy groups. ^{13}C NMR spectrum gave 18 absorption peaks representing the presence of 18 carbons. The presence of a carbonyl group was shown by peak at δ 192.6, while three methoxy groups were represented by peaks at δ 55.4, δ 55.6, δ 55.8. The HMQC indicated that three methoxy were assigned to three methyls, respectively. Besides, H-α and H-β were assigned to C-α and C-β, H-3 and H-5 to C-3 and C-5, H-2 and H-6 to C-2 and C-6, respectively.

The HMBC spectrum showed that hydroxyl groups correlated to C-3', C-2' and C-1'. Based on these spectral data the compound **3** was identified as 2'-hydroxy-4,4',6'-trimethoxychalcone, which was previously isolated from [10], [11].



(1) R_1=CH_3
(2) R_1= Ph



(3)

All crude extracts were examined their cytotoxicity properties using CEM-SS cell line (T-Lymphoblastic Leukaemia) [12] (Table 1). Chloroform and ethyl acetate extracts of *C. xanthorrhiza* exhibited the strongest inhibitory activity with IC_{50} less than 10 μg/ml (IC_{50} 2.8 μg/ml and 3.7μg/ml), while crude extracts of *K. angustifolia*, *K. rotunda* and *C. heyneana* were not cytotoxic towards CEM-SS cell line, with IC_{50} values more than 30μg/ml.

The crude extracts were all subjected to antimicrobial test against 4 bacteria: Methicillin Resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* (ATCC 60690), *Salmonella typhimurium* (S974) and *Bacillus subtilis* (wild) (B_{29}), using disc diffusion method [13] (Table 1). All the extracts of *C. xanthorrhiza* and *C. heyneana* produced inhibition zones against all bacterial tested, with the hexane extract of *C. xanthorrhiza* gave the most outstanding activity. The antimicrobial activities of crude extracts of *Kaempferia* species were rather low; with only PE extracts from *K. rotunda* was active against certain microbes.

Table 1. Bioactivities results of plants extracts

Plant	Extract	Cytotoxic (μg/ml)	Antimicrobial Activity (mm)			
			MRSA	60690	B_{29}	B_{28}
K. angustifolia	PE	>30	N.A	10	7	N.A
	$CHCl_3$	>30	N.A	N.A	N.A	N.A
	MeOH	>30	N.A	N.A	N.A	N.A
K. rotunda	PE	>30	13	14	14	N.A
	$CHCl_3$	>30	N.A	N.A	N.A	N.A
	MeOH	>30	N.A	N.A	N.A	N.A
C. xanthorrhiza	Hex	>30	11	15	10	15
	$CHCl_3$	2.8	11	12	10	14
	EtOAc	3.7	11	12	10	12
C. heyneana	PE	>30	9	9	10	N.A
	$CHCl_3$	>30	7	10	9	N.A
	MeOH	>30	8	9	9	N.A

N.A= Not active

CONCLUSION

In this studies, *Kampferia angustifolia* has yielded 3 pure compounds (crotepoxide, boesenboxide and 2'-hydroxy-4,4',6'-trimethoxychalcone). *Curcuma xanthorrhiza* showed strong activity in both bioassay activities.

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