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学 位 論 文 要 旨
Dissertation Abstract

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学位論文題目：
Title of Dissertation　Studies on Bioactive Compounds Found in *Litsea cubeba* Fruits (*Litsea cubeba* 果実に含まれる生理機能性物質に関する研究)

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Litsea cubeba (Lauraceae) is a plant distributed in Himalaya, southern China, Taiwan, Japan and South East Asia. All part of this plant has been used as traditional medicines. *L. cubeba* is also well known as an essential oil source. The fruit essential oil, May Chang oil, is an important aromatherapy component and a raw material for citral, ionones and vitamin A production. In this study, we determined antioxidant activities of *L. cubeba* fruit essential oil components, anticancer activity of methanol extract of *L. cubeba* fruits residue from essential oil extraction. Finally, anticancer bioactive compounds were isolated and analyzed by spectroscopic methods.

For the antioxidant activity, the essential oil components which exhibited radical scavenging activity (DPPH assay) are geranial, neral, geraniol, *(-)*α-pinene, β-citronellol, β-pinene, citronellal, linalool, ocimene and α-terpinene. The highest activities were exhibited by ocimene and α-terpinene. For the activity to prevent lipid oxidation (β-carotene bleaching assay), most of the compounds exhibited the activity but geranial neral geraniol nerol *(-)*α-pinene β-caryophyllene and α-terpinene were significantly more effective than other components.

The essential oil extracted from *L. cubeba* fruits was found to be less effective than the fruit residue methanol extract in cytotoxicity against HeLa cells. Therefore, the methanol extract was then fractionated by Amberlite^{TM} XAD-7 column chromatography (mobile phase: 20-100% methanol). Only four fractions eluted by 80-100% methanol (4B, 5A, 5B and 5C) were effective against viability of HeLa cells and they also exhibited activity to promote G_1-S phase cell cycle arrest (BrdU assay). Late apoptosis was observed according to leakage of LDH in cell population treated with these samples. Finally, these fractions were proven to be effective in induction of effector caspase-3/-7 activation in HeLa cells.

However, the effective fractions isolated by using XAD-7 resin as stationary phase were highly complex. So, fractionation of the methanol extract was repeated by using silica gel (45~75μm particle size) as stationary phase instead. Elution was done

by using mobile phase gradient of hexane and diethylether to give 20 fractions. Fraction 11 and 12 eluted by 50% diethylether were cytotoxic against HeLa cells. The major cytotoxic compound in these fractions [*(+)*-6-(4-hydroxy-4-methyl-2-pentenoyl)-4,6-dimethyl-5-(3-methyl-2-butenyl)-1,3-cyclohexadienecarbaldehyde, (Compound **1**)] was successfully isolated by preparative HPLC and identified for chemical structure by spectroscopic methods (1-D, 2-D NMR, IR, UV, HR-ESI-MS). This compound structure has not been reported elsewhere, and it was named as cubelin ($C_{20}H_{28}O_3$). This compound exhibited activity against HeLa cell viability and proliferation (IC_{50}: 34.43, 30.49 μM). The changes in nuclear morphology which indicates apoptosis were clearly observed. The presence of cleaved caspase-3/-7, caspase-8 and caspase-9 in the cubelin treated population indicated the potential of the compound to induce apoptosis in HeLa cells via both intrinsic and extrinsic pathways.

Other than cubelin, there are several other cytotoxic compounds isolated from methanol extract of *L. cubeba* fruits that are not yet successfully identified. Firstly, compound **2**, **3** and **4** from the same fractions as cubelin exhibited cytotoxicity with IC_{50} of 41.91, 52.50 and 60.18 μg/mL. Another two compounds (**5** and **6**) were obtained by washing the column, after elution of hexane/diethylether gradient, with 10% acetic acid in ethanol. The compounds were isolated by using preparative HPLC. Compound **5** and **6** exhibited activity against HeLa cell viability (IC_{50}: 1.58, 2.25 μg/mL) and cell proliferation (IC_{50}: 0.77, 0.89 μg/mL). They also exhibited caspase-8, -9 and -3/-7 inducing activity at the similar level as camptothecin. However, because of very low yield percentage of **5** and **6**, the obtained amount is not possible for structure analysis by NMR spectroscopy.