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Isolation and Characterization of a New Compound from *Prunus mume* Fruit that Inhibits Cancer Cells

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An active compound that inhibits cancer cells was isolated from the fruit of *Prunus mume*, and its structure and in vitro activities were characterized. The *n*-hexane fraction obtained from methanol extracts exhibited the strongest inhibitory effect on the growth of cancer cells. From the *n*-hexane fraction, a new compound named **B-1** was purified through preparative thin-layer chromatography, ODS column chromatography, and reverse phase high-performance liquid chromatography and its structure was analyzed by fast atom bombardment mass spectrometry and ^{1}H and ^{13}C NMR. The molecular formula of **B-1** was $C_{19}H_{22}O_6$ {2-hydroxy-1-[(7-hydroxy-2-oxo-2H-chromen-6-yl)methyl]-2-methylpropyl-(2*Z*)-3-methyl-but-e-enoate:prunate}, and the IC_{50} value was in the range of 39−58 μg/mL in descending order of the cancer cell lines Hep-2, SW-156, HEC-1-B, and SK-OV-3. **B-1** exhibited 81−96% inhibition at a concentration level of 100 μg/mL against all cells, based on an 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide assay. However, **B-1** showed little effect against normal cells with only 23% or less growth inhibition at 100 μg/mL. Thus, **B-1** has a highly specific inhibitory effect against cancer cells but little effect against normal cells. When the cancer cell lines Hep-2 and SK-OV-3 were incubated with **B-1** for 72 h, most of the tested cells suffered strong growth inhibition. The compound has the potential to be developed as a nutraceutical.

KEYWORDS: *Prunus mume* fruit; cancer cell-inhibiting compound; MTT assay; structure analysis

INTRODUCTION

Elderly people are the fastest growing part of the population in developed countries, and control of circulatory disease is an important aspect of aging (*1*, *2*). Much more attention is being paid to foods and nutraceuticals because immune system functions are closely related to dietary factors. According to mortality statistics, cancer has become the most common cause of death in Korea (*3*). It is well-known that various carcinogens derived from environmental pollutants are key factors that induce mutations and cancers via damage to DNA (*4*). Numerous reports have focused on attempts to cure cancer using functional compounds isolated from plants (*5*−*9*). Vitamins A and C and the dietary fibers abundant in fruits and vegetables were reported to decrease the incidence of cancer (*10*, *11*). Citrus fruits are especially effective in lowering the incidence of gastric and colon cancers (*12*), and sitosterol was reported to decrease the development of colon cancer (*13*).

The fruit of *Prunus mume* has been widely used in folk remedies and as an herbal medicine having antifatigue, antidiarrheal, and antifever effects (*14*). *P. mume*, a deciduous tree of the genus *Rosaceae*, originated from central China. It is now widely distributed with more than 400 varieties worldwide. However, the raw fruit of this tree is poisonous because the fruit contains prunasin and amygdalin (*15*, *16*). Therefore, it is necessary to process the fruit by pickling in vinegar, liquor, or syrup or to prepare the fruit as a juice concentrate. In this study, *P. mume* fruit was extracted using organic solvents. A compound that inhibits the proliferation of cancer cells was isolated from the extracts and was characterized.

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MATERIALS AND METHODS

Sample Extraction and Column Chromatography. Samples of *P. mume* fruit were supplied by Bohae Maesil Farm in Korea (Maesil, Korean name of *P. mume* fruit). Ten kilograms of fruit was macerated in 10 L of pure methanol (MeOH) for 3 days. The residue was then extracted with 10 L of MeOH. This procedure was repeated four times, and the extract solutions were gathered. The combined solution was filtered through a no. 5A filter paper (Advantec, Japan), followed by concentration using a rotary vacuum evaporator.

A crude sample (732 g) was dissolved in water (1 L) and then serially fractionated with *n*-hexane, chloroform ($CHCl_3$), and ethyl acetate (EtOAc). An 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay using the Hep-2 and SK-OV-3 cell lines was used for measurement of anticancer activity during purification process. The

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n-hexane fraction was subjected to preparative thin-layer chromatography (TLC). Samples were loaded on a 200 mm × 200 mm Kieselgel 60 F_{254} plate (Merck) and developed with a mixture of *n*-hexane/$CHCl_3$/MeOH (6:2:1; v/v). Spots were visualized with UVL-56 (UV-366 nm, Ultra-Violet Products, Inc., United States), and the fraction with an R_f value of 0.6 was isolated. The TLC fractionation was repeated with another developing solvent of *n*-hexane/$CHCl_3$/EtOAc/MeOH (6:2:1:1; v/v).

The active fraction was subjected to chromatography on a 550 mm × 20 mm YMC-Gel ODS column (YMC Co., Kyoto, Japan) that was successively stepwise eluted with H_2O/MeOH (20:80, 10:90, and 0:100; v/v) and MeOH/acetone (95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 40:60, and 0:100; v/v). Further purification using a reverse phase high-performance liquid chromatography (HPLC) with a 150 mm × 4.6 mm Symmetry C_{18} column (Waters, United States) was performed. The elution was isocratically performed with 60% MeOH at a flow rate of 1.0 mL/min and detected at 210 nm.

Structural Analysis. Fast atom bombardment (FAB) mass spectra of the **B-1** compound were obtained using a JMSHX110A/110A tandem mass spectrometer (JEOL, Tokyo, Japan) with a mass resolution of 1000 (10% valley). The ion source was operated at an accelerating voltage of either 10 or −10 kV, depending on the ion mode. Ions were produced by FAB using a cesium ion gun operated at 22 kV. Approximately 10 μg of sample was dissolved in $CHCl_3$/MeOH (1:1; v/v). Then, 1 μL of 3-nitrobenzyl alcohol (3-NBA) (Sigma) in a positive ion mode or triethanolamine (BDH, Poole, Dorset, United Kingdom) in a negative ion mode was added to 1 μL of the sample solution. ^{1}H (500 MHz), ^{13}C (125 MHz), and two-dimensional (2D) NMR spectra were measured in CD_3OD using a ^{UNIT}INOVA 500 spectrometer (Varian, CA) with tetramethylsilane (TMS, $\delta = 0$) as an internal standard.

Cell Lines and Cultivation. The cancer cell lines SW-156 (human kidney hypernephroma cells), Hep-2 (human larynx carcinoma cells), SK-OV-3 (human ovary adenocarcinoma cells), and HEC-1-B (human uterus adenocarcinoma cells) were used. WRL-68 (human liver cells) and NIH/3T3 (mouse embryo cells) were used as normal cells. All cell lines were obtained from the Korean Cell Line Bank (College of Medicine, Seoul National University, Korea).

The cell lines SW-156, SK-OV-3, and HEC-1-B, which were cultivated in RPMI 1640 medium (fetal bovine serum free), contained 1% penicillin−streptomycin (100 units/μL) (Gibco BRL, Gaithersburg, MD) and 10% FBS (fetal bovine serum) (Hyclone, Logan, UT). The Hep-2 cells were cultivated in minimum essential medium (MEM) (Gibco, Grand Island, NY) and contained 10% FBS. The cell lines WRL-68 and NIH/3T3 were cultivated in DMEM/F12 (Gibco) and contained 10% FBS. All cells were cultivated in a 5% CO_2 incubator at 37 °C.

MTT Assay. The inhibition of cell growth was measured by an MTT assay. After 100 μL of each cell culture was placed in a 96 well plate at a concentration of 2×10^4 cells/mL, cells were cultivated in a 5% CO_2 incubator at 37 °C for 24 h. Extract samples of *P. mume* fruit were added to the cell cultures. Each culture was incubated for 48 h in a 5% CO_2 incubator at 37 °C, and then, 10 μL of 5 μg/mL MTT (Sigma Chemical Co., St. Louis, MO) was added to the wells. Controls were cultivated under the same conditions without addition of *P. mume* fruit extracts. After an additional 4 h of incubation, the medium of each well culture was carefully removed, followed by addition of 100 μL of DMSO. Then, the absorbance values were read with an enzyme-linked immunosorbent assay reader (Bio-Rad, Tokyo, Japan) at 540 nm to confirm the number of living cells.

Inhibition of cell growth was expressed as follows: % inhibition = [1 − (absorbance of treated cells − absorbance of blank)/(absorbance of untreated cells − absorbance of blank)] × 100. The IC_{50} values of samples indicating 50% growth inhibition were estimated by an MTT assay. Data are expressed as the average of three analyses.

Microscopic Observation of Cells. After the cell lines Hep-2, SK-OV-3, HEC-1-B, and SW-156 were incubated with or without the **B-1** compound for 72 h in a 5% CO_2 incubator at 37 °C, cells were observed using an inverted microscope (Olympus, Japan) (*17*).

Statistical Analysis. Data were analyzed by the SPSS package for Windows (Version 10). Values were expressed as mean standard error (SE). The data were evaluated by one-way, and the differences between the means were assessed by Duncan's multiple-range test. The differences were considered significant at $p < 0.05$.

Table 1. Effect of Fractions Isolated from Methanol Extracts of *P. mume* Fruit on Proliferation of Hep-2 and SK-OV-3 Cell Lines by MTT Assay

fraction	concentration (μg/mL)	inhibition rate (%)	
		Hep-2	SK-OV-3
n-hexane	100	10.11	13.84
	300	82.99	60.02
	500	94.21	87.50
chloroform	100	13.67	8.15
	300	42.82	15.13
	500	83.49	80.36
ethyl acetate	100	15.74	12.88
	300	44.21	41.05
	500	76.62	69.76
water	100	11.31	6.71
	300	47.17	37.44
	500	80.09	68.18

RESULTS AND DISCUSSION

Isolation of a Compound Inhibiting Cancer Cells from *P. mume* Fruit. *P. mume* fruit (10 kg) was first extracted with methanol and then fractionated serially with various solvents at room temperature. An MTT assay using the Hep-2 and SK-OV-3 cell lines was used for measurement of anticancer activity during the purification process. The inhibitory effects of each solvent fraction on the growth of human cancer cells were examined (**Table 1**). The *n*-hexane fraction showed the highest growth inhibitory effect against the cancer cell lines Hep-2 and SK-OV-3. The activity was observed in a descending order of *n*-hexane, chloroform, water, and ethyl acetate. The inhibitory activities of *n*-hexane extract against Hep-2 and SK-OV-3 cells increased greatly up to 94.2 and 87.5% with an increasing extract concentration up to 500 μg/mL. The IC_{50} values of the *n*-hexane fraction for Hep-2 and SK-OV-3 were estimated to be 240 and 279 μg/mL, respectively.

The *n*-hexane fraction (26.7 g) was selected and subjected to preparative TLC. One gram of active fraction ($R_f = 0.7$, 1.0 g) was obtained and subjected to ODS column chromatography with successive elution of H_2O/MeOH and MeOH/acetone. The resulting fraction (15.9 mg) was further purified by HPLC on a Symmetry C_{18} column. A white-yellowish powder (0.9 mg) with a retention time of 20.06 min on HPLC and $[\alpha]_D^{29} = -50.8°$ ($c = 0.05$) was obtained. The pure active compound was named **B-1**.

B-1 Structural Determination. Structural analysis of **B-1** was performed using FAB-MS and NMR. As shown in **Table 2**, the ^{13}C NMR (125 MHz, CD_3OD) and DEPT spectra of **B-1** showed 19 carbon signals including two carbonyl carbon signals [δ 163.5 (C-2), 167.2 (C-1″)], 10 sp^2 carbon signals (δ 160−105.1), one oxygenated quarternary carbon signal (δ 78.2, C-3′), one oxygenated methine carbon signal (δ 70.8, C-2′), one methylene carbon signal (δ 28.9, C-1′), and four methyl carbon signals [δ 25.4 (C-4′), 23.7 (C-5′), 27.6 (C-4″), 20.6 (C-5″)]. The ^{1}H NMR (500 MHz, CD_3OD) spectrum of **B-1** was consistent with the carbon signals that appeared in the ^{13}C NMR spectrum. The ^{1}H NMR spectrum showed the presence of five sp^2 proton signals (δ 7.83−5.65), an oxygenated methine proton signal [δ 5.12, (1H, *dd*, 5.0, 5.5, H-3′)], a methylene carbon signal [δ 2.91 (1H, *dd*, 5.0, 17.5, H-1′a), 3.24 (1H, *dd*, 5.0, 17.5, H-1′b)], and four methyl carbon signals (δ 2.12−1.37). These data indicate that **B-1** is a coumarin conjugated with

Table 2. Chemical Shifts of ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Analyses for **B-1** (CD_3OD, TMS)

position	^{1}H, δ (multi, Hz)	^{13}C, δ
2		163.5
3	6.22 (1H, *d*, $J = 9.5$)	113.7
4	7.83 (1H, *d*, $J = 9.5$)	145.8
5		114.5
6	7.37 (1H, *s*)	130.7
7		118.0
8		158.2
9	6.74 (1H, *s*)	105.1
10		155.6
1′a	2.91 (1H, *dd*, $J = 5.0, 17.5$)	28.9
1′b	3.24 (1H, *dd*, $J = 5.0, 17.5$)	
2′	5.12 (1H, *dd*, $J = 5.0, 5.0$)	70.8
3′		78.2
4′	1.37 (3H, *s*)	25.4[a]
5′	1.37 (3H, *s*)	23.7[a]
1″		167.2
2″		160.0
3″	5.65 (1H, *dd*, $J = 1.5, 1.5$)	116.5
4″	1.89 (3H, *d*, $J = 1.5$)	27.6
5″	2.12 (3H, *d*, $J = 1.5$)	20.5

[a] Chemical shifts may be vertically interchangeable.

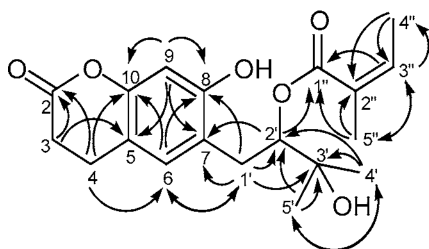

Figure 1. Structure of compound **B-1** and HMBC NMR correlations.

methylbutenoic acid and methylpentanediol. In particular, proton signals of δ 6.22 (1H, *d*, 9.5, H-3) and 7.83 (1H, *d*, 9.5, H-4) in the ^{1}H NMR spectrum exhibited the same coupling constant (J) of 9.5 Hz, indicating that these protons are the AB system of the 3 and 4 positions of coumarin. In addition, the singlet proton signals of δ 7.37 (1H, *s*, H-5) and 6.74 (1H, *s*, H-8) apparently indicate protons of the para type on the benzene ring. Therefore, four sp^2 of the δ 6.22−7.83 protons suggests that the coumarin is linked with the carbonyl carbon (δ 163.5, C-2) that is observed in the ^{13}C NMR spectrum. On the other hand, the coupling constant ($J = 1.5$ Hz) between the upfield shifted sp^2 proton of δ 5.65 (1H, *dd*, 1.5, H-3″) and the two methyl groups [δ 1.89 (3H, *s*, 1.5, H-4″) and 2.12 (3H, *s*, 1.5, H-5″)] suggests that the methylbutenoic acid is connected with another carbonyl group (δ 167.2, C-1″). In addition, the coupling constant ($J = 5.0$ Hz) between the oxygenated methine proton signal [δ 5.65 (1H, *dd*, 5.0, 5.0, H-2′)] and the methylene proton signal [δ 2.91 (1H, *dd*, 5.0, 17.5, H-1′a) and 3.24 (1H, *dd*, 5.0, 17.5, H-1′b)] indicates that these protons are located in methylpentanediol linked with two methyl groups [δ 1.37 (6H, *s*, H-4′, 5′)].

The structure of **B-1** was further analyzed using 2D NMR, including HSQC, ^{1}H−^{1}H COSY, NOESY, and HMBC. Results indicate that the partial structures of **B-1** consist of 7-hydroxycoumarin, 2-methylbutenoic acid, and 2-methyl-pentane-2,3-diol. In the HMBC spectrum of **B-1** (**Figure 1**), the presence of cross-peaks from the methylene proton signals [δ 2.91 (H-1′a) and 3.24 (H-1′b)] to δ 130.7 (C-4) and 158.2 (C-6) and from the oxygenated methine proton signal (δ 5.65, H-2′) to δ 118.0 (C-6) indicates that 2-methyl-pentane-2,3-diol is conjugated with the 6 position of 7-hydroxycoumarin. The signals, which correspond to 6-(2′-methyl-pentane-2′,3′-diol)-7-hydroxycoumarin (peucedanol) as the partial structure of **B-1** in ^{1}H NMR spectrum, agree with the signal patterns of a compound isolated from *Evodia belahe* Baillon (*18*). In the NOESY spectrum, the cross-peak between the methine proton at δ 5.65 (H-3″) and the methyl protons at δ 2.12 (3H, *d*, 1.5, H-5″) indicates that the double bond of the 2 position on 2-methyl-butenoic acid is a *Z* form. Furthermore, the cross-peak between the oxygenated methine proton at δ 5.65 (H-2′) and the carbonyl carbon at δ 167.2 (C-1″) in the HMBC spectrum indicates that (2*Z*)-2-methyl-but-2-enoic acid is esterified at the 2′ position of peucedanol (**Figure 1**).

Table 3. IC_{50} Values of **B-1** on Various Cancer Cell Lines

sample	IC_{50}[a] (μg/mL) Hep-2	SK-OV-3	SW-156	HEC-1-B
B-1	39	58	45	47

[a] IC_{50} is the concentration required for 50% growth inhibition of cancer cells.

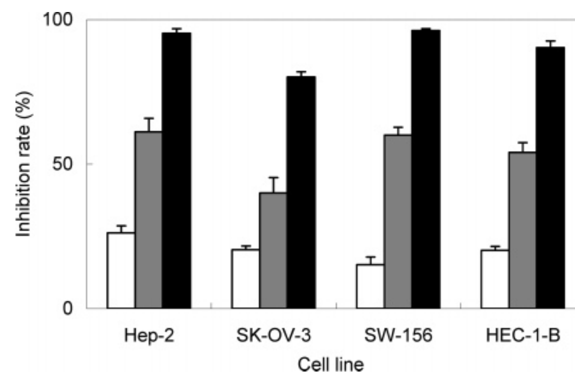

Figure 2. Growth inhibition of **B-1** against various cancer cell lines by MTT assay. The concentration of compound **B-1** was 10 (white), 50 (gray), and 100 (black) μg/mL.

The molecular weight of **B-1** was analyzed by FAB-MS. In the FAB-MS spectrum, pseudo-molecular ion peaks were observed at 384.82 $[M + K]^+$, consistent with the molecular weight (MW 346) of **B-1**. Consequently, the structure of **B-1** was unambiguously determined to be 2-hydroxy-1-[(7-hydroxy-2-oxo-2H-chromen-6-yl)methyl]-2-methylpropyl-(2*Z*)-2-methyl-but-2-enoate ($C_{19}H_{22}O_6$, named “prunate”), which is reported here for the first time.

Inhibitory Effect of B-1 against the Growth of Cancer Cells. The inhibitory effect of **B-1** against various human cancer cell lines was observed. As shown in **Table 3**, the IC_{50} value of **B-1** was 39 μg/mL against Hep-2 cells, 45 μg/mL against SW-156 cells, 47 μg/mL against HEC-1-B cells, and 58 μg/mL against SK-OV-3 cells. These values are much lower than the values of the hexane fraction, indicating that the compound was refined. Genistein, an isoflavone of soybean, has recently received attention as an anticancer-active agent (*20*, *21*). Considering that the IC_{50} value of genistein against human breast cancer cells (MDA-MB-435) is 140 μg/mL (*19*), **B-1** is considered to have high inhibitory activities against the growth of cancer cells.

The concentration of applied **B-1** varied. As shown in **Figure 2**, when **B-1** was added at 10 μg/mL, inhibition rates of less than 20% were observed against all of the cancer cell lines. At 50 μg/mL, the inhibition rate was higher than 50% against Hep-2, SW-156, and HEC-1-B and 40% against SK-OV-3. At 100 μg/mL, growth inhibition was 81−96% in ascending order of

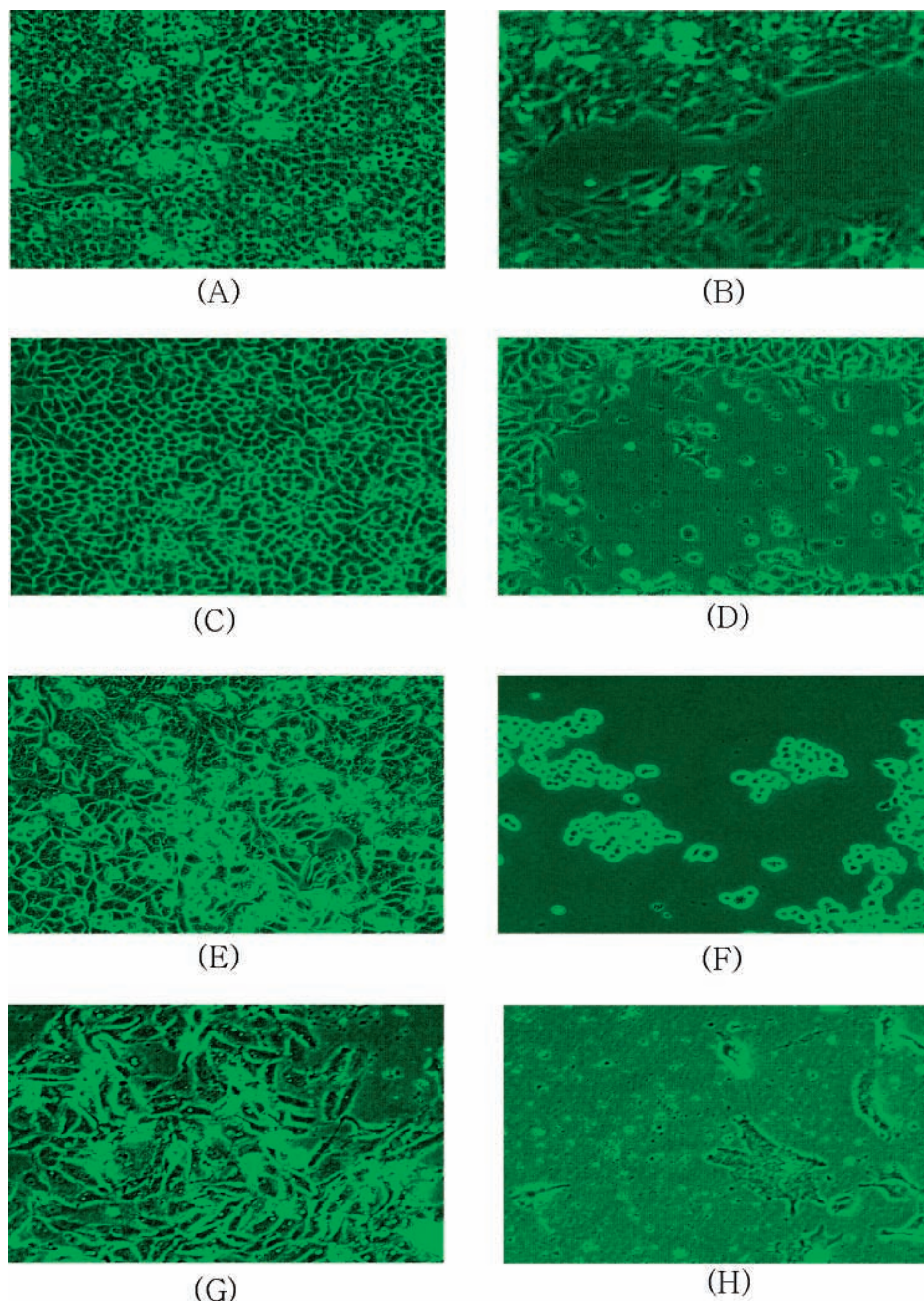

Figure 3. Photomicrographs of the cancer cells Hep-2, SK-OV-3, HEC-1-B, and SW-156 cultivated without and with **B-1** (100 μg/mL) for 72 h. (**A**) Hep-2 (control) × 100; (**B**) Hep-2 (treated) × 100; (**C**) SK-OV-3 (control) × 100; (**D**) SK-OV-3 (treated) × 100; (**E**) HEC-1-B (control) × 100; (**F**) HEC-1-B (treated) × 100; (**G**) SW-156 (control) × 100; and (**H**) SW-156 (treated) × 100.

the SW-156, Hep-2, HEC-1-B, and SK-OV-3 cell lines. Growth inhibition results indicated that **B-1** has significant growth inhibitory activities against the tested cell lines.

Inhibitory Effect of B-1 against the Growth of Normal Cells. The human liver cell line WRL-68 and the mouse embryo cell line NIH/3T3 were selected as a model for normal cells and used for inhibition tests of **B-1** against the growth of normal cells. **B-1** showed little inhibition against either WRL-68 or NIH/3T3 cells. When the applied concentration of **B-1** was increased to 50 μg/mL, the growth of normal cells was little affected. Even at 100 μg/mL, growth inhibitions of only 17 and 23% were observed for WRL-68 and NIH/3T3 cells, respectively. This result indicates that the compound **B-1** from *P. mume* fruit shows only a little growth inhibition against normal cells. **B-1** showed significant inhibitory effects against cancer cells but little against normal cells.

Microscopic Observation of Cancer Cells Treated with B-1. After cells of the cancer cell lines Hep-2, SK-OV-3, HEC-1-B, and SW-156 were cultivated for 72 h in the presence or absence of **B-1**, the cells were observed using an inverted microscope (**Figure 3**). When the Hep-2 cells were cultivated without **B-1**, they proliferated normally forming cell clumps (**Figure 3A**). However, when **B-1** was added at 100 μg/mL, most of the cells suffered strong growth inhibition (**Figure 3B**). Similar results were obtained for SK-OV-3 (**Figure 3C**,**D**), HEC-1-B (**Figure 3E**,**F**), and SW-156 (**Figure 3G**,**H**) cells.

Compound **B-1** is a phenolic compound that is present in *P. mume* fruit. There are several thousand phenolic compounds in

plants, most abundant in fruits, vegetables, and plant-derived beverages such as tea and red wine (*22*). These compounds have a variety of anticancer activities. Many fruits and onions are rich in phenolic compounds that have antitumorigenic properties (*23*, *24*). Naringen and quercetin inhibit phase I carcinogen-metabolizing enzymes (*25*, *26*). Specific fruits and vegetables, including phenolic compounds, can reduce the risk of lung cancer by 40−50% (*27*).

P. mume fruit contains various phenolic compounds such as phenolic acids and flavonoids. Various biological activities of the *P. mume* fruit have been reported. Chuda et al. (*28*) reported that mumefural, a citric acid derivative, markedly improved blood fluidity. The *n*-hexane extract of *P. mume* fruit has antimutagenic activites. The effective substances were determined to be fatty acids, such as oleic acid, linoleic acid, and linolenic acid (*29*). *P. mume* fruit juice concentrate inhibits the growth signals of vascular smooth muscle cells induced by angiotensin II (*30*). Benzyl glucoside and chlorogenic acid from *P. mume* fruit may contribute to reducing the tension of M-rats caused by ether stress via regulation of the adrenocorticotropic hormone and catecholamine levels (*31*). A new polyacylated sucrose, prunose III, was isolated from flowers of *P. mume* as a scavenging constituent of the 1,1-diphenylpicryl-2-hydrazyl radical and a superoxide (*32*). A methanol extract from flowers of *P. mume* exhibited inhibitory effects against aldose reductase and platelet aggregation (*33*).

There have been only a few reports regarding the anticancer effects of the phenolic compounds obtained from *P. mume* fruit. We isolated the novel compound **B-1** that exhibits an inhibitory effect against human cancer cells. The compound is similar to phenolic compounds from other plants that have anticancer effects. **B-1** was effective against cancer cells but exhibited little effect against normal cells. On the basis of HPLC analysis, the amount of **B-1** in *P. mume* fruit is approximately 1.47−1.70 g/kg. It can be used as a nutraceutical compound for alcohol beverages and fruit juices of *P. mume* fruit, which are currently sold in the market. Further research regarding the bioavailability and the metabolic fate of **B-1** in the human body will be performed.

LITERATURE CITED

(1) Ettinger, W. H.; Fried, L. P.; Harries, T.; Shemanski, L.; Schulz, R.; Robbins, J. Self-reported causes of physical disability in order people: Cardiovascular health study. *J. Am. Geriatr. Soc*. **1994**, *42*, 1035−1044.

(2) Tamara, B. H.; Peter, J. S.; Grethe, S. T.; Mary, H.; Shiriki, K.; James, C. L. Carrying the burden of cardiovascular risk in old age: Associations of weight and weight change with prevalent cardiovascular disease, risk factors, and health status in the cardiovascular health study. *Am. J. Clin. Nutr*. **1997**, *66*, 837−844.

(3) Yoon, Y. D. Deaths by major causes, Annual Report on the Cause of Death Statistical Office, 2001.

(4) Doll, R.; Peto, R. The causes of cancer: Quantitative estimates of avoidable risk of cancer in the United States today. *J. Natl. Cancer Inst*. **1981**, *66*, 1191−1198.

(5) Yang, K. Y.; Yang, J. H.; Ryu, B. H. Antitumor effects of extracts obtained from *Daedalea dickinsil*. *Korean J. Appl. Microbiol. Biotechnol*. **1997**, *25*, 178−182.

(6) Kim, S. J.; Yi, M. A. Growth inhibition of cancer cells by rice bran extract. *Natl. Sci. Inje Univ*. **1998**, *2*, 191−198.

(7) Son, H. S.; Hwang, W. I. A study on the cytotoxic activity of galic (*Allium sativum*) extract against cancer cell (in Korean). *Korean J. Nutr*. **1990**, *23*, 135−147.

(8) Hwang, W. I.; Oh, S. K. Effects of petroleum ether extract of ginseng root on some enzyme activity in human colon cancer cells. *Korean J. Ginseng Sci*. **1984**, *8*, 153−166.

(9) Ito, Y.; Maeda, S.; Sugiyama, T. Suppression of 7,12-dimethylbenzo[*a*]-anthracene-induced chromosome aberrations in rat bone marrow cells by vegetable juices. *Mutat. Res*. **1986**, *172*, 55−60.

(10) Yang, E. J.; Kim, W. Y. A study on dietary factors related to the incidence of stomach cancer and colon cancer in Korean. *Korean J. Nutr*. **1993**, *26*, 603−614.

(11) Weisburger, J. H. Nutritional approach to cancer prevention with emphasis on vitamins, antioxidants and carotenoids. *Am. J. Clin. Nutr*. **1991**, *53*, 226−237.

(12) Steinmetz, K. A.; Potter, J. D. Vegetables, fruit and cancer prevention: A review. *J. Am. Diet. Assoc*. **1996**, *96*, 1027−1039.

(13) Raicht, R. F.; Cohen, B. I.; Fazzini, E. P.; Sarwal, A. N.; Takahashi, M. Protective effect of plant sterols against chemically induced colon tumors in rats. *Cancer Res*. **1980**, *40*, 403−405.

(14) Jee, H. J. *Health Foods from Herbs*; Seoul National University Press: Seoul, South Korea, 1999; 52 pp.

(15) Terada, H.; Sakabe, Y. High-performance liquid chromatographic determination of amygdalin in Ume extract. *J. Hyg. Chem.* **1988**, *34*, 36−40.

(16) Ohtsubo, T.; Ikeda, F. Seasonal changes of cyanogenic glycosides immune seeds. *J. Jpn. Soc. Hortic. Sci*. **1994**, *62*, 695−700.

(17) Mosman, T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **1983**, *65*, 55−58.

(18) Rondest, J.; Das, B. C.; Ricroch, M. N.; Kan-Fan, C.; Potier, P.; Polonsky, J. Plantes malgaches-III. Etude des constituents de *Evodia belahe* Baillon (Rutacees). *Phytochemistry* **1968**, *7*, 1019−1026.

(19) Felicia, V. S.; Najla, G.; Ann, F. C.; Madeleine, M.; Kenneth, K. C. Inhibition of human breast cancer cell proliferation and delay of mammary tumorigenesis by flavonoids and citrus juices. *Nutr. Cancer* **1996**, *26*, 167−181.

(20) Messina, M.; Erdman, J. W. Modern applications for an ancient bean: Soybeans and the prevention and treatment of chronic disease. *J. Nutr*. **1995**, *125*, 567s−569s.

(21) Barnes, S. Effest of genistein on *in vitro* and *in vivo* models of cancer. *J. Nutr*. **1995**, *125*, 777−783.

(22) Harbone, J. Nature, distribution and function of plant flavonoids. *Plant Flavonoids in Biology and Medicine: Biochemical Pharmacological and Structure−Activity Relationships*; Alan, R. Liss, Inc.: New York, 1986; 17 pp.

(23) Lampe, J. W. Health effects of vegetables and fruit: Assessing mechanisms of action in human experimental studies. *Am. J. Clin. Nutr*. **1999**, *70*, 475s−490s.

(24) Boyle, S. P.; Dobson, V. L.; Duthie, S. J.; Kyle, J. A.; Collins, A. R. Absorption and DNA protective effect of flavonoid glycosides from an onion meal. *Eur. J. Nutr*. **2000**, *39*, 213−223.

(25) Rahden-Staron, I.; Czeczot, H.; Szumilo, M. Induction rat liver cytochrome p450 isoenzymes CYP 1A and CYP 2B by different fungicides, nitrofurans, and quercetin. *Mutat. Res*. **2001**, *498*, 57−66.

(26) Obermeier, M. T.; White, R. E.; Yang, C. S. Effects of bioflavonoids on hepatic p450 activities. *Xenobiotica* **1995**, *25*, 575−584.

(27) LeMarchand, L.; Murphy, S. P.; Hankin, J. H.; Wilkens, L. R.; Kolonel, L. N. Intake of flavonoids and lung cancer. *J. Natl. Cancer Inst*. **2000**, *92*, 154−160.

(28) Chuda, Y.; Ono, H.; Ohnishi-Kameyama, M.; Matsumoto, K.; Nagata, T.; Kikuchi, Y. Mumefural, citric acid derivative improving blood fluidity from fruit-juice concentrate of Japanese apricot. *J. Agric. Food Chem*. **1999**, *47*, 828−831.

(29) Dogasaki, C.; Murakami, H.; Yamamoto, K.; Miyazaki, T. Antimutagenic activities of *n*-hexane extracts of the fruit extract and the kernels of *Prunus mume* Sieb. Et Zucc. *Yakugaku Zasshi* **1992**, *112*, 577−584.

(30) Utsunomiya, H.; Takekoshi, S.; Gato, N.; Utatsu, H.; Motley, E. D.; Eguchi, K.; Fitzgerald, T. G.; Mifune, M.; Frank, G. D.; Eguchi, S. Fruit-juice concentrate of Asian plum inhibits growth signals of vascular smooth muscle cells induced by angiotensin II. *Life Sci*. **2002**, *72*, 659−667.

(31) Ina, H.; Yamada, K.; Matsumoto, K.; Miyazaki, T. Effects of benzyl glucoside and chlorogenic acid from *Prunus mume* on adrenocorticotropic hormone (ACTH) and catecholamine levels in plasma of experimental menopausal model rats. *Biol. Pharm. Bull*. **2004**, *27*, 136−137.

(32) Matsuda, H.; Morikawa, T.; Ishiwada, T.; Managi, H.; Kagawa, M.; Higshi, Y.; Yoshikawa, M. Medicinal flowers. VIII. Radical scavenging constituents from the floweres of *Prunus mume*: Structure of prunose III. *Chem. Pharm. Bull*. **2003**, *51*, 440−443.

(33) Yoshikawa, M.; Murakami, T.; Ishiwada, T.; Morikawa, T.; Kagawa, M.; Higashi, Y.; Matsuda, H. New flavonol oligoglycosides and polyacylated sucrose with inhibitory effects on aldose reductase and platelet aggregation from the flowers of *Prunus mume*. *J. Nat. Prod*. **2002**, *65*, 1151−1155.

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