Anti-metastatic Properties of the Leaves of *Eriobotrya japonica*

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The leaves of *Eriobotrya japonica* Lindl. have been widely used as a traditional medicine for the treatment of many diseases including gastroenteric disorders, diabetes mellitus, chronic bronchitis and asthma. In the present study, the anti-metastatic action of the EtOAc fraction of the leaves of *E. japonica* (LEJ) was investigated. LEJ showed potent inhibitory effects on MMP-2 and MMP-9 activities and expressions via down-regulation of NF-κB translocation to the nucleus in B16F10 cells. In addition, the cell migration and invasion were down-regulated by LEJ. LEJ also significantly suppressed lung metastasis in vivo. Moreover, we isolated the compounds ursolic acid and 2α-hydroxyursolic acid from LEJ and both compounds also significantly suppressed MMP-2 and MMP-9 activities, indicating that they are the active components of LEJ. The present results demonstrate that LEJ may be used as valuable anti-metastatic agent for the treatment of cancer metastasis.

Key words: *Eriobotrya japonica*, Matrix metalloproteinase, Cancer metastasis

INTRODUCTION

Cancer metastasis, a hallmark of malignancy, is defined as the spread and growth of cancer cells from the primary neoplasm to distant sites (Weiss, 1990). It is the principal cause of mortality among cancer patients and to date, no therapeutic option is available. Thus, it is critical to develop effective anti-metastatic agents.

Metastasis of cancer cells is generally described as a cascade of events including primary tumor dissociation, migration, invasion, adhesion and proliferation at a target site (Arvelo and Cotte, 2006). Throughout the metastatic process, the rate-limiting step is the breakdown of connective tissue barriers such as the extracellular matrix (ECM) and basement membrane (BM) (Yoon et al., 2003). Therefore, the degradation of ECM and BM is a crucial event in the process of metastasis.

Matrix metalloproteinases (MMPs) are a multigene family of zinc-dependent endopeptidases that play a crucial role in the proteolysis of ECM and BM, which are essential physiological barriers that help prevent the invasion, metastasis and angiogenesis of tumors (McCawley and Matrisian, 2000). Although other MMPs are also involved in the metastatic process, the two gelatinases, MMP-2 and MMP-9, are recognized as key enzymes in tumor invasion and metastasis (Liabakk et al., 1996). They are abundantly expressed in various cancer cells and have been shown to play a crucial role in tumor invasion and metastasis (Johnsen et al., 1998). Therefore, inhibitors of MMP-2 or MMP-9 are attractive therapeutic targets against tumor invasion and metastasis.

*Eriobotrya japonica* Lindl., also known as 'loquat', belongs to the Rosaceae family. It originated in southeastern China and later became naturalized in Korea, Japan, India and many other countries. The leaves of *E. japonica* has been widely used as a traditional medicine with beneficial effects in numerous diseases, including asthma, gastroenteric disorders, diabetes mellitus and chronic bronchitis (Ito et al., 2000).

Various triterpenes, sesquiterpenes, flavonoids, tannins and megastigmane glycosides have been analyzed from the leaves of *E. japonica*, and some of them have been found to possess antitumor, antiviral, hypoglycemic and anti-inflammatory properties (Shimizu et al., 1986; De Tommasi et al., 1991; Ito et al., 2000;
Taniguchi et al., 2002; Kim and Shin, 2009). Recently, Kim et al. (2009) have shown that the methanol extract of *E. japonica* suppresses the adhesion, migration and invasion of a human breast cancer cell line. However, studies on the anti-metastatic activity of the leaves of *E. japonica* are extremely limited. Thus, the present studies were undertaken to investigate the inhibitory effect of the EtOAc fraction of the leaves of *E. japonica* (LEJ) and compounds isolated from LEJ on MMP-2 and MMP-9 activity and expression in B16F10 cells. We also describe its inhibitory effects on migration, invasion and lung metastasis *in vivo*.

**MATERIALS AND METHODS**

**General experimental procedures**

1H- and 13C-NMR spectra were determined on a JEOLJMN-EX 400 spectrometer. TLC was carried out on Merck precoated silica gel F254 plates and the spots were detected under UV and by spraying with 10% H2SO4 in ethanol followed by heating at 100–120°C for 3 min. The deionised water used was obtained from a Millipore ultra-pure water system (Millipore). All other chemicals and solvents used for extraction were analytical grade and used without further purification.

**Plant materials**

The plant materials were purchased from Hainyakupsa in October 2009. A voucher specimen (WOPE057) has been deposited at the Department of Oriental Pharmacy, College of Pharmacy, Woosuk University.

**Extraction and isolation**

The dried sample (2000 g) was extracted twice with 12,000 mL of MeOH by sonication for 2 h. The resultant methanolic extract was concentrated into 60.7 g (Yield: 3.035%) using a rotary evaporator. The sample was then subjected to successive solvent partitioning to give n-hexane (0.83 g), CH2Cl2 (16.4 g), EtOAc (21 g) and n-BuOH (15.4 g) soluble fractions. Each of the fractions was lyophilized and then stored at −20°C until use. The ethyl acetate soluble extract was separated by chromatography on a silica gel column using CHCl3-MeOH, 30:1 as an eluent to give four fractions (E1–E4). Fraction E2 (160 mg) was separated by chromatography on a silica gel column chromatography using CHCl3-EtOAc-MeOH, 80:50:1 as an eluent to give two subfractions (E21–E22). Subfraction E22 (50 mg) was purified by recrystallization from methanol to give compound 1 (9 mg). Compound 2 was obtained by recrystallization of fraction E4 (20 mg) from methyl alcohol.

**Compound 1 (Ursolic acid)**

Colorless needles, m.p. 255-258°C; 1H-NMR (400 MHz, DMSO-d6 and pyridine-d5); δ ppm: 5.30 (1H, m, H-2), 3.22 (1H, dd, J = 10.0, 6.0 Hz, H-3), 2.60 (1H, d, J = 11.0 Hz, H-18), 1.13 (3H, s, H-23), 1.07 (3H, s, H-27), 0.94 (3H, s, H-26), 0.92 (3H, s, H-24), 0.90 (3H, d, J = 7.0 Hz, H-29), 0.89 (3H, d, J = 6.0 Hz, H-30), 0.85 (3H, s, H-5); 13C-NMR (100 MHz, DMSO-d6 and pyridine-d5); δ ppm: 179.65 (C-28), 139.00 (C-13), 125.45 (C-12), 77.83 (C-3), 55.64 (C-5), 53.25 (C-18), 47.86 (C-17), 47.74 (C-9), 42.40 (C-14), 39.75 (C-8), 39.64 (C-19), 39.28 (C-4), 39.16 (C-20), 38.99 (C-1), 37.23 (C-10), 37.19 (C-22), 33.47 (C-7), 30.94 (C-21), 28.91 (C-23), 28.40 (C-15), 27.75 (C-2), 24.65 (C-16), 23.90 (C-29), 23.56 (C-27), 21.61 (C-30), 18.70 (C-6), 17.61 (C-26), 17.59 (C-11), 16.70 (C-24), 15.79 (C-25).

**Compound 2 (2α-Hydroxyursolic acid)**

Colorless needles, m.p. 255-258°C; 1H-NMR (400 MHz, pyridine-d5); δ ppm: 5.45 (1H, t, J = 3.5 Hz, H-12), 4.10 (1H, dt, J = 3.9, 9.7 Hz, H-2β), 3.39 (1H, d, J = 9.7 Hz, H-3α), 2.61 (1H, d, J = 10.8 Hz, H-18), 1.26 (3H, s, H-23), 1.194 (3H, s, H-27), 1.06 (3H, s, H-24), 1.03 (3H, s, H-29), 0.99 (3H, d, J = 6.2 Hz, H-30), 0.96 (3H, s, H-25), 0.93 (3H, d, J = 6.6 Hz, H-29); 13C-NMR (100 MHz, pyridine-d5); δ ppm: 180.71 (C-28, COOH), 139.27 (C-13), 125.52 (C-12), 83.78 (C-3), 68.55 (C-2), 55.89 (C-5), 53.50 (C-18), 48.07 (C-17), 47.97 (C-1), 42.52 (C-14), 40.01 (C-8), 39.81 (C-4), 39.46 (C-20), 39.38 (C-19), 38.41 (C-10), 37.42 (C-22), 33.48 (C-7), 31.05 (C-21), 29.35 (C-23), 28.62 (C-15), 24.88 (C-16), 23.88 (C-27), 23.71 (C-11), 21.38 (C-29), 18.81 (C-6), 17.68 (C-24), 17.48 (2C, C-26, C-30), 16.95 (C-25).

**Animals**

Male C57BL/6 mice (5 weeks old) weighing 16-20 g were supplied by Damul Science. All animals were housed at 22 ± 1°C with a 12 h light/dark cycle and fed a standard pellet diet with tap water ad libitum.

**Cell culture**

B16F10 murine melanoma cells and HT-1080 human fibrosarcoma cells were obtained from the Korean cell line bank (KCLB) and cultured in DMEM containing 10% heat-inactivated FBS supplemented with penicillin (100 U/mL), streptomycin (100 µg/mL), and sodium bicarbonate (2.2 g/L) at 37°C in a 5% CO2 and humidified air atmosphere. Cultures used in subsequent experiments were at < 50 passages.