CYTOTOXICITY OF *ALPINIA GALANGA* RHIZOME EXTRACTS AND ISOLATED COMPOUNDS AGAINST SMALL CELL LUNG CANCER NCI-H187 CELL LINE

Anchulee Pengsook[1] Parinthorn Temyarasilp[2*] Wanchai Pluempanupat[3]

Received : February 21, 2023
Revised : June 10, 2024
Accepted : July 25, 2024

Abstract

The purpose of this research was to study the cytotoxicity of the extracts from *Alpinia galanga* rhizome and isolated compounds against NCI-H187 small cell lung cancer cells. 1'*S*-1'-acetocavical acetate **(1)**, *p*-coumaryl diacetate **(2)**, 1'*S*-1'-acetoxy-eugenol acetate **(3)**, (*S*)-hydroxycavicol acetate **(4)**, 4-hydroxycinnamaldehyde **(5)** and *trans*-*p*-acetoxycinnamyl alcohol **(6)** were isolated from the rhizomes of Alpinia galangal. The structures of the isolated compounds were characterized by spectroscopic techniques and comparison with the literature. Among them, the extracts, and compounds 1-5 displayed potent cytotoxic activity against human small cell lung cancer (NCI-H187) cells exhibiting >98% at the highest concentration (50 μg). Compound 1 showed the highest cytotoxicity against NCI-H187 cells with 99.69% inhibition and IC_{50} of 4.61 μM, 1.68-fold higher than ellipticine.

Keywords: *Alpinia galanga*, Cytotoxicity, NCI-H187

[1] *Student, Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, and Special Research Unit for Advanced Magnetic Resonance, Kasetsart University, e-mail: anchulee.peng@ku.th*

[2] *Dr. Lecturer, Chemistry Program, Faculty of Science and Technology, Valaya Alongkorn Rajabhat University under the Royal Patronage Pathum Thani Province, e-mail: parinthorn@vru.ac.th*

[3] *Associate Professor Dr., Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, and Special Research Unit for Advanced Magnetic Resonance, Kasetsart University, e-mail: fsciwcp@ku.ac.th*

** Corresponding author, e-mail: parinthorn@vru.ac.th*

Introduction

Small cell lung cancer (SCLC) is a rapidly spreading cancer. This type of cancer can recur even after chemotherapy. SCLC cells are also small and the cytoplasm surrounding the nucleus is limited. Cells tend to grow accordingly floating clumps or spheres, which are often challenging to achieve separately. SCLC is a type of lung cancer that has no effective treatment over recent years and it affects to more than 200,000 people worldwide yearly with a very high death rate. SCLC has unique biological and chromosomal changes, abnormal regulation of tumor suppressor genes, oncogenes, and signaling pathways, upregulation of tyrosine kinase receptor, growth factors and cell markers and an active early development path. Treatment for SCLC depends on many factors including age, overall health and cancer stage. The options include surgery, radiation therapy, chemotherapy and immunotherapy.

A. galanga (L.) Wild (Figure 1) is a herb in the family *Zingiberaceae* that distribute in Asia including Thailand, Malaysia, China and India. The rhizome of *A. galanga* is used as spices in Southeast Asian cuisine and as a traditional medicine (Anirban & Santanu, 2018). Currently, it is reported that *A. galanga* has various biological activities such as anti-tumor, antimicrobial (Wong, 2009), antiallergic (Matsuda *et al.*, 2003), antioxidant. and anti-inflammatory (Ghosh *et al.*, 2008).

Figure 1 *Alpinia galanga* (L.) Wild

Several studies on the phytochemicals of A. galanga have been reported over the past decades, in which A. galanga has various medicinal properties such as antibacterial, antifungal, cancer cytotoxicity, etc. The examples of chemical compositions and related biological activities are shown in Table 1

Table 1 The examples of the chemical compositions from *A. galanga* and their biological activities

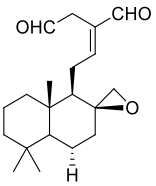
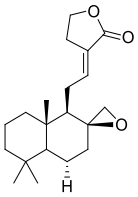
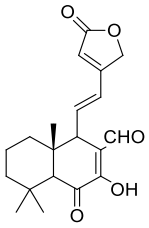
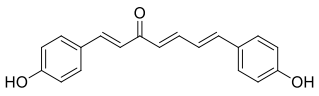
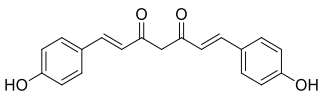
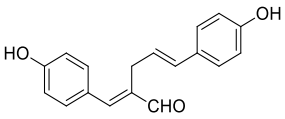
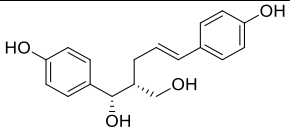
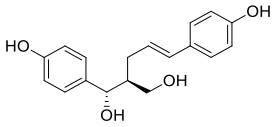
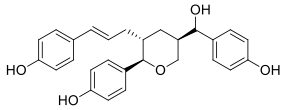
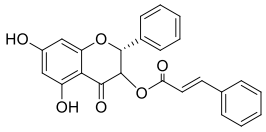
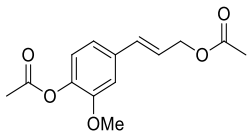
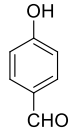
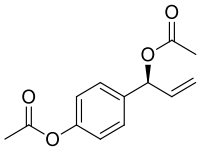
Structure	Name	Biological activity
	(*E*)-8β,17-epoxylabd-12-ene-15,16-dial	Antibacterial against *Staphylococcus aureus* and *Bacillus cereus* and α-glucosidase inhibitory (Itokawa et al., 1980 & Sivasothy et al., 2013)
	Galanolactone	Antifungal activity to *Candida guilliermondii* PW44 and *Candida tropicalis* PW30 (Morita & Itokawa, 1988)
	Galanganin	Cytotoxicity towards various cancer cells (Ma et al., 2017)
	1,7-bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one	Effectively inhibited the proliferation of melanoma cells (Lo et al., 2013)
	Bisdemethoxy-curcumin	Effectively inhibited the proliferation of melanoma cells (Lo et al., 2013)
	Galanganal	Cytotoxicity against Hela cells and inhibit nitric oxide production (Morikawa et al., 2005 & Xu et al., 2013)

Table 1 The examples of the chemical compositions from *A. galanga* and their biological activities (Continued)

Structure	Name	Biological activity
	Galanganol A	Cytotoxicity against Hela cells (Xu et al., 2013)
	Galanganol B	Inhibit nitric oxide production (Morikawa et al., 2005)
	Galanganol C	Inhibit nitric oxide production (Morikawa et al., 2005)
	(2*R*,3*S*)-Pinobaksin-3-cinnamate	Exhibited neuroprotective effect against PC12 cells. (Ma et al., 2017)
	trans-Coniferyl diacetate	Xanthine oxidase inhibitory (Noro et al., 1988)
	4-Hydroxybenzaldehyde	Xanthine oxidase inhibitory (Noro et al., 1988)
	1'S-1'-Acetoxychavicol acetate	Antileishmanial, antiallergic, gastroprotective, nitric oxide production inhibitory, anti-HIV, antibacterial, anticancer (Matsuda et al., 2003; Morikawa et al., 2005; Niyomkam et al., 2010; Ye & Li, 2006; Zeng et al., 2015)

Based on the above information, a group of interesting compounds with diverse biological activity has been identified. However, the biological activity of *A. galanga* extract on SCLC is less reported. In this works, the researcher is interested in studying the biological activity of *A. galanga* extracts against NCI-H187 lung cancer cells and the isolated compounds in the *A. galanga* extract as well.

Objectives

To extract and characterize the chemical constituents from *A. galanga* rhizomes and test their cytotoxic activity against NCI-H187 lung cancer cells

Materials and methods

1. Plant preparation

A. galanga rhizomes were collected from Prachinburi province, Thailand. Fresh *A. galanga* rhizomes (20 kg) were cut into small pieces and air-dried for 7 days.

2. Extraction and isolation

The air-dried *A. galanga* rhizomes (1.6 kg) were ground and sequentially extracted with n-hexane, EtOAc, and EtOH by Soxhlet extraction over 7 days, respectively. The extracted solutions were filtered and evaporated under reduced pressure at 40– 45 °C to obtain 6.83 g from hexane extract, 19.11 g from EtOAc extract and 82.86 g from MeOH extract. All crude extracts had a significant effect on cells, whereas in this experiment only EtOAc extract was selected to determine the active ingredients. This is because EtOAc extract has been reported to contain many bioactive compounds (Datt *et al.*, 2015).

The EtOAc extract (9.0 g) was fractionated by column chromatography, using a gradient solvent system of n-hexane, n-hexane-EtOAc, and EtOAc with increasing the amount of polar solvents. The eluents were examined by TLC and 12 groups of fractions were obtained. Fraction 2 gave 1'*S*-1'-Acetoxychavicol acetate **(1)** as colorless oil (2.0 g). Fraction 6 was chromatographed by isocratic elution with n-hexane:EtOAc (4:1) to afford *p*-Coumaryl diacetate **(2)** as pale yellow oil (205 mg) in fraction 6-1. Fraction 7 was further fractionated by preparative thin layer chromatography (PTLC) using an isocratic solvent system of n-hexane:EtOAc (4:1) to give three

fractions (fr. 7-1 to 7-3). Fraction 7-2 was chromatographed two times with PTLC using n-hexane:EtOAc (5:1) as eluting solvent, then the darkest fraction 7-2-1, was collected. Fraction 7-2-1 was separated with PTLC twice, using n-hexane:EtOAc (5:1), to give two fractions (fr. 7-2-1-1 and 7-2-1-2). Fraction 7-2-1-2 was subjected to PTLC, using n-hexane:EtOAc (3:1) to give 1'*S*-1'-acetoxy-eugenol acetate **(3)** as yellow liquid (5 mg) in fraction 7-2-1-2-2. Fraction 8 was further fractionated by column chromatography, using an isocratic solvent system of n-hexane:EtOAc (4:1), to give four fractions (fr. 8-1 to 8-4). Fraction 8-3 was separated with PTLC by of n-hexane:EtOAc (3:1) to yield (*S*)-Hydroxychavicol acetate **(4)** as yellow oil (160 mg) in fraction 8-3-2. Fraction 9 was further fractionated by column chromatography, using a gradient solvent system from 100% hexane to n-hexane:EtOAc (3:1), to give fraction 9-1, 9-2 and 9-3. Fraction 9-2 was isolated three times by PTLC using n-hexane-EtOAc (2:1) followed by n-hexane-EtOAc (2:1) and n-hexane-EtOAc (3:1) to yield 4-Hydroxycinnamaldehyde **(5)** as yellow solid (20 mg). Fraction 10 was further fractionated by column chromatography, using an isocratic solvent system of n-hexane-EtOAc (4:1), to give *trans-p*-Acetoxycinnamyl alcohol **(6)** as pale yellow solid (380 mg). The isolation scheme off all bioactive compounds is shown in Figure 2.

1'*S*-1'-Acetoxychavicol acetate **(1)** colorless oil; $[\alpha]_D^{20}$ -50 (*c*=0.5, CH_2Cl_2): Colorless solid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.36 (d, *J* = 8.6 Hz, 2H), 7.07 (d, *J* = 8.6 Hz, 2H), 6.26 (d, *J* = 5.8 Hz, 1H), 5.98 (ddd, *J* = 17.1, 10.4, 5.8 Hz, 1H), 5.30 (dt, *J* = 17.2, 1.3 Hz, 1H), 5.25 (dt, *J* = 10.5, 1.2 Hz, 1H), 2.29 (s, 3H), 2.10 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.8, 169.3, 150.4, 136.4, 136.0, 128.4, 121.7, 117.0, 75.5, 21.1, 21.0. HRMS (ESI) Calculated for $C_{13}H_{14}NaO_4$ 257.0790 ($[M+Na]^+$), Found 257.0825. The Physical and spectral data were in agreement with Noro *et al*. (1988).

p-Coumaryl diacetate **(2)** pale yellow oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.39 (d, *J* = 8.6 Hz, 2H), 7.05 (d, *J* = 8.6 Hz, 2H), 6.62 (d, *J* = 16.1 Hz, 1H), 6.22 (dt, *J* = 15.9, 6.4 Hz, 1H), 4.75 (dd, *J* = 6.4, 1.3 Hz, 2H), 2.30 (s, 3H), 2.10 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.8, 169.4, 150.4, 134.0, 133.1, 127.6, 123.4, 121.7, 64.9, 21.1, 21.0. HRMS (ESI) Calculated for $C_{13}H_{14}NaO_4$ 257.0790 ($[M+Na]^+$), Found 257.0796. (Noro *et al*., 1988) The Physical and spectral data were in agreement with Noro *et al*. (1988).

1'*S*-1'-acetoxy-eugenol acetate **(3)** yellow liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.01 (d, *J* = 8.5 Hz, 1H), 6.95-6.93 (m, 2H), 6.25 (d, *J* = 5.8 Hz, 1H), 5.99 (ddd, *J* = 17.2, 10.5, 5.8 Hz, 1Hz), 5.31 (dt, *J* = 17.2, 1.2 Hz, 1H), 5.26 (dt, *J* = 10.5, 1.3 Hz, 1H), 3.84 (s, 3H), 2.31 (s, 3H), 2.12 (s, 3H).

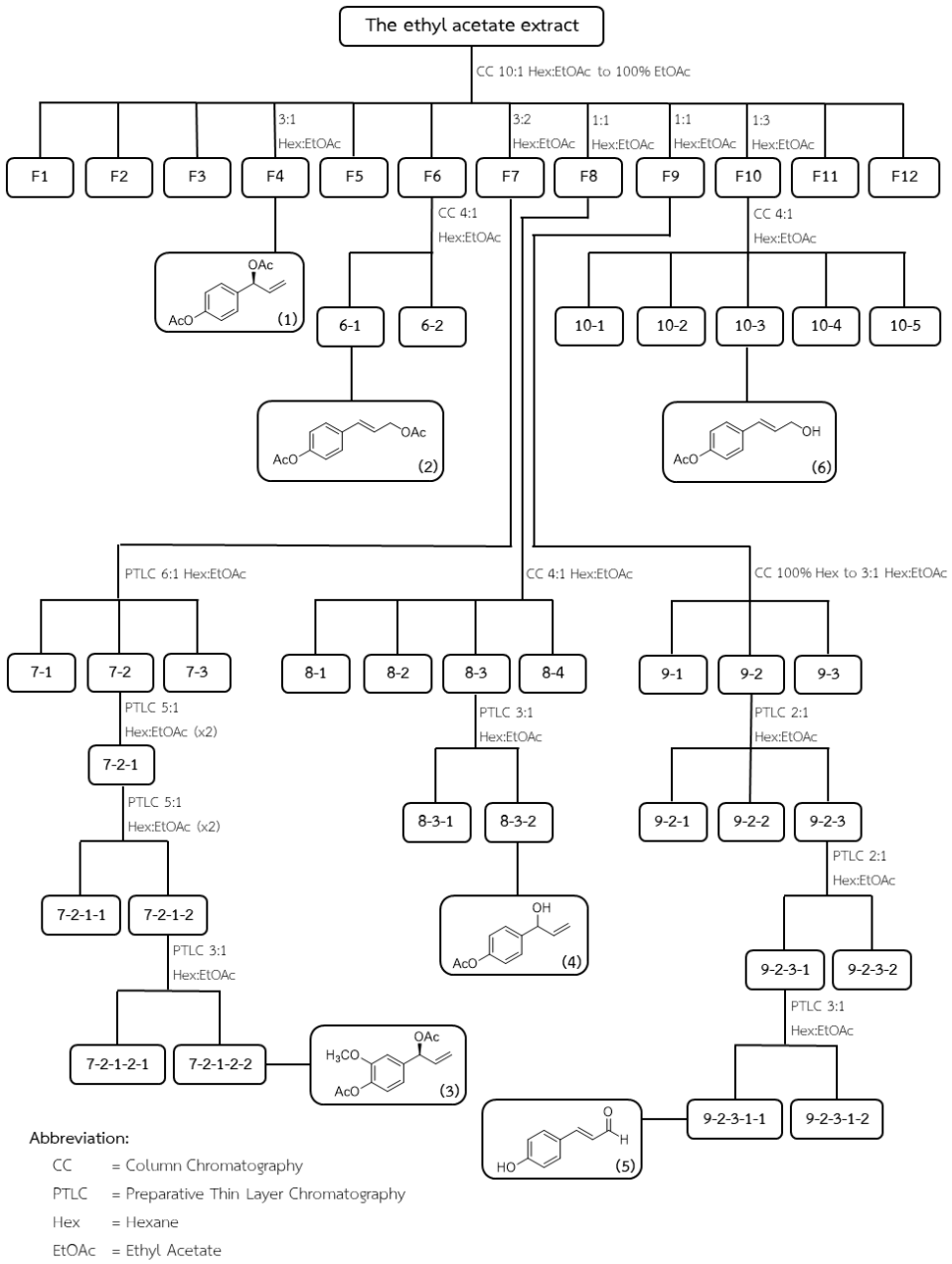


Figure 2 Schematic representation of the separation of active compounds using chromatographic technique

^{13}C NMR (100 MHz, $CDCl_3$) δ 170.04, 169.12, 151.21, 139.68, 137.81, 136.07, 122.92, 119.75, 117.17, 111.62, 75.83, 56.04, 21.38, 20.81. HRMS (ESI) Calculated for $C_{14}H_{16}NaO_6$ 287.0895 ($[M+Na]^+$), Found 287.0908. The Physical and spectral data were in agreement with Janssen & Scheffer (1985)

(*S*)-Hydroxychavicol acetate **(4)** yellow oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.37 (d, *J* = 8.5 Hz, 2H), 7.06 (d, *J* = 8.6 Hz, 2H), 6.00 (ddd, *J* = 17.1, 10.3, 6.1 Hz, 1H), 5.33 (dt, *J* = 17.1, 1.4 Hz, 1H), 5.20-5.17 (m, 2H), 2.28 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.6, 150.0, 140.2, 140.0, 127.5, 121.6, 115.4, 74.7, 21.1. HRMS (ESI) Calculated for $C_{11}H_{12}NaO_3$ 215.0684 ($[M+Na]^+$), Found 215.0679. The Physical and spectral data were in agreement with Janssen & Scheffer (1985)

4-Hydroxycinnamaldehyde **(5)** yellow solid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.67 (d, *J* = 7.8 Hz, 1H), 7.51 (d, *J* = 8.7 Hz, 2H), 7.44 (d, *J* = 15.8 Hz, 1H), 6.92 (d, *J* = 8.7 Hz, 2H), 6.63 (dd, *J* = 15.8, 7.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 194.1, 158.8, 153.1, 130.8, 127.0, 126.6, 116.3. HRMS (ESI) Calculated for $C_9H_8NaO_2$ 171.0422 ($[M+Na]^+$), Found 171.0421. The Physical and spectral data were in agreement with Stange *et al.* (1999).

trans-p-Acetoxycinnamyl alcohol **(6)** pale yellow solid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.36 (d, *J* = 8.6 Hz, 2H), 7.03 (d, *J* = 8.6 Hz, 2H), 6.57 (d, *J* = 15.9 Hz, 1H), 6.28 (dt, *J* = 15.9, 5.7 Hz, 1H), 4.29 (dd, *J* = 5.7, 1.3 Hz, 2H), 2.28 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.6, 150.0, 134.4, 129.9, 128.7, 127.3, 121.6, 63.4, 21.0. HRMS (ESI) Calculated for $C_{11}H_{12}NaO_3$ 215.0684 ($[M+Na]^+$), Found 215.0681. The Physical and spectral data were in agreement with Zhu *et al.* (2013).

3. Cytotoxic activity

All extracts and isolated compounds of *A. galanga* were evaluated for cytotoxicity against NCI-H187 small lung cancer cell line via Resazurin microplate assay (O'Brien *et al.*, 2000). Ellipticine and 0.5% DMSO were used as positive and negative controls, respectively.

The NCI-H187 cell line was cultured in RPMI-1640 supplemented with 15% non-thermal activated FBS, 1 mM sodium pyruvate, 2.5 g/L glucose and 2.2 g/L $NaHCO_3$). The growth cell was harvested and diluted to $6.7x10^4$ cells/mL in the complete medium before the test. The REMA testing was triplicate performed in 384-well plates. Each well contained 5 μL of sample and 45 μL of cell suspension. The plates were then incubated at 37 °C for 5 days in a humidified incubator with 5% CO_2. Then, 0.0625 mg/mL resazurin solution (12.5 μL) was added

to each well and further incubated at 37 ^{o}C for 4 hours. For determination of cytotoxicity, the fluorescence was measured at 530 nm excitation and 590 nm emission wavelength using the bottom reading mode and the noise of the signal was removed by a blank before calculation.

Percent cytotoxicity was calculated by the following equation, where FU_T and FU_C are mean fluorescent units from cells treated with the test compound and 0.5% DMSO, respectively.

$$\% \text{ Cytotoxicity} = [1-(FU_T/ FU_C)] \times 100$$

The sample was considered active if percent cytotoxicity was more than 50.

Results and discussion

From the assessment of cytotoxicity with biological approaches human small cell lung cancer (NCI-H187), column chromatography of EtOAc extract from *A. galanga* rhizomes has led to the isolation of six compounds such as 1'S-1'-acetocavical acetate **(1)**, *p*-coumaryl diacetate **(2)**, 1'S-1'-acetoxy-eugenol A acetate **(3)**, (S)-hydroxycavicol acetate **(4)**, 4-hydroxycinnamal-dehyde **(5)** and *trans-p*-Acetoxycinnamyl Alcohol **(6)**. The structures of the isolated compounds were shown by comparison of spectroscopic and physical data with literary value (Figure 3). The cytotoxic activities of *A. galanga* rhizome extracts and isolated compounds from EtOAc extract against the NCI-H187 are summarized in Table 2.

The results indicated that the cytotoxicity of *A. galanga* rhizome extracts against small cell lung cancer (NCI-H187) was excellent inhibition (>99%) at the concentrations, 50 µg/mL. In addition, the isolated compounds, compound **(1)** presented high activity with 99.69%, followed by compound **(3)** (99.49%), compound **(4)** (99.46%), compound **(2)** (99.41%) and compound (**5)** (98.84%). Compound (**6)** exhibited weak activity with 52.01% cytotoxicity. Then, the compound **1** and Ellipticine were determined theirs IC_{50} values. The results showed that the IC_{50} of compound **1** was 4.61 µM, 1.68-fold higher than ellipticine ($\mathbf{IC_{50}}$ 7.75 µM), the reference anticancer drug.

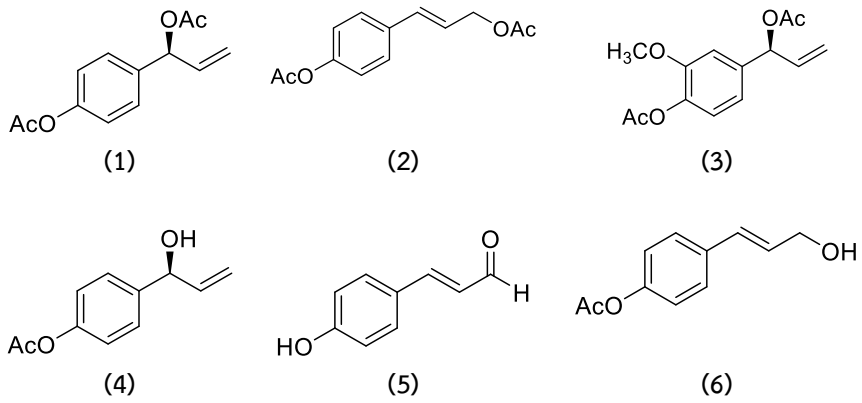


Figure 3 Structures of compound **1-6** isolated from EtOAc extract of *A. galanga* rhizomes

Table 2 Cytotoxicity of *A. galanga* rhizome extracts and isolated compounds against NCI-H187 small lung cancer cells

Crude extract / Compound	% Cytotoxicity[1]	IC_{50} (μM)
n-Hexane extract	99.15	-
EtOAc extract	99.27	-
EtOH extract	99.96	-
1'*S*-1'-acetocavical acetate **(1)**	99.69	4.61
p-coumaryl diacetate **(2)**	99.41	-
1'*S*-1'-acetoxy-eugenol A acetate **(3)**	99.49	-
(*S*)-hydroxycavicol acetate **(4)**	99.46	-
4-hydroxycinnamal- dehyde **(5)**	98.84	-
trans-p-Acetoxycinnamyl Alcohol **(6)**	52.01	-
Ellipticine	-	7.75

[1]Final concentration 50 μg/mL

In comparing the activity of compounds **(2)** and **(6)**, it can be suggested that the acetyl group of compound **(2)** should play an important role in its cytotoxicity, while the presence of free hydroxyl groups appears to reduce the cytotoxic effect of compound **(6)**. Compound **(1)** should be selected as a potential lead molecule for the development of anticancer drug.

Conclusions

The purpose of this research was to study the cytotoxicity of the extracts and its isolated compounds of EtOAc extract from *A. galanga* rhizomes against NCI-H187 small cell lung cancer. The isolated compounds were 1'*S*-1'-acetocavical acetate **(1)**, *p*-coumaryl diacetate **(2)**, 1'*S*-1'-acetoxy-eugenol A acetate **(3)**, (*S*)-hydroxycavicol acetate **(4)**, 4-hydroxycinnamaldehyde **(5)** and *trans-p*-Acetoxycinnamyl Alcohol **(6)**. The structures of all compounds were characterized by spectroscopic techniques and comparison with the literature. Compound **(1)** showed the highest cytotoxicity against NCI-H187 cells with 99.69% inhibition and IC_{50} of 4.61 μM, 1.68-fold higher than ellipticine.

Acknowledgements

Anchulee pengsook and colleagues thank to Department of Chemistry, Faculty of Science, Kasetsart University for providing facilities, chemicals and equipment for this research. This work was supported by the Center of Excellence for Innovation in Chemistry (PERCH-CIC), Ministry of Higher Education, Science, Research and Innovation and the Kasetsart University Research and Development Institute (KURDI).

References

Anirban, C., & Santanu, P. (2018). A Review on Phytochemical and Pharmacological Potential of *Alpinia galanga*. **Pharmacognosy Journal**, 10(1), 9-15.

Datta, R., Kaur, A., Saraf, I., Singh, I. P., & Kaur, S. (2019). Effect of crude extracts and purified compounds of *Alpinia galangal* on nutritional physiology of a polyphagous lepideopteran pest, *Spodoptera litura* (Fabricus). **Ecotoxicology and environmental Safety**, 168, 324-329.

Ghosh, B., Saha, K., Ghosh, A., & Dhar, S. (2008). Cardiovascular Evaluation in Patients with Systemic Lupus Erythematosus-a Cross Sectional Study. **Chronicles of Young Scientists**, 2(3), 139-143.

Itokawa, H., Watanabe, K., Mihashi, S., & Iitaka, Y. (1980). Isolation of Agarofuran-Type Sesquiterpenes from *Alpinia japonica* (Thunb.) Miq. **Chemical and Pharmaceutical Bulletin**, 28(2), 681-682.

Janssen, A.M., & Scheffer, J.J. (1985). Acetoxychavicol Acetate, an Antifungal Component of **Alpinia galanga. Planta Med***,* 51(6), 507-511.

Lo, C.Y., Liu, P.L., Lin, L.C., Chen, Y.T., Hseu Y.C., Wen Z.H., & Wang H.M. (2013). Antimelanoma and Antityrosinase from *Alpinia galangal* Constituents. **The Scientific World Journal**, 2013(3), 186505.

Ma, X.N., Xie, C.L., Miao, Z., Yang, Q., & Yang X.W. (2017). An Overview of Chemical Constituents from Alpinia Species in the Last Six Decades. **RSC Advances***,* 7(23), 14114-14144.

Matsuda, H., Morikawa, T., Managi, H., & Yoshikawa, M. (2003). Antiallergic Principles from *Alpinia galanga*: Structural Requirements of Phenylpropanoids for Inhibition of Degranulation and Release of Tnf-**α** and Il-4 in Rbl-2h3 Cells. **Bioorganic & Medicinal Chemistry Letters**, 13(19), 3197-3202.

Morita, H., & Itokawa, H. (1988). Cytotoxic and Antifungal Diterpenes from the Seeds of *Alpinia galanga*. **Planta Med***,* 54(02), 117-120.

Morikawa, T., Ando, S., Matsuda, H., Kataoka, S., Muraoka, O., & Yoshikawa M. (2005). Inhibitors of Nitric Oxide Production from the Rhizomes of A*lpinia galanga*: Structures of New 8-9' Linked Neolignans and Sesquineolignan. **Chemical and Pharmaceutical Bulletin***,* 53(6), 625-630.

Niyomkam, P., Kaewbumrung, S., Kaewnpparat, S., & Panichayupakaranant, P. (2010). Antibacterial Activity of Thai Herbal Extracts on Acne Involved Microorganism. **Pharmaceutical Biology**. 48(4), 375-380.

Noro, T., Sekiya, T., Katoh, M., Oda, Y., Miyase, T., Kuroyanagi, M., Ueno, A., & Fukushima, S. (1988). Inhibitors of Xanthine Oxidase from *Alpinia galanga*. **Chemical & Pharmaceutical Bulletin***,* 36(1), 244-248.

O'Brien, J., Wilson, I., Orton, T., & Pognan, F. (2000). Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity. **Eur. J. Biochem**, 267(17), 5421–5426.

Sivasothy, Y., Ibrahim, H., Paliany, A.S., Alias, S.A., Md Nor, N.R., & Awang, K. (2013). A New Bis-Labdanic Diterpene from the Rhizomes of *Alpinia pahangensis*. **Planta Med**, 79(18), 1775-1780.

Stange, R.R.Jr., Sims, J.J., Midland, S.L., & McDonald, R.E. (1999). Isolation of a Phytoalexin, *trans-p*-Coumaryl Aldehyde, from *Cucurbita maxima*, Cucurbitaceae. **Phytochemistry**, 52(1), 41-43.

Wong, L.F., Lim, Y.Y., & Omar, M. (2009). Antioxidant and Antimicrobial Activities of Some Alpinia Species. **Journal of Food Biochemistry**, 33(6), 835-851.

Xu, J.J., Zeng G.Z., Yang S.C., Shen Y., & Tan N.H. (2013). 8-9' Linked Neolignans with Cytotoxicity from *Alpinia conchigera.* **Fitoterapia**, 91, 82-86.

Ye, Y., & Li, B. (2006). 1'S-1'-Acetoxychavicol Acetate Isolated from *Alpinia galanga* Inhibits Human Immunodeficiency Virus Type 1 Replication by Blocking Rev Transport. **Journal of General Virology**, 87(7), 2047-2053.

Zeng, Q.H., Lu, C.L., Zhang, X.W., & Jiang, J.G. (2015). Isolation and Identification of Ingredients Inducing Cancer Cell Death from the Seeds of *Alpinia galanga*, a Chinese Spice. **Food & Function**, 6(2), 431-443.

Zhu, Y., Regner, M., Lu, F., Kim, H., Mohammadi, A., Pearson, T.J., & Ralph, J. (2013). Preparation of Monolignol **γ**-Acetate, **γ**-p-Hydroxycinnamate, and **γ**-p-Hydroxybenzoate Conjugates: Selective Deacylation of Phenolic Acetates with Hydrazine Acetate. **RSC Advances**, 3(44), 21964-21971.