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# Antileukemic Activity and Chemical Constituents of Some Zingiberaceae Species

M.A. SUKARI*, S.W. TANG, B.K. NEOH†, G.C.L. EE and M. RAHMANI
*Department of Chemistry, Faculty of Science, University Putra Malaysia, 43400 Serdang, UPM, Selangor Darul Ehsan, Malaysia*
*Fax: (60)0389435380; Tel: (60)0389466797*
*E-mail: aspollah@fsas.upm.edu.my; swjune@hotmail.com*

Crude extracts of various solvents from six species (genus *Alpinia*, *Zingiber*, *Kaempferia*) of Zingiberaceae family have been chosen for the *in vitro* cytotoxic activity screening against the inhibition of HL-60 cell line (Human promyelocytic leukemia). Eight extracts (44 %) were found to have cytotoxic activity with $IC_{50}$ values less than 30 µg/mL, with *Alpinia galanga* showing the most prominent antileukemic activity. Further isolation work on *Kaempferia galanga* has yielded ethyl *p*-methoxy-*trans*-cinnamate (**1**), *p*-methoxy-*trans*-cinnamic acid (**2**) besides β-sitosterol.

**Key Words: Zingiberaceae, Antileukemic, *Alpinia*, *Zingiber*, *Kaempferia*.**

## INTRODUCTION

Zingiberaceae is one of the largest rhizomatous herbs families which comprise about 52 genera with 1500 species in the world and about 18 genera with more than 160 species occur in Peninsular Malaysia. It has been long widely used as spices, traditional medicine, perfume, dyes and as ornamental plants in tropical region. *Alpinia*, *Zingiber* and *Kaempferia* are three of the genus from the Zingiberaceae family. *Alpinia galanga* is commonly known as lengkuas or galangal. *Zingiber cassumunar* is locally known as lempoyang, *Zingiber officinale* var. rubrum as halia bara and *Zingiber officinale* as halia biasa. *Kaempferia rotunda* is also known as temu putri while *Kaempferia galanga* is recognized as cekur.

*Alpinia* is a genus of about 200 species of ginger-scented, rhizomatous perennials natives to Asia and Australia. The thick rootstock is used as medicines as well as food condiments[1]. Twenty three species of *Alpinia* were found in Peninsular Malaysia which includes *A. mutica*, *A. galanga*, *A. conchigera* and *A. rafflesiana*. In recent years, *Alpinia galanga* has been reported as possessing antitumour, antibacterial, antiulcer, antifungal and insecticidal properties. In Thailand for example, galangal is used for carminative, stomachic, antispasmodic, antiphlogistic and antibacterial drugs[2].

†Sime Darby Technology Centre, 2, Jalan Tandang, 46050 Petaling Jaya, Selangor, Malaysia.

Genus of *Zingiber* has about 85 species of aromatic herbs mostly distributed in East Asia and tropical Australia. *Zingiber* species are rich in volatile oils and are used in traditional medicine and as spices. *Z. officinale* Roscoe or 'ginger' has been used in Chinese and Indian traditional medicine for relief from arthritis, rheumatism, sprains, muscular aches and pains, congestion, coughs, dementia, sinusitis, sore throats, diarrhea, cramps, indigestion, loss of appetite, motion sickness, fever, flu, chills, *etc*.[3]. *Zingiber cassumunar* is a traditional medicinal plant in Southeast Asia, especially in Thailand and Indonesia. Previous reports have demonstrated that the rhizomes of *Z. cassumunar* showed antioxidant and antifungal activities[4].

*Kaempferia* is a genus of herbs of about 55 species which are chiefly native to the tropics and subtropics of Asia and Africa. It is small rhizomatous herbs with usually thick aromatic tuberous roots and short rhizome. *Kaempferia galanga*, an Indian folk medicinal plant, providing aromatic edible tubers for expectorant, carminative and diuretic uses and also for curing malaria, rheumatism, dyspepsia, coughs, pectoral infections and inflammatory tumours[5]. *Kaempferia rotunda* is traditionally used to treat abdominal pain, dysentery, diarrhea, cold, obesity, astringent (cosmetic) and after childbirth. Interestingly, the dried powder of *K. rotunda* rhizomes is famous for traditional prevention and treatment for cancer diseases recently[6].

Zingiberaceae species have long been consumed as food or flavouring and known to possess many classes of phytochemicals such as flavonoids, chalcones, phenylpropanoids, diarylheptanoids, *etc*. Cancer is one of the major health problems in Malaysia and it has become increasingly important as a public health concern with the development and progress that has been achieved in this country[7]. Previously our group has reported the chemical constituents and bioactivities studies of *Curcuma*, *Alpinia* and *Boesenbergia* species[8-11] and here we wish to report the antileukemic activity of some other ginger species together with the isolation work.

## EXPERIMENTAL

Plant materials used in this study-Rhizomes of *Zingiber cassumunar* and *Kaempferia rotunda* were collected from Yogyakarta, Indonesia; whereas *Zingiber officinale*, *Zingiber officinale* var. *rubrum*, *Kaempferia galanga* and *Alpinia galanga* were collected from Selangor, Malaysia. Finely ground air-dried rhizomes of each plant material were extracted three times with different solvents (petroleum ether/ hexane, chloroform/dichloromethane, ethyl acetate and methanol) sequentially for 72 h for each extract. The extracts were then concentrated under reduced pressure using rotary evaporator.

**Isolation of constituents from *Kaempferia galanga*:** Air-dried rhizomes of *K. galanga* (847.40 g) were extracted by using conventional soaking method to give dichloromethane extract (82.20 g) and methanol extract (53.10 g). Ethyl-*p*-methoxy-*trans*-cinnamate (**1**) (23.33 g) was yielded from both extracts while β-sitosterol was obtained as minor compound from dichloromethane extract. Another batch of

samples (565.00 g) from same source was extracted similarly to afford hexane extract (6.40 g), chloroform extract (20.00 g) and methanol extract (87.34 g). Fractionation and purification of non-polar extract has yielded β-sitosterol while *p*-methoxy-*trans*-cinnamic acid (**2**) (0.054 g) was furnished from semi-polar extract of *K. galanga*.

**Ethyl-*p*-methoxy-*trans*-cinnamate (1):** Colourless crystal, $C_{12}H_{14}O_3$, m.p. 41-43 °C (Lit.[12] 43-48 °C). IR (KBr disc, $\nu_{max}$, $cm^{-1}$): 1706, 1630, 1512, 1174, 830. EI-MS m/z (rel. int. %): 206 ($M^+$, 63), 161 (100), 134 (29), 89 (20), 77 (21), 63 (15). The $^1H$ and $^{13}C$ NMR data are in good agreement with the published data[12].

***p*-Methoxy cinnamic acid (2):** Colourless crystal, $C_{10}H_{10}O_3$, m.p. 171-172 °C (Lit.[12] 175-177 °C). IR (KBr disc, $\nu_{max}$, $cm^{-1}$): 3438, 2974, 1686, 1624, 1512, 1029. EI-MS m/z (rel. int. %): 178 ($M^+$, 100), 161 (32), 133 (14), 89 (14), 77 (15). The $^1H$ and $^{13}C$ NMR data are in good agreement with the published data[12].

**Cytotoxic assay:** The cytotoxic assay was carried out according to the methods described previously[11]. The HL-60 leukemic cancer cells was maintained in RPMI 1640 media, supplemented with 10 % fetal calf serum (FCS) and 1 % antibiotic penicillin-streptomycin in an atmosphere of 5 % $CO_2$ at 37 °C. The test was performed in 96-flat bottom microwell plates. Briefly, 100 µL of varying concentration of the extracts and isolated pure compounds prepared from the stock solutions by serial dilution in RPMI-1640 medium were added into each well. The various concentration used were 30, 15, 7.5, 3.75, 1.875, 0.9375 and 0.46875 µg/mL. Subsequently, each well was filled with 100 µL of exponentially growing cell suspensions at the concentration of $5 \times 10^5$ cells/mL and incubated for 3 days at 37 °C, 5 % $CO_2$, 90 % humidity. The assay of each concentration of extracts or isolated compounds was performed in triplicates and the control wells of untreated well population were also included.

After 3 days, the fraction of surviving cells were determined relative to the untreated cell population by the colorimeter MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) (Sigma, USA) method. 20 µL of MTT solution (5 mg in 1 mL PBS, freshly prepared before assay) was then added to 100 µL of cell suspension of cell monolayer in each microtiter well followed by 4 h incubation at 37 °C. To solubilize the formazan crystals formed after the incubation with MTT, 100 µL of DMSO was added to each well. The plate was left at room temperature for 0.5 before reading the absorbance for each well at 550 nm with ELISA reader. A graph of percentage of cell viability *versus* the concentration of crude extracts or pure compounds tested was plotted and the $IC_{50}$ values were determined. The cytotoxic index used was $IC_{50}$, which is the concentration that yields 50 % inhibition of the treated cell compared with untreated control.

## RESULTS AND DISCUSSION

Eight (44 %) extracts were found to have cytotoxic activity with $IC_{50}$ values less than 30 µg/mL. It was found that non-polar and semi-polar extracts of *Alpinia*

species exhibited the most active cytotoxic activity against HL-60 (human promyelocytic leukemic) cell line. The petroleum ether and chloroform extracts of *Alpinia galanga* were strongly active against HL-60 cell line with $IC_{50}$ values of 4.7 and 5.6 µg/mL, respectively. Non-polar and semi-polar extracts of *Zingiber cassumunar* and *Z. officinale* var. *rubrum* demonstrated moderate activity in this assay. All methanol extracts were found to be inactive in this screening with the $IC_{50}$ values were more than 30 µg/mL, except for *Zingiber cassumunar* which was weakly active ($IC_{50}$ value = 27 µg/mL) against HL-60 leukemic cell. Extracts of *Kaempferia* species were not active towards HL-60 cell. The cytotoxic data for screening of Zingiberaceous species against HL-60 (human promyelocytic leukemic) cell line is shown in Table-1.

TABLE-1
CYTOTOXIC SCREENING RESULTS OF ZINGIBERACEOUS SPECIES

| Plant | Extracts | $IC_{50}$ (µg/mL) |
|---|---|---|
| *Alpinia galanga* | Petroleum ether | 4.7 |
| | Chloroform | 5.6 |
| | Methanol | > 30 |
| *Zingiber cassumunar* | Hexane | 21.7 |
| | Ethyl acetate | 22.6 |
| | Methanol | 27.0 |
| *Zingiber officinale* var. *rubrum* | Petroleum ether | 12.2 |
| | Chloroform | 20.5 |
| | Methanol | > 30 |
| *Zingiber officinale* | Petroleum ether | > 30 |
| | Chloroform | 20.8 |
| | Methanol | > 30 |
| *Kaempferia rotunda* | Petroleum ether | > 30 |
| | Chloroform | > 30 |
| | Methanol | > 30 |
| *Kaempferia galanga* | Hexane | > 30 |
| | Chloroform | > 30 |
| | Methanol | > 30 |
| Standard (Goniothalamin) | | 1.4 |

Indication of $IC_{50}$ (µg/mL) values: < 10 = strong activity, 10-20 = moderate activity, 20-30 = low activity.

From a study by Azuma *et al.*[13], (1'S)-1'-acetoxychavicol acetate, a bioactive constituent from rhizomes and seeds of *A. galanga*, induced high apoptotic activity against HL-60 cell line. This compound also exerts its antitumor activity by similar mechanism in various cancer cells such as Ehrlich ascites, rat and human hepatocellular carcinoma cells, human colon cancer cells and human myeloid leukemic cells from other findings. 1'-Acetoxychavicol acetate which was also obtained from non-polar and semi-polar extracts of *Alpinia conchigera*[10], could explain the good cytotoxic activity of petroleum-ether and chloroform extracts of *A. galanga* against HL-60 cell in this study.

To the best of our knowledge, there were no previous reports regarding the cytotoxicity of the extracts from *Zingiber cassumunar* against HL-60 cancer cell line. Park *et al.*[14] showed that the chloroform extract of the rhizome of *Zingiber cassumunar* showed moderate cytotoxicity ($ED_{50}$ < 20 μg/mL) towards human lung carcinoma (A549) and stomach (SNU-638) cancer cells but not active against human colon cancer (Col2) cell line. Extracts of *Kaempferia rotunda* rhizomes were not cytotoxic against the tested cell lines[14]. Further study by Han *et al.*[15] revealed that curcumin was the active principle of the chloroform extract of *Z. cassumunar* while (*E*)-4-(3'4'-dimethoxyphenyl)but-3-en-1-ol and (*E*)-4-(3',4'-dimethoxyphenyl) -but-3-en-1-yl acetate were not active in the assay. Curcumin demonstrated significant cytotoxicity against several human cancer cell lines in the study (Col2; 2.30, A549; 12.30, SNU638; $IC_{50}$ 18.80 μg/mL).

Diarylheptanoids have been reported as components of both fresh and dried ginger. Wei *et al.*[16] reported significant cytotoxic and apoptotic activities against human promyelocytic leukemia cells (HL-60) of several ginger (*Zingiber officinale* Roscoe) constituents, including some diarylheptanoids and gingerol-related compounds. It was found that six of these compounds exhibited significant activity ($IC_{50}$ < 50 μM) on the cell proliferation of HL-60 cells, with 1, 7-*bis*(4-hydroxy-3-methoxyphenyl)hept-4-en-3-one (**3**) demonstrated the most prominent activity, displayed $IC_{50}$ value of 9.6 μg/mL (27.1 ± 3.4 μM).

Ethyl *p*-methoxycinnamate (**1**) and *p*-methoxy cinnamic acid (**2**) (Fig. 1) are two main compounds reported for *Kaempferia galanga*. The NMR spectra for these two compounds were very similar with the exception of signals responsible for substituents at the carbonyl group. Ethyl *p*-methoxycinnamate is an ester which showed a quartet signal at δ 4.21 ($J$ = 7.32 Hz) and a triplet signal at δ 1.30 ($J$ = 7.32 Hz) which is responsible for the ethoxyl group while these signals were not observed in the $^1$H NMR spectrum of *p*-methoxy cinnamic acid, a carboxylic acid derivatives. These two phenylpropanoid derivatives have been known with its strong larvicidal properties (Kiuchi *et al.*[17]) and have been reported to be cytotoxic against HeLa cervical cancer cell lines with $IC_{50}$ values of 35 μg/mL. The antileukaemic property of ethyl *p*-methoxy-*trans*-cinnamate (**1**) is not reported yet, it is not active towards HL-60 cells (up to 30 μg/mL) in present study.

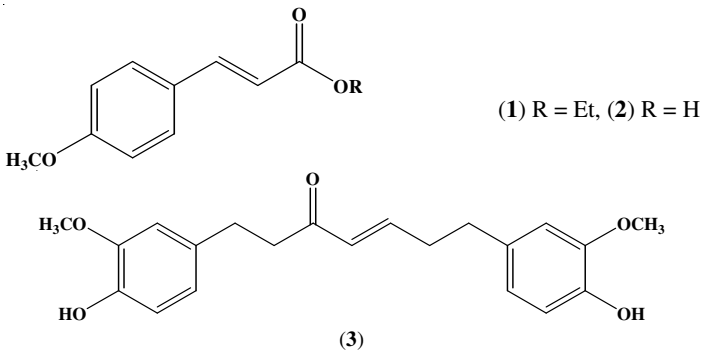


Fig. 1. Structures of isolated phytochemicals and reference compound

## Conclusion

Screening of antileukemic properties of Zingiberaceae extracts have revealed the promising activity of *Alpinia* and *Zingiber* species. The study of *Zingiber cassumunar*, *Kaempferia rotunda* and *K. galanga* extracts against human promyelocytic leukaemia (HL-60) cell line is firstly described in this paper. Phytochemical isolation work on *K. galanga* has afforded two phenylpropanoid derivatives besides common phytosterol.

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