SHORT REPORT

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Cytotoxic Activity of β-Caryophyllene Oxide Isolated from Jeju Guava (*Psidium cattleianum* Sabine) Leaf

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Abstract: β-caryophyllene oxide was isolated from the leaf of the Jeju guava (*Psidium cattleianum* Sabine) for the first time in this study. The chemical structure was confirmed with the help of various spectroscopic analyses, including NMR data, by comparison to that of the published data. An MTT assay was conducted against several cancer cell lines to resolve an ongoing controversy regarding the cytotoxic effects of β-caryophyllene oxide. β-caryophyllene oxide evidenced potent cytotoxic activity against HepG2, AGS, HeLa, SNU-1, and SNU-16 cells, with IC_{50} values of 3.95, 12.6, 13.55, 16.79, and 27.39 µM, respectively. The results also showed that β-caryophyllene oxide evidenced cytotoxicity in both a dose-dependent and time-dependent manner.

Keywords: β-caryophyllene oxide; cytotoxicity; Jeju guava; *Psidium cattleianum*

1. Plant Source

The Jeju guava (*Psidium cattleianum* Sabine), also known as the strawberry guava or cattley guava, is a medicinal and aromatic plant belonging to the Myrtaceae family. It has been cultivated in various parts of the world, and specifically on the Jeju Island of South Korea. The leaves were collected from a farmer's field near the National Institute of Subtropical Agriculture in Jeju Province,

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Korea in January, 2010. A voucher specimen (16/2010) was deposited in the Herbarium of the Subtropical Research Institute of Jeju National University for further reference.

2. Previous Studies

Plants belonging to the *Psidium* genus have been shown to exhibit several therapeutic properties, including antibacterial, hypoglycemic, anti-inflammatory, analgesic, antipyretic, spasmolytic, and central nervous system-depressant activities, and are used as a popular medicine [1]. A survey of the relevant literature revealed that previously, isoflavonoids [2] and volatile compounds [3] were detected from the leaf and fruit oil, respectively, of *P. cattleianum*. The chemical compositions of the leaf oils of *P. cattleianum* Sabine from different geographical areas were assessed via GC and GC/MS. Depending on the location from which the samples were collected, the oil was composed of different percentages of the following primary components: β-caryophyllene, α-pinene, myrcene α-thujene, 1,8-cineole, epi-α-muurolol, α-cadinol, epi-α-cadinol and caryophyllene oxide, at different percentages [4-6].

The leaf extract was shown to exert antimicrobial [7-8] and anti-caries effects in rats [9]. However, there remains some controversy as to whether or not one of the main components of guava leaf, caryophyllene oxide, is cytotoxic. Sibanda *et al.* 2004 [10] found that caryophyllene oxide exhibits modest cytotoxic activity, consistent with an earlier report [11], whereas some reports found it to be inactive against tumor/cancer cell lines [12, 14-15].

3. Present Study

The Jeju guava (*P. cattleianum*) leaf samples (1.2 kg) were air-dried, chopped, and extracted three times with 80% MeOH (12 L) for 14 days at room temperature. The 80% methanol solvent was evaporated to dryness under reduced pressure at a temperature below 40°C with a rotary evaporator to obtain the crude methanol extract (25.3 g). A portion of the crude methanol extract (10.0 g) was then suspended in H_2O (2 x 300 mL) and fractionated with the organic solvents hexane, $CHCl_3$, and *n*-BuOH to yield the hexane-soluble fraction (2.4 g), the $CHCl_3$-soluble fraction (1.1 g), the *n*-BuOH-soluble fraction (3.2 g), and finally the H_2O-soluble fraction (2.8 g). The hexane fraction (1.0 g) was then subjected to column chromatography over silica gel using increasing polarities of *n*-hexane: EtOAc to generate seven fractions (P1-7). The second fraction (P2) (223 mg) was subjected to another chromatogram with a solvent system of *n*-hexane and diethyl ether ranging from (100 : 1 → 14 : 1), followed by the combination of fraction 11-15 of P2 on the basis of TLC and evaporation to obtain pure β-caryophyllene oxide (**1**) (35 mg). The structure of compound **1**(figure 1) was confirmed after comparison of its NMR and Mass with published data [13, 16]. ^{1}H-NMR and ^{13}C-NMR at 400 and 100 MHz, respectively, were conducted on a Bruker AM 400 spectrometer in $CDCl_3$. This study was the first to isolate β-caryophyllene oxide from this guava species.

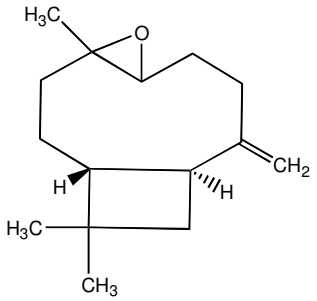

Figure 1. Structure of β-caryophyllene oxide isolated from Jeju guava leaf.

Cytotoxic activities against several human cancer cell lines were evaluated by an MTT based assay [17] to determine whether or not β- caryophyllene oxide is a cytotoxic compound. Cancer cell lines, including HeLa human cervical adenocarcinoma cells, HepG2 human leukemia cancer cells, AGS human lung cancer cells, SNU-1 human gastric cancer cell and SNU-16 human stomach cancer cells were obtained from the Korean Cell Line Bank (KCLB, Seoul, Korea). The cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) or RPMI RPMI 1640 containing 10% (v/v) heat-inactivated fetal bovine serum (FBS), 100 units/ml of penicillin, and 100 μg/ml of streptomycin. Cells were maintained in a humidified incubator at 37 °C in a 5% CO_2 atmosphere. The cells were subsequently incubated for four days in the presence of various concentrations of β-caryophyllene oxide. After incubation, 0.1 mg of MTT (Sigma, St. Louis, MO) was added to each well and the cells were incubated for 4 h at 37°C. The plates were subsequently centrifuged for 20 min at 2,500 rpm at RT, and the medium was carefully removed. DMSO (150 μL) was then added to each well to dissolve the formazan crystals. The plates were read immediately at 570 nm on a Sunrise microplate reader (Sunrise, Tecan, Salzburg, Austria). All cell inhibition was determined in triplicate for each concentration. All results were analyzed and presented using Sigma Software (Chicago, IL, USA). All assays were conducted in triplicate.

The effects of various concentrations of β-caryophyllene oxide on the growth of HeLa, HepG2, AGS, SNU-1, and SNU-16 cells are shown in Fig 2 and Table 1. Our results demonstrated that the inhibition of cell growth occurred in a dose-dependent and cell-specific manner. β-caryophyllene oxide evidenced the most potent cytotoxic activity against HepG2, AGS, HeLa, SNU-1, and SNU-16 cells, with IC_{50} values of 3.95, 12.6, 13.55, 16.79, and 27.39 μM (Table 1), respectively. Compared to natural standard quercetin, its cytotoxic activity is quite promising. Additionally, β-caryophyllene oxide evidenced time-dependent cytotoxicity, as shown in Fig 2B, where HeLa and AGS cells evidenced the highest sensitivity over the experimental time course.

Table 1. Cytotoxic activity of the isolated β-caryophyllene oxide and quercetin.

Cell lines	IC_{50} (μM)	
	β-caryophyllene oxide	**Quercetin**
HepG2	3.95 ± 0.23	97.3 ± 2.17
HeLa	13.55 ± 0.45	166.3 ± 2.23
AGS	12.6 ± 0.86	126.03 ± 1.94
SNU-1	16.79 ± 1.2	19.64 ± 1.3
SNU-16	27.39 ± 1.4	62.53 ± 1.8

Inhibitory activity was expressed as the mean of 50% inhibitory concentration of triplicate determinations

The findings of this study demonstrate that β-caryophyllene oxide exhibited significant cytotoxicity against HepG2 and excellent activity against other tested human cancer cell lines compared to the activities reported in previous studies. We also determined that the cytotoxicity was both dose- and time-dependent. By way of contrast, our results contradict those of other research groups, who either did not detect activity or detected only low activity.

This may be the consequence of a problem with the solubility of β-caryophyllene oxide, which we also experienced during our study. Thus, based on the results of this study, it can be concluded that β-caryophyllene oxide was in fact isolated for the first time from the leaves of Jeju guava (*P. cattleianum*), which are an excellent candidate for cytotoxic study. However, more extensive studies on molecular mechanisms will be required for the further development of new cytotoxic agents; some of this research is currently underway in our lab.

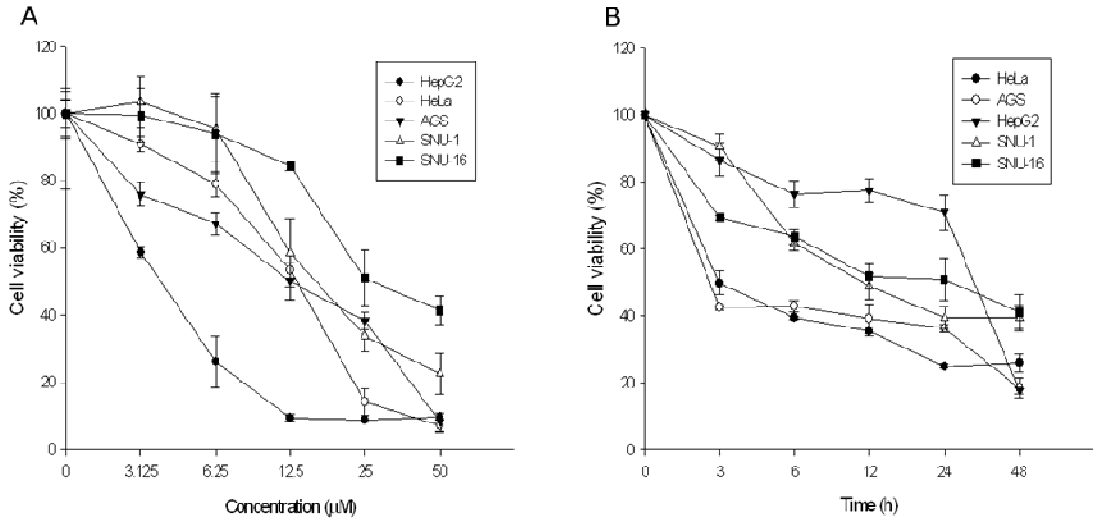

Figure 2. Cytotoxic effect of β-caryophyllene oxide against various human cancer cell lines. (A) Cells were treated with different concentration for 24 h. (B) Cells were treated with 25 μM for indicated period of time. Cell viability without treatment was taken as 100%. Data are expressed as the mean ± SD (n = 4).

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