

In vitro Cytotoxic Activities and Molecular Mechanisms of *Angelica shikokiana* Extract and its Isolated Compounds

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ABSTRACT

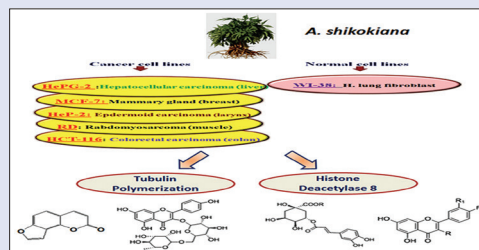
Background: *Angelica shikokiana* is a Japanese medicinal herb that is included among food and drug preparations protecting against cancer; however, there is no previous report about the cytotoxicity of *A. shikokiana* or its bioactive compounds. **Objective:** This study was designed to investigate the cytotoxic activities of *A. shikokiana* methanol extract (AME) and its isolated compounds and to identify the molecular mechanisms of the cytotoxicity. **Materials and Methods:** Cytotoxicity and selectivity was investigated by measuring the IC₅₀ values on five cancer cell lines; human hepatocellular carcinoma, rhabdomyosarcoma (RD), colorectal carcinoma, human epithelioma and human breast adenocarcinoma and one normal cell line; human lung fibroblasts. The effects on tubulin polymerization and histone deacetylase 8 (HDAC8), were examined to determine the mechanism of cytotoxicity. Docking study was designed to examine the binding affinity to the target molecules. **Results:** Methanol extract and some of its isolated coumarins and flavonoids showed potent, selective cytotoxicity against cancer cell lines. AME and all isolated compounds inhibited tubulin polymerization. Angelicin and kaempferol-3-*O*-rutinoside were the most active compounds. Phenolic compounds and furanocoumarins showed binding affinity to colchicine binding site rather than the vinblastine binding site of tubulin microtubules. On the other side, quercetin, kaempferol, luteolin, chlorogenic acid, and methyl chlorogenate exhibited the strongest activity against HDAC8 and the highest affinity to trichostatin A binding site. **Conclusion:** These findings provide the first scientific evidence of the cytotoxicity of AME through inhibition of tubulin polymerization and HDAC8 activity through its coumarin and flavonoid content.

Key words: *Angelica shikokiana*, cytotoxicity, histone deacetylase 8, tubulin polymerization

SUMMARY

- The present study provides for the first time a clue for the cytotoxic activities

of the AME. Our results indicate that the cytotoxic activities are partially related to the ability of AME to inhibit tubulin polymerization and HDAC8 activity. Isolated compounds; Angelicin and kaempferol-3-*O*-rutinoside showed the strongest inhibition of tubulin polymerization through binding to colchicine binding domain of tubulin microtubules. Phenolic compounds; quercetin, luteolin, kaempferol, chlorogenic acid and methyl chlorogenate exhibited a strong inhibition of HDAC8 through binding to TSA binding site. This, however, further detailed pharmacological and *in vivo* studies should be the next step in evaluating the cytotoxic activities of AME and its active compounds that are currently ongoing.



Abbreviations used: AME: Methanol extract of the aerial part of *A. shikokiana*, HDACs: Histone deacetylases, HDAC8: Histone deacetylase 8

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INTRODUCTION

Cancer as a one of the leading causes of death worldwide, being the main reason for searching for anti-neoplastic agents, especially from natural origin, which has become an important research aspect.^[1] Tubulin microtubules destabilization and histone deacetylases (HDACs) inhibition are two of the most promising strategies for cancer treatment.^[2] Microtubules polymerization is a highly dynamic process and is crucial to mitosis and cell division.^[3] Chemotherapeutic agents targeting microtubules destabilization bind to one of the three binding sites; paclitaxel, vinca domain or the colchicine domain.^[4] On the other hand, HDACs have been shown to regulate the acetylation of the histones and nonhistone proteins that regulate the expression and activity of numerous proteins involved in both cancer initiation and cancer progression.^[5] Inhibition of HDACs leads to changes in the expression of genes involved in the regulation of apoptosis, proliferation, and the cell cycle.^[6] Histone deacetylase inhibitors (HDACis) are an emerging class of epigenetic anticancer drugs that possess selective cytotoxicity to cancer cells. HDACis was evidenced to inhibit tumor cell growth and induce their apoptosis.^[7] Several studies suggested that the cytotoxicity of HDACis may be due primarily to the inhibition of Class I

HDACs (HDACs 1–3 and HDAC8) which are overexpressed in various tumors.^[8] HDAC8 is one of the most resistant isoforms of HDACs and has been implicated in the pathogenesis of several types of cancers such as neuroblastoma, acute myeloid leukemia, and T-cell lymphomas.^[9]

Angelica shikokiana (*Apiaceae*) is a Japanese traditional herb known as Inutoki, and it is widely marketed as a dietary food supplement as a health tea for treating digestive and circulatory systems diseases.^[10] Previous studies incorporated the methanol extract of *A. shikokiana* among

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food and drink additives preparations used for tumor inhibition.^[11,12] Our previous study confirmed that the alcoholic extract of the leaves and stems had strong antioxidant, anti-inflammatory activities.^[13] A following-up study included the isolation of bioactive compounds of the aerial part^[14] resulted in isolation of 19 compounds; two triterpenes; α -glutinin and β -amyirin, five flavonoids; kaempferol, luteolin, quercetin, kaempferol-3-*O*-glucoside and kaempferol-3-*O*-rutinoside, two phenolic acids; methyl chlorogenate and chlorogenic acid, seven coumarins; hyuganin E, hyuganin C, isoeoxypteryxin, isopteryxin, angelicin, bergapten and psoralen and other compounds; 5-(hydroxymethyl)-2-furaldehyde, β -sitosterol-3-*O*-glucoside and adenosine. To the best of our knowledge, there is no previous report about the bioactive compounds contributed to the cytotoxic activity of *A. shikokiana*. Hence, the present study investigated the *in vitro* cytotoxicity of the methanol extract of the aerial part as well as the isolated compounds against different cancer and normal cell lines. Additionally, the molecular mechanisms of cytotoxicity were examined by testing the effect of the extract, as well as the isolated compounds on tubulin polymerization and HDAC8. Molecular docking study illustrated the binding affinity of the active compounds to the active site of tubulin protein and HDAC8.

MATERIALS AND METHODS

Plant material

The dried powder of the aerial part of *A. shikokiana* (voucher specimen No. Y-1) was obtained from Joy Plus (Kurume, Japan). Twenty grams of the dried powder of the aerial part of *A. shikokiana* was extracted with methanol (150 ml \times 3) at room temperature (25°C) to prepare *A. shikokiana* methanol extract (AME). Isolation of active compounds was done as previously described^[14] and as shown in Flowchart 1 in supplementary material.

Cell lines

Human hepatocellular carcinoma (HepG₂), rhabdomyosarcoma (RD) and colorectal carcinoma (HCT116) cells were cultured in Dulbecco's Modified Eagle's medium (DMEM) (Wako Pure Chemical Industries, Osaka, Japan) supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 U/ml streptomycin (Gibco BRL, Tokyo, Japan). Human epithelioma (Hep₂), human breast adenocarcinoma (MCF7), and human lung fibroblasts (WI-38) were cultured in Eagle's Minimum Essential Medium (Nissui Pharmaceutical, Tokyo, Japan) under the same conditions as with DMEM medium. All cell lines were obtained from Riken Bioresource Center of Japan (Ibaraki, Japan).

General experimental procedures

A tubulin polymerization assay kit was purchased from Cytoskeleton (Denver, CO, USA). HDAC8 Deacetylase Fluorometric kit was obtained from CycLex (Nagano, Japan). Vinblastine, paclitaxel, and 5-fluorouracil (5-FU) were purchased from Sigma (St. Louis, MO, USA). WST-1 was purchased from Wako Pure Chemical Industries (Osaka, Japan). All other chemicals were purchased from Sigma (St. Louis, MO, USA).

Cytotoxicity to cancer and normal cell lines

HepG₂, Hep2, MCF7, RD, HCT116 and WI-38 were cultured in 96-well plates at densities of 1×10^4 , 0.5×10^4 , 2.5×10^4 , 3×10^4 , 3.5×10^4 and 2×10^4 cells/well, respectively, in a humid atmosphere of 5% (v/v) CO₂ and 95% (v/v) air at 37°C. After 24 h, the cells were treated with five different concentrations of the methanol extract (6.25, 12.5, 25, 50, and 100 μ g/ml, dissolved in DMSO) and the isolated compounds (6.25, 12.5,

25, 50, and 100 μ M, dissolved in DMSO). 5-FU was used as a positive control.^[15] After 48 h, cell viability was determined using WST-1 reagent as follows: 10 μ L of WST-1 reagent was added to each well, followed by 4 h incubation at 37°C, after which the absorbance was measured at 450 nm using a Microplate Reader (Biotek, Winooski, VT, USA).

Tubulin polymerization assay

The abilities of the methanol extract (25 and 50 μ g/ml) and isolated compounds at a concentration of 10 μ M to either inhibit or enhance tubulin polymerization were tested according to the manufacturer's protocols. Briefly, the reaction mixture in a final volume of 10 μ L in PEM buffer (80 mM PIPES, 0.5 mM EGTA, 2 mM MgCl₂, pH 6.9) contained 2 mg/ml bovine brain tubulin, 10 μ M fluorescent reporter, and 1 mM GTP was mixed in 96 well-plate at 37°C with test compounds, vinblastine or paclitaxel (3 μ M) as positive controls^[16] for the inhibition or enhancement, of tubulin polymerization, respectively. Tubulin polymerization was determined by measuring the fluorescence emission at $\lambda = 420$ nm (excitation $\lambda = 360$ nm) for 1 h at 1 min. Intervals using flexstation 3 Microplate Reader (Molecular Devices, LLC, Sunnyvale, CA, USA), and data were managed by SoftMax Pro[®] 5.4.1 software (Molecular Devices, LLC, Sunnyvale, CA, USA).

Histone deacetylase 8 inhibitory assay

The inhibitory effects of the methanol extract (30 μ g/ml), isolated compounds (10 μ M) and trichostatin A (TSA) (5 μ M) as a positive control^[17] on HDAC8 were examined according to the manufacturer's protocols. Briefly, 35 μ L of the assay buffer (5 μ L, (20 mM Tris-HCl, pH 8.0, 125 mM NaCl, 1% glycerol) was mixed with 1 μ L of 1 mM fluoro-substrate peptide, 2.5 μ L lysyl endpeptidase (5 mAU/ml), 26.5 μ L distilled water with or without 5 μ L of samples or TSA. The reaction is initiated by addition of 10 μ L of X5 diluted recombinant HDAC8. HDAC8 activity was measured by monitoring the fluorescence emission at $\lambda = 440$ nm (excitation $\lambda = 340$ nm) for 1 h at 1 min intervals at 37°C using flexstation 3 Microplate Reader.

Molecular docking

Docking experiment was used to investigate the binding affinity of the active compounds to the binding residues of the active site of tubulin protein (at the inhibitory binding domains sites; colchicine and vinblastine) and HDAC8. The crystal structures of tubulin protein complexed with colchicine or vinblastine (code: 4O2B and 4EB6, respectively) and HDAC8 complexed with TSA (code: 3F0R) were downloaded from protein data bank and imported into the workplace of Molegro Virtual Docker (MVD 6.0, Molegro, Aarhus, Denmark) software. Chain D of 4O2B and chain C of 4EB6 were used as a template for docking experiments at the binding sites of colchicine and vinblastine, respectively. All other ligands and cofactors were removed from the protein structure prior to perform the docking simulation. Docking parameters were adjusted as following: Mmaximum iterations of 1500, a maximum population size of 100, neighbor distance factor of 1, grid resolution of 0.7 Å and 100 runs). Chain B805 of 3F0R protein was used for docking experiment at the binding site of the ligand TSA B-805. Docking parameters were the same as that of the docking with tubulin microtubules. Rank scores were used as an indicative for the binding affinity of isolated compounds to the active site of tubulin microtubules and HDAC8.^[18] The three-dimensional structures of ligands were drawn using Chemscketch 12.0 software (Cambridge Soft Corporation, Cambridge, MA, USA) and saved as mol2 formats. Steps of docking experiment were done as previously described.^[19] The binding residues were determined using LigPlot⁺ V.1.4 program.

Table 1: Cytotoxicity and selectivity of AME and its isolated compounds

Compounds	<i>In vitro</i> cytotoxicity IC ₅₀ (μM for the compounds and μg/ml for the extract)					
	HePG2	Hep2	HCT116	RD	MCF7	WI-38 (normal cell line)
5-FU ^a	21.4±0.23 (2.9) ^b	9.3±0.32 (6.8)	9.5±0.47 (6.8)	21.1±0.63 (3)	3.1±1.27 (20.5)	63.4±3.11
Extract	30.7±0.81 (3.7)	18.1±1.21 (6.4)	32.6±0.91 (3.5)	55.4±2.30 (2.1)	19.5±1.59 (5.9)	115.9±1.81
α-glutinol	50.9±0.42 (1.2)	48.1±2.16(1.3)	66.5±2.21 (0.9)	79.8±1.17 (0.7)	28.4±0.52 (2.2)	62.9±0.72
β-amyrin	82.8±0.78 (1.2)	85.6±0.15 (1.2)	91.3±15 (1.1)	93.7±0.63 (1.1)	63.6±0.34 (1.6)	102.8±2.15
Bergapten	18.1±1.81 (4.7)	23.4±2.44 (3.7)	29±0.97 (1.4)	31.8±1.14 (2.7)	34.5±0.74 (2.5)	86.3±1.44
Psoralen	17.2±0.21 (5.6)	20.5±0.01 (4.7)	26.1±0.51 (3.7)	28.3±0.22 (3.4)	32.4±0.43 (3)	97.2±0.92
Angelicin	13.8±0.64(7.4)	11.5±2.28 (8.9)	8.7±0.72 (11.8)	12.9±1.33 (7.9)	7±0.91 (14.7)	102.9±1.35
Isoeopxyterixin	20.7±2.42 (4.8)	36.4±0.54 (2.7)	49.9±0.87 (1.9)	42.8±0.42 (2.3)	25.1±1.40 (3.9)	99.6±0.48
Isopteryxin	50.9±1.55 (2.3)	53.5±1.14 (2.2)	55.8±2.25 (2.1)	44.4±0.75 (2.7)	39.1±0.25 (3.1)	120.4±1.49
Hyuganin E	24.4±0.34 (3.6)	11.3±0.15 (7.9)	16.1±0.44 (5.5)	31.8±1.22 (2.8)	6.1±2.32 (14.7)	89.6±2.25
Hyuganin C	19.1±4.52 (3.7)	20.8±0.78 (3.4)	60.7±5.12 (1.2)	36.5±3.48 (1.9)	41.2±4.18 (1.7)	70.8±6.34
luteolin	15.2±1.41 (5.9)	10.7±2.17 (8.4)	11.07±0.56 (8.2)	16±0.34 (5.7)	13.4±4.24 (6.8)	90.8±1.52
Quercetin	10.1±1.92 (8.6)	10.9±0.34 (8.1)	9.1±0.73 (9.6)	29.8±2.51 (2.9)	11.7±0.42 (7.5)	87.6±0.75
Kaempferol	34.4±1.47 (2.7)	30.3±0.22 (3.1)	44.7±0.52 (2.1)	54.1±0.41 (1.7)	27.2±3.11 (3.5)	95.4±1.86
Kaempferol-3-O-rutinoside	20.27±3.34 (5.4)	10.3±0.91 (10.6)	28.1±1.89 (3.8)	36.6±2.75 (2.9)	5±1.12 (21.9)	109.5±5.17
Kaempferol-3-O-glucoside	37.8±1.90 (3.1)	23.9±2.43 (4.7)	36.6±0.84 (3.1)	48.8±1.73 (2.3)	15.7±2.65 (7.2)	113.4±3.48
Chlorogenic acid	24.6±0.31 (3.7)	5.1±3.11 (17.8)	27.9±2.55 (3.3)	30.1±1.66 (3)	9.5±0.44 (9.6)	91.2±2.97
Methyl chlorogenate	30.9±2.24 (3.5)	21.2±0.71 (5.1)	30.8±0.68 (3.5)	36.1±0.41 (3)	10.2±0.61 (10.8)	109.9±0.65
5-(hydroxymethyl)-2-furaldehyde	77.7±0.97 (1)	78.9±0.32 (0.9)	83.1±1.32 (0.9)	60.6±0.93 (1.29)	46.2±0.32 (1.7)	78.3±1.31
Adenosine	55.1±1.46 (2.3)	49.1±2.82 (2.6)	41.8±0.95 (3.1)	66.5±1.42 (1.9)	48.3±0.28 (2.7)	130.8±5.92

^aIs used as a positive control; ^b() is the SI=IC₅₀ of WI-38/IC₅₀ of each cancer cell line. SI: Selectivity index; IC₅₀: Half-maximal inhibitory concentration; AME: *Angelica shikokiana* methanol extract; HepG2: Human hepatocellular carcinoma; Hep2: Human epithelioma; HCT116: Human colorectal carcinoma; RD: Rhabdomyosarcoma; MCF7: Human breast adenocarcinoma; 5-FU: 5-Fluorouracil; WI-38: Human lung fibroblasts

Data analysis

IC₅₀ (concentration resulting in 50% cytotoxicity) values were calculated using Probit Analysis (Version 16.0 for Windows; SPSS, Chicago, IL, USA) for five different concentrations in three independent experiments. Data are expressed as means ± standard deviations. Fluorescence readings for tubulin polymerization and HDAC8 assays are expressed in relative fluorescence units (RFU). From the mean values, percentage inhibition was calculated using the following equation:

$$\% \text{inhibition} = \frac{\text{RFU control} - \text{RFU sample}}{\text{RFU control}} \times 100$$

RESULTS AND DISCUSSION

Cytotoxicity and selectivity

Cytotoxic activities of the methanol extract and isolated compounds [Supplementary Material Figure 1] were evaluated on five different cancer cell lines [Table 1]. Selectivity was evaluated by measuring the cytotoxicity against a normal cell line (WI-38) and expressed in selectivity index (SI) [IC₅₀ of WI-38/IC₅₀ of each cancer cell line, Table 1]. The methanol extract of *A. shikokiana* exhibited strong cytotoxicity against tested cancer cell lines with IC₅₀ values <60 μg/ml. It had SI values >3 for all tested cancer cell lines except RD cells (SI = 2.1). The results of cytotoxicities and selectivities of isolated compounds confirmed that the activity of the methanol extract is due to its coumarin and flavonoid contents. Among isolated furanocoumarins; angelicin exhibited the strongest cytotoxicity (IC₅₀ < 20 μM) against cancer cell lines and the highest selectivity (SI >7). On the other hand, among the isolated pyranocoumarins; hyuganin E exhibited the strongest cytotoxicity against most tested cancer cell lines (Hep2, HCT116, and MCF7) with IC₅₀ values <25 μM and good selectivity (SI >3). Luteolin and quercetin showed the strong cytotoxicity (IC₅₀ <20 μM) and high selectivity (SI > 5.9) except for the cytotoxicity and selectivity of quercetin against RD cells. Kaempferol showed lower cytotoxicity and selectivity while O-glycosylation at C-3

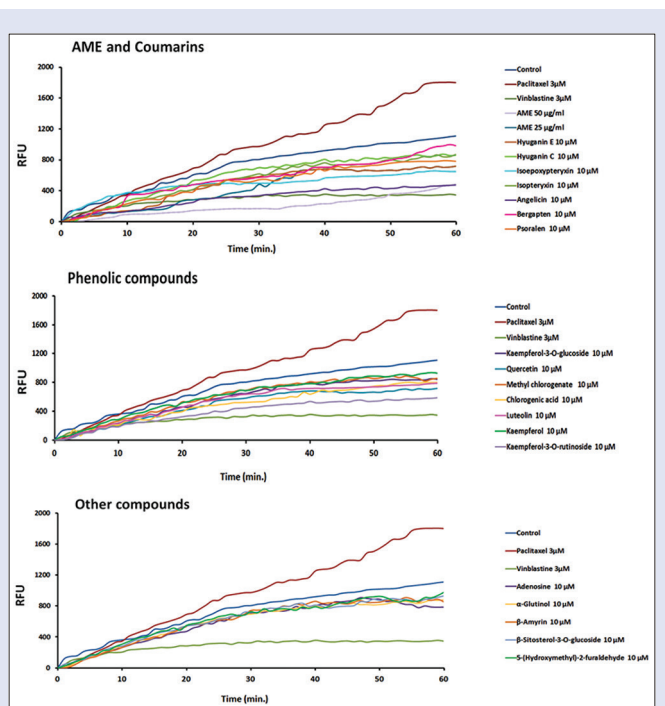


Figure 1: Effects of the methanol extract of *Angelica shikokiana* and its isolated compounds on tubulin polymerization. Fluorescence signal was monitored at Ex/Em = 360/420 nm at 37°C every minimum for 60 min

with 6-O-α-L-rhamnosyl-D-glucose (rutinose) sugar showed a marked improve in both selectivity and cytotoxicity. Chlorogenic acid and methyl chlorogenate showed specific cytotoxicity against certain types of tested cancer cell lines such as Hep2 and MCF7. Other compounds showed lower cytotoxicity and selectivity against all tested cancer cell lines.

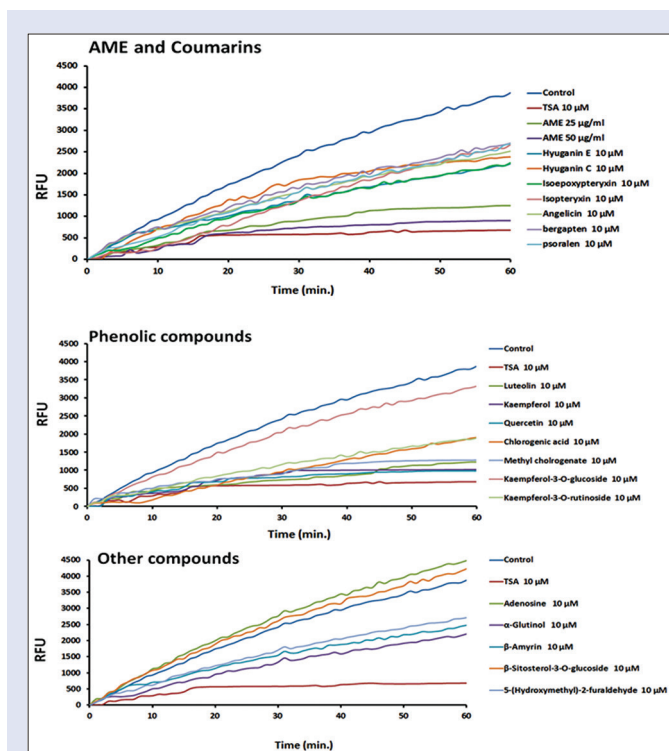


Figure 2: Effects of the methanol extract of *Angelica shikokiana* and its isolated compounds on HDAC8 activity. Fluorescence signal was monitored at Ex/Em = 340/440 nm at 37°C every minimum for 60 min

Effect on tubulin polymerization

To gain some insights into the molecular mechanisms of the cytotoxicity, the activity of the AME and its isolated compounds were evaluated for their effect on the tubulin polymerization [Figure 1] and HDAC8 [Figure 2] activity as two targets of cytotoxic activity. AME and its isolated compounds inhibited tubulin polymerization in a different degree of inhibition [Table 2]. AME had a concentration-dependent inhibition of tubulin polymerization at the tested concentrations; 25 and 50 µg/ml. Angelicin showed the strongest inhibition ($57.1 \pm 6.3\%$) among the isolated compounds at the tested concentration of 10 µM. Other coumarins showed weak (15–30% inhibition, e.g. isopteryxin, hyuganin C and bergapten) to moderate inhibition (30–50% inhibition, e.g. hyuganin E, psoralen, and isoeopoxypteryxin). From those results, some features of structure-activity relationship could be concluded. Angular furanocoumarin (angelicin) is more active than the linear ones (bergapten and psoralen). Epoxide ring at C-2''' and C-3''' in the side chain at C-3' substituted ester of pyranocoumarin ring (isoeopoxypteryxin) showed similar activities as the presence of vicinal diol (hyuganin E) and higher activity than double bond (isopteryxin) at the same carbon atoms. In addition, changing the position of acetoxy group from C-4' to C-3' along with the presence of more nonpolar side chain ester at the C-4' (hyuganin C) showed a marked decrease in the activity. On the other side, among the phenolic compounds; kaempferol-3-O-rutinoside showed the strongest inhibition of tubulin polymerization ($45.93 \pm 2.1\%$). Quercetin and chlorogenic acid showed moderate inhibition while other phenolic compounds showed weak activities. Docking experiment was performed to identify the putative binding sites of isolated compounds on tubulin microtubules

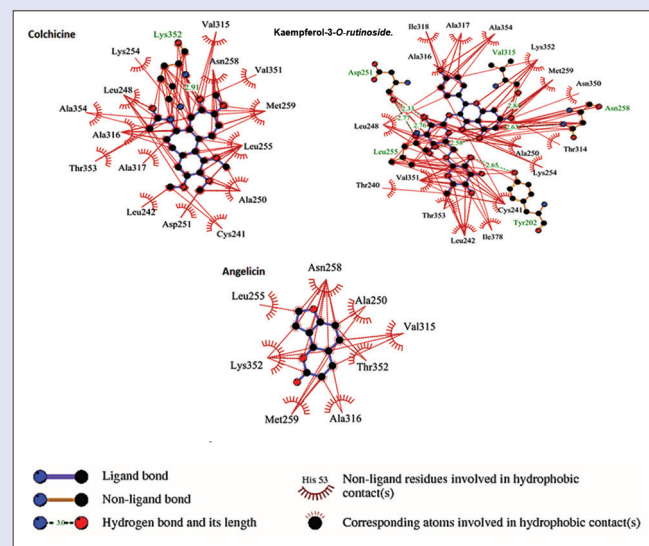


Figure 3: Binding residues of colchicine, kaempferol-3-O-rutinoside and angelicin on tubulin microtubules using LigPlot^v 1.4 program

Table 2: Inhibition of tubulin polymerization and HDAC8 activity at 10 µM concentration

Sample	Percentage inhibition tubulin polymerization ^a	Percentage inhibition HDAC8 activity ^b
AME (25 µg/ml)	40.36±3.98	63.6±5.21
AME (50 µg/ml)	72.17±4.5	72.30±3.7
	(IC ₅₀ = 32.71±3.12)	(IC ₅₀ = 17.31±5.8)
α-glutininol (10 µM)	17.04±1.83	44.82±6.30
β-amyryn (10 µM)	15.48±5.44	34.93±3.98
Isoeopoxypteryxin (10 µM)	32.8±7.69	43.52±1.72
Isopteryxin (10 µM)	24.8±5.55	40.9±2.44
Hyuganin E (10 µM)	33.84±3.75	41.2±4.32
Hyuganin C (10 µM)	18.46±2.51	30.62±1.10
Psoralen (10 µM)	30.13±6.13	30.70±3.52
Bergapten (10 µM)	22.50±1.54	34.4±0.94
Angelicin (10 µM)	57.10±6.3	33.86±2.86
	(IC ₅₀ = 7.92±1.9)	
Luteolin (10 µM)	26.17±4.28	67.25±7.1
		(IC ₅₀ = 6.91±0.61)
Kaempferol (10 µM)	14.99±2.34	66.29±1.5
		(IC ₅₀ = 7.80±2.32)
Quercetin (10 µM)	32.04±1.79	67.95±1.7
		(IC ₅₀ = 7.72±1.41)
Kaempferol-3-O-glucoside (10 µM)	21.16±2.92	14.79±0.80
Kaempferol-3-O-rutinoside (10 µM)	45.93±2.11	46.71±4.74
Chlorogenic acid (10 µM)	30.90±5.19	58.21±3.8
		(IC ₅₀ = 8.62±1.21)
Methyl chlorogenate (10 µM)	16.9±2.85	60.47±2.5
		(IC ₅₀ = 8.22±0.40)
5-(hydroxymethyl)-2-furaldehyde (10 µM)	13.4±1.74	2.59±4.74
β-Sitosterol-3-O-glucoside (10 µM)	14.38±6.50	-8.57±1.98
Adenosine (10 µM)	17.92±5.74	-15.92±0.72

^aVinblastine (3 µM) induced 60.02±4.8% inhibition of tubulin polymerization, and paclitaxel (3 µM) showed 34.27±5.2% enhancement of tubulin polymerization;

^bTSA (5 µM) showed 76.9±6.2% inhibition of HDAC8 activity. TSA: Trichostatin A; HDAC8: Histone deacetylase 8; IC₅₀: Half-maximal inhibitory concentration; AME: *Angelica shikokiana* methanol extract

were more active than furanocoumarins (psoralen, bergapten, and angelicin). Finally, similar to tubulin polymerization assay, changing the position of acetoxy group from C-4' to C-3' together with more nonpolar side chain ester at the C-4' in pyranocoumarins (hyuganin C) showed about $10 \pm 2.2\%$ decrease in the inhibitory activity. Some studies reported the HDACs activity of dietary flavonoids; luteolin,^[22] quercetin,^[23] kaempferol^[24] and chlorogenic acid;^[25,26] however to the best of our knowledge, this study is the first to report the effect of AME, isolated coumarins and kaempferol-3-O-rutinoside on HDAC8 activity.

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Nil.

Conflicts of interest

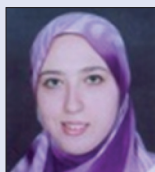
There are no conflicts of interest.

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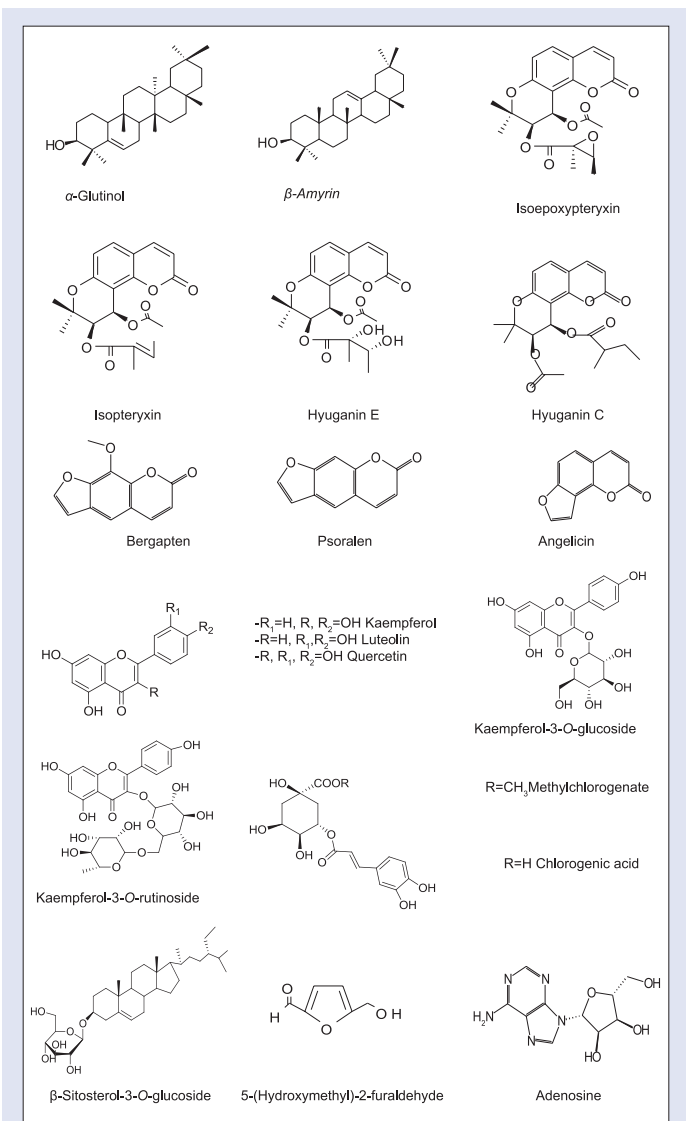


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Supplementary Material Figure 1: Structures of isolated compounds from the aerial parts of *Angelica shikokiana*