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Isolation and characterization of new lactam compounds that inhibit lung and colon cancer cells from adlay (*Coix lachryma-jobi* L. var. *ma-yuen* Stapf) bran

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Abstract

Five active compounds that inhibit cancer cells were isolated from adlay bran (*Coix lachryma-jobi* L. var. *ma-yuen* Stapf), and their structures and activities *in vitro* were characterized. The ethyl acetate-soluble fraction of methanol extracts of adlay bran (ABM-EtOAc) exhibited a stronger anti-proliferative effect on human lung cancer cell A549, human colorectal carcinoma cell HT-29, and COLO 205 than other fractions by MTT (3-4,5-dimethylthiazol-2-yl-2,5-diphenyl-tetrazolium bromide) assay. Assay-guided isolation gave five lactams including three that were previously undocumented; coixspirolactam A (**1**), coixspirolactam B (**2**), and coixspirolactam C (**3**); one isolated from the natural plant for the first time, coixlactam (**4**); and one known compound, methyl dioxindole-3-acetate (**5**). Pure active compounds were identified by spectral analysis including IR, ^{1}H and ^{13}C NMR, UV–vis, MS and 2D NMR techniques. All the compounds were tested for their anti-proliferative effect on A549, HT-29 and COLO 205 cells. These compounds showed anti-cancer activities with IC_{50} values between 28.6 and 72.6 μg/mL.

Keywords: Adlay bran; Anti-proliferative; Lung cancer; Colorectal carcinoma; Lactam

1. Introduction

Adlay (Chinese pearl barley, soft-shelled Job's tears, *Coix lachryma-jobi* L. var. *ma-yuen Stapf*) is a grass crop that has long been used in traditional Chinese medicine and as a 'nourishing' food. Adlay is mainly planted in Taiwan, China, and Japan, where it is considered a health food supplement. According to the ancient Chinese medical book Pen-Tsao-Kang-Mu, Li (1596), the seed of adlay was used in China for the treatment of warts, chapped skin, rheumatism, and neuralgia, and as an anti-inflammatory or anti-helmintic agent. Numerous recent reports have indicated that the consumption of adlay seed is beneficial to the human body (Hidaka et al., 1992; Huang and Chiang, 1999; Tsai et al., 1999; Chiang et al., 2000a,b; Hsu et al., 2003).

In the past, a few scientific studies have identified active components in adlay. Kuo et al. (2002) used DPPH-directed fractionation and identified six phenolic compounds including coniferyl alcohol, syringic acid, ferulic acid, syringaresinol, 4-ketopinoresinol, and a lignan known as

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mayuenolide. All compounds isolated from adlay hulls showed strong free radical scavenging activity. Nagao et al. (1985) isolated benzoxazinones from adlay seeds that showed anti-inflammatory activity. Takahashi et al. (1986) reported that coixans A, B, and C isolated from adlay seeds have hypoglycemic activity in rats. Coixenolide was isolated from adlay seeds, and it exhibited anti-tumor activity towards Ehrlich ascites sarcoma in mice (Tanimura, 1961; Ukita and Tanimura, 1961). The anti-tumor constituents of adlay include α-monolinolein (Tokuda et al., 1990), and free fatty acids such as palmitic, stearic, oleic, and linoleic acids (Numata et al., 1994).

In addition, recent studies indicate that adlay has anti-tumor effects. Chiang et al. (2000a,b) found that adlay inhibited sarcoma-180 tumor in mice. Kuo et al. (2001) indicated that a methanolic extract of adlay hull has anti-proliferative activity against human histolytic lymphoma U937 monocytic cells via apoptosis. Chang et al. (2003) reported that a methanolic extract of adlay has an anti-proliferative effect on A549 lung cancer cells by inducing cell cycle arrest and apoptosis. Feeding mice a diet containing adlay reduced the number of surface lung tumors in mice. Shih et al. (2004) showed that dehulled adlay suppressed early events in colon carcinogenesis and it also reduced COX-2 protein expression.

Recent studies have shown that adlay bran may inhibit cancer cell growth, but only a few anti-tumor specific compounds have been identified. In this study, adlay bran was extracted using organic solvents. Compounds that inhibit the proliferation of lung and colon cancer cells were isolated from the methanolic extracts.

2. Materials and methods

2.1. General experimental procedures

IR spectra were recorded on a Perkin–Elmer 983 G infrared spectrometer. ^{1}H NMR and ^{13}C NMR spectra were obtained on Bruker AM-300 and Bruker AMX-500 instruments, COSY, HMQC, HMBC and NOESY spectra were obtained on a Bruker AMX-500 instrument and recorded using standard pulse sequences. MS analysis was taken on a JEOL JMS-HX300 mass spectrometer. The measurement of melting points was performed with Yanaco MP-S3 micro melting point apparatus (Yanagimoto, Kyoto, Japan). Ultraviolet absorption spectra of the purified active fractions were recorded on a U-2000 spectrophotometer (Hitachi) in methanol. Thin-layer chromatography was performed on silica gel 60 F_{254} TLC plates (Merck, Darmstadt, Germany), with compounds visualized by spraying with 10% (v/v) H_2SO_4 in an ethanol solution. Silica gel (230–400 mesh) (Macherey-Nagel, Germany) was used for column chromatography. High performance liquid chromatography (HPLC) was performed with a WATERS 510 instrument and an IOTA-2 RI detector (Precision Instruments, UK). A 10 × 250-mm-i.d., 7-μm, Lichrosorb Si-60 column (Merck, Darmstadt, Germany) was used for analysis. All solvents used for chromatographic isolation were of analytical grade and purchased from Tedia Co. (Fairfield, OH).

2.2. Plant material

Adlay was purchased (October, 2004) from a local farmer who planted Taichung Shuenyu No. 4 (TCS4) variety of *Coix lachryma-jobi* L. var. *mayuen* Stapf in Taichung, Taiwan, in March 2003 and harvested it in July of the same year. After the harvest, the seeds were dried at ambient temperature with ventilation and dehulled by a grinder. The samples were divided into hull, testa, and dehulled adlay by gentle blowing using an electric fan. The dehulled adlay was separated into bran and polished adlay. The adlay bran was blended into powder form, and screened through a 20-mesh sieve (aperture, 0.94 mm).

2.3. Extraction and isolation procedure

In order to isolate, purify, and identify the active components that demonstrate inhibition of cancer cell proliferation, a large amount of methanolic extracts were prepared from adlay bran as follows. Adlay bran powder (20 kg) was extracted three times with 200 L of methanol at room temperature for 2 weeks (5 day for each time). To minimize methanol consumption during adlay bran methanol extraction, we prolonged the extraction time to replace the use of methanol. The plant residue was filtered off, and the methanolic extracts were combined and concentrated under reduced pressure by a rotary vacuum evaporator. The methanolic extracts of adlay bran were named ABM. The dry extract (ABM, 1864 g) was suspended in 18.6 L of H_2O, followed by an extraction with the same volume of *n*-hexane, ethyl acetate, and 1-butanol, yielding four subfractions named: ABM-Hex (*n*-hexane soluble fraction), ABM-EtOAc (ethyl acetate soluble fraction), ABM-BuOH (1-butanol soluble fraction), and ABM-H_2O (water soluble fraction). ABM-EtOAc (380 g) was coated with 380 g silica gel (230–400 mesh), then subjected to column chromatography on silica gel (230–400 mesh) with successive elution by a Hex/EtOAc and EtOAc/MeOH gradient. Subfractions with the same TLC pattern were combined into one fraction, thus 15 fractions were obtained. The fractions which showed greater inhibition of cancer cell proliferation were chromatographed with 20–40% Hex/EtOAc on a silica gel column using a $CHCl_2$/EtOAc gradient system to yield subfractions. Subfractions were further purified by HPLC on a Lichrosorb Si-60 column at 3 mL/min, using 30% or 50% or 65% or 80% EtOAc/$CHCl_2$ as the eluent to yield five compounds, coixspirolactams A (**1**) (12.3 mg), B (**2**) (7.8 mg), C (**3**) (7.5 mg), and coixlactams (**4**) (9.1 mg), methyl dioxindole-3-acetate (**5**) (11.3 mg). Their structures are shown in Fig. 1. Each compound was collected manually and concentrated at 40 °C under reduced pressure and checked for purity by TLC and HPLC. Their structures are shown in Fig. 1.

Compounds **1**–**5** were identified by spectroscopic methods, including IR, ^{1}H NMR, ^{13}C NMR, 2D NMR and MS analytical data.

Coixspirolactam A (**1**): pale yellow oil. $[\alpha]_D^{25} = +9.7$ ($c = 0.70$, MeOH). UV (MeOH) λ_{max} nm (log ε): 250 (3.72). EIMS *m*/*z* (%): 203 (M^+, 8), 167 (5), 146 (2), 145 (22), 137 (100), 117 (8), 107 (7), 84 (14). HREIMS *m*/*z*: 203.0580 calcd 203.0582 for $C_{11}H_9NO_3$. IR ν_{max} (film)/cm^{-1}: 3289, 1785, 1730, 1619, 1505, 1367, 1178. ^{1}H and ^{13}C NMR data are presented in Table 1.

Coixspirolactam B (**2**): pale yellow oil. $[\alpha]_D^{25} = -4.3$ ($c = 0.40$, MeOH). UV (MeOH) λ_{max} nm (log ε): 251 (3.78). EIMS *m*/*z* (%): 217 (M^+, 9), 174 (3), 173 (26), 146 (8), 145 (100), 144 (5), 117 (34), 107 (5), 90 (9). HREIMS *m*/*z*: 217.0738 calcd 217.0739 for $C_{12}H_{11}NO_3$. IR ν_{max} (film)/cm^{-1}: 3268, 1789, 1726, 1621, 1521, 1360, 1189. ^{1}H and ^{13}C NMR data are presented in Table 1.

Coixspirolactam C (**3**): pale yellow oil. $[\alpha]_D^{25} = +5.9$ ($c = 0.39$, MeOH). UV (MeOH) λ_{max} nm (log ε): 252 (3.61). EIMS *m*/*z* (%): 217 (M^+, 9), 174 (3), 173 (30), 146 (11), 145 (100), 144 (6), 117 (62), 90 (19), 89 (14). HREIMS *m*/*z*: 217.0738 calcd 217.0739 for $C_{12}H_{11}NO_3$. IR ν_{max} (film)/cm^{-1}: 3269, 1795, 1736, 1625, 1471, 1200. ^{1}H and ^{13}C NMR data are presented in Table 1.

Coixlactam (**4**): pale yellow oil. $[\alpha]_D^{25} = +5.1$ ($c = 0.21$, MeOH). UV (MeOH) λ_{max} nm (log ε): 248 (3.81). EIMS: *m*/*z* (%) 205 (M^+, 46), 173 (18), 146 (33), 145 (100), 128 (10), 117 (28). HREIMS *m*/*z*: 205.0736 calcd 205.0739 for $C_{11}H_{11}NO_3$. IR ν_{max} (film)/cm^{-1}: 3376, 1732, 1690, 1626, 1490, 1228, 1062. ^{1}H and ^{13}C NMR data are presented in Table 1. Compound **4** had been synthesized by Crotti et al. (1986), but here was isolated from a natural source for the first time.

Methyl dioxindole-3-acetate (**5**): pale yellow oil. $[\alpha]_D^{25} = -1.4$ ($c = 0.45$, MeOH). UV (MeOH) λ_{max} nm (log ε): 252 (3.25). EIMS *m*/*z* (%): 221 (M^+,