PHYTOCHEMICAL STUDY ON SOME *CURCUMA* SPECIES FROM INDONESIA

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INTRODUCTION

Cancer or tumor is one of the dangerous diseases nowadays. And until know, the medicine of this disease have not been found yet. So, the efforts to find the medicinal compounds which can be used to prevent and therapy cancer are needed. Zingiberaceae family constitutes a vital group of rhizomatous medicinal and aromatic plants characterised by the presence of volatile oils and oleoresins of export value. Generally, the rhizomes and fruits are aromatic, tonic and stimulant; occasionally they are nutritive. Some are used as food as they contain starch in large quantities while others yield an astringent and diaphoretic juice. The important genera coming under Zingiberaceae are *Curcuma, Kaempferia, Hedychium, Amomum, Zingiber, Alpinia, Elettaria* and *Costus*. *Curcuma* is widely distributed in tropical and subtropical regions of Asia,especially Indonesia,Thailand, and Malaysia. The rhizome of *C. heyneana* has been used extensively in indigenous medicine in Indonesia as an is used in the treatment of skin diseases and is extensively used in vanishing creams. *C aeruginosa* has been used as disinfectant, expectorant, anthelmentic, antifungal, febrifuge, antiinflammatory and tonic (Heyne K, 1987; Morikawa, 2002; Wu, 2002). The aim of this research is to examine the cytotoxic effect against human cancer cell lines and to isolate bioactive compounds from temu giring (*Curcuma heyneana*) and temu ireng (*Curcuma aeruginosa*).

EXPERIMENTAL METHOD

General Experimental Procedure

UV and IR spectra were measured with Varian Cary 100 Conc and Shimadzu 8300 FTIR, respectively. ^{1}H and ^{13}C NMR spectra were recorded with Jeol JNM A-5000 spectrometers, operating at 500.0 MHz (^{1}H) and 125.0 MHz (^{13}C) using residual and deuterated solvent peaks as internal standards. Vacuum liquid chromatography (VLC) was carried out using Si gel Merck 60 GF_{254} (230-400 mesh), column chromatography using Si-gel Merck 60 (200-400 mesh), and TLC analysis on precoated Si gel plates Si-gel Merck Kieselgel 60 F_{254} 0.25 mm, 20 x 20 cm.

Plant Material

Samples of the rhizoma of *C. heyneana* and *C. aeruginosa* were collected in Juni 2010 from the market of Yogyakarta, Indonesia. The plant was identified by the staff at the Faculty of Biology, UGM Yogyakarta, a voucher specimen had been deposited at the Herbarium.

Extraction and Isolation

Each the milled dried rhizome of *C. heyneana* (3 Kg) and *C. aeruginosa* (3 Kg) was extracted exhaustively with methanol. Each of methanolic extract from the dried rhizomes of *Curcuma heyneana* and *Curcuma aeruginosa* was partitioned with n-hexane, chloroform, and ethyl acetate.

The isolation of methabolit compounds from chloroform fraction of *C. aeroginosa* was done by using the chromatography. A portion (50 g) of the total chloroform fraction was fractionated by vacuum liquid chromatography (VLC) and purified by repeated column chromatography on silica gel eluted with various solvent systems. From this method we obtained two sesquiterpen compounds, a new sesquiterpen lacton, aeruginon (1) (60 mg) and curcumenon (2) (120 mg). The structures of these compounds were established on the basis of their spectral data, including UV, IR, and NMR spectra.

Aeruginon (1) was obtained as a brown oil, UV (MeOH) $\lambda_{max.}$ 229 and 250 nm, IR (KBr) $\upsilon_{max.}$: 3322; 2933; 1713;1650; 1598; 1441; 1373; 1313; and 1019 cm^{-1}, ^{1}H and ^{13}C NMR (Me_2CO-d_6, 500.0 and 125 MHz) see Table 2. Curcumenon (2) was obtained as a brown oil, UV (MeOH) $\lambda_{max.}$: 214 and 238 nm, IR (KBr) $\upsilon_{max.}$: 2922; 2870; 1713; 1678; 1600; 1453; 1369; and 1270 cm^{-1}, ^{1}H and ^{13}C NMR (Me_2CO-d_6, 500.0 and 125 MHz) see Table 2.

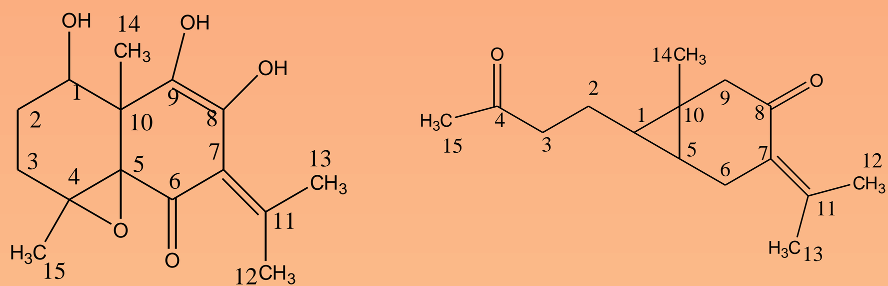

Fig. 2. Structure of aeruginon (1) and curcumenon (2)

Table 2. . ^{1}H and ^{13}C NMR data of compounds (**1** and **2**)* in chloroform

No carbon	Aeruginon (1)		Curcumenon (2)	
	δ C ppm	δ H (∑ H; m; *J* Hz)	δ C ppm	δ H (∑ H; m; *J* Hz)
1	60.72	1,95 (1H; m)	24,23	0,63 (1H; m)
2	37.35	2.03 (2H; m)	23.48	2.07 (2H; m)
3	27.32	2.46 (2H; dd; 15.3; 10,7)	43.99	2.40 (2H; m)
4	86.25	-	208.95	-
5	83.19	-	24.19	1.63 (1H; m)
6	194.87	-	28.09	2.77 (2H; d;
7	144.00	-	128,16	-
8	152.30	-	201,84	-
9	133.12	-	49.01	2,40 (2H, br s)
10	37.03	-	20.19	-
11	127.95	-	147.56	-
12	22.34	1.87 (3H, s)	23.56	1,76 (3H, s)
13	22.42	1.84 (3H,s)	23.51	0.63 (3H,s)
14	23.92	1.27 (3H, s)	19.14	1.09 (3H, s)
15	23.10	1.77 (3H,s)	30.13	2.12 (3H,s)

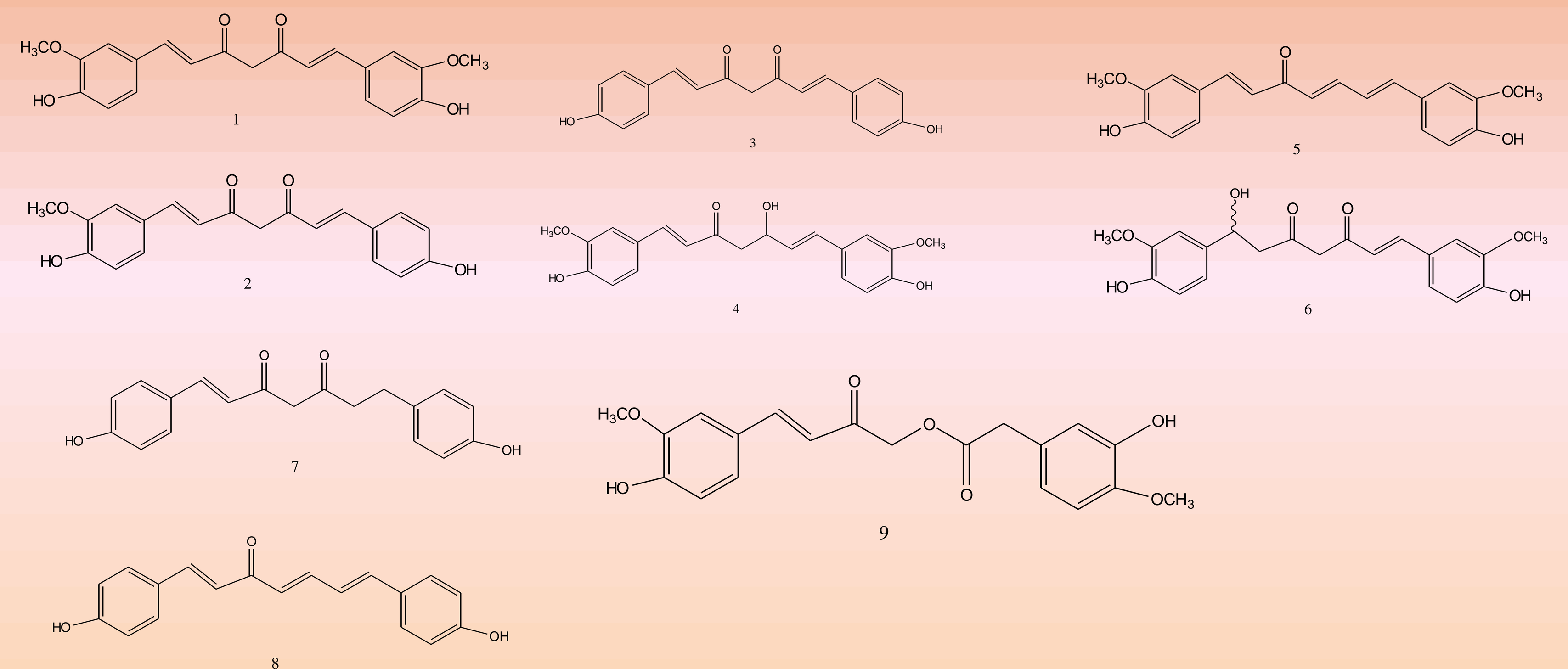

Figure 1. Some isolated compounds from Curcuma : curcumin (1), demetoxycurcumin (2), bis(4-hydroxysinamoil)-metan (3), dihydrocurcumin (4), 1,7-bis(4-hydroxy-3-metoxyphenyl)-1,4,6-heptatrien-3-on (5), 1-hydroxy-1,7-bis(4-hydroxy-3-metoxyphenyl)-6-hepten-3,5-dion (6), 1,7-bis(4-hydroxyphenyl)-1-hepten,3,5-dion (7), 1,7-bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-on (8), and calebin A (9).

Measurement of cytotoxic activity

Each extract and partitionated was measured cytotoxic activity test against human cancer cell lines, such as *Breast carcinoma (MCF-7); Cervical carcinoma (Ca Ski); T47-D;* and *Hela S3.* The invitro cytotoxicity test was investigated using plate with 96 wells, with cell density $2x10^4$ cells per ml. Into each well was added 100 µl cells in culture medium (87,5% RPMI 10,4 g/L; 2% penstrep; and 10% FBS) which was then incubated in CO_2 incubator for 12-24 hours at 37^0C. Each sample was dissolved in culture medium containing 0,05% DMSO, and 100 µl of each sample in different concentrations was added into each well in triplicate and was then incubated in CO_2 incubator for 12-24 hours at 37^oC. MTT solution (10 µl per 100 µl medium) was added to all wells of an assay, and plates were incubated for 4 hours at 37^oC in CO_2 incubator. As much as 100µl formazon (10% SDS and 0, 01 N hydrochloric acid) was added into each well and mixed on a shaker for 5 minutes. The wells were incubated in the dark room for 12-24 hours at room temperature. The absorbance was measured using multiwell scanning spectrophotometers (ELISA reader) at 595 nm. The absorbance is directly proportional to the number of living cells. So the dead cell could be calculated to determine LC_{50}. Activity test was also measured its cytotoxic activity on Vero cell lines as normal cell control comparison. The cytotoxic activity of the samples against as cancer cell line measured as LC_{50} were provided in Table 1.

RESULTS AND DISCUSSION

Table 1. Activity test of methanol extract and this fraction of *C. heyneana* and *C. aeroginosa* againt as cancer cell lines

No	Rhizoma of	Extract/ fraction	LC_{50} (µg/ml)				
			MCF-7	Ca Ski	Hela S3	T-47D	Vero
1	*C. aeroginosa*	Methanol	> 100	95,73 3,06	> 500 (not active)	> 500 (not active)	> 500 (not active)
		Hexane	69,47 2,16	66,02 0,45	> 500 not active)	> 500 (not active)	> 500 (not active)
		Chloroform	92,60 4,10	94,87 1,94	> 500 (not active)	> 500 (not active)	> 500 not active)
2	*C. heyneana*	Methanol	61,63 1,76	59,27 2,67	133,72 14,23	82,84 9,36	>100
		Hexane	69,00 2,25	65,6 2,16	111,98 13,47	77,69 7,84	91,44 2,08
		Chloroform	80,77 2,51	83,33 1,96	> 100	> 100	>100
		Ethyl acetate	67,17 0,42	67,98 0,53	118,81 27,25	86,53 13,20	>100

Conclusion

In this paper we concluded that the hexane and chloroform fraction from *C aeroginosa* had cytotoxic activity againt MCF-7 and Ca-ski, but not toxic against Hela S3 and T-47D. Cytotoxic activity of extract and fraction from *C. heyneana* showed more active than *C. aeroginosa*. This research showed that extract and fraction from *C. aeroginosa* had not toxic (LC_{50} > 500), but hexane fraction from *C. heyneana* showed toxic (LC_{50} = 91,44 µg/ml) against as Vero cell lines. From the chloroform fraction of *C. aeroginose* after separated and repeatedly purification by extensive chromatography resulted two compounds, aeruginon (1) and curcumenon (2)

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