Chemical Components from Aloe and their Inhibition of Indoleamine 2, 3-dioxygenase

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ABSTRACT

Background: In Korea, Aloe is routinely ingested as a traditional medicine or as a component of health beverages. Objective: To research the inhibition of indoleamine 2, 3-dioxygenase (IDO) activities of components from Aloe. Materials and Methods: the compounds were isolated from a combination of silica gel and YMC Rp-18 column chromatography, and their structures were identified by analysis of spectroscopic data (1D, 2D-NMR, and MS). All of the isolated compounds were examined for their ability to inhibit IDO, which actively suppresses immune functions by catalyzing the rate limiting reaction in the conversion of tryptophan to kynurenine. Results: In this phytochemical study, 18 known compounds were isolated from aqueous dissolved Aloe exudates. All of the isolated compounds were examined for their ability to inhibit IDO activities for a series of anthraquinone derivatives (1–7) isolated from the Aloe extract; the IC₅₀ values of these compounds ranged from 39.41 to 52.93 μM. Enzyme kinetic studies of their modes of inhibition indicated that all of the compounds were uncompetitive inhibitors. Conclusion: The aqueous dissolved Aloe exudate can be used as a source of novel natural IDO inhibitors and merit testing as therapeutic agents in the treatments of cancer and immunopathologic diseases, such as autoimmune, inflammatory, and allergic disorders.

Key words: Aloe, anthraquinone derivatives, Asphodelaceae, indoleamine 2, 3-dioxygenase (IDO)

SUMMARY

• In this study, 18 known compounds were isolated from aqueous dissolved Aloe exudates. All of the isolated compounds were examined for their ability to inhibit indoleamine 2, 3-dioxygenase (IDO) activities for a series of anthraquinone derivatives (1–7) isolated from the Aloe extract.

Abbreviation used: IDO: inhibit indoleamine 2, 3-dioxygenase, TMS: tetramethylsilane, HMGC: heteronuclear multiple quantum correlation, HMBC: heteronuclear multiple bond correlation, COSY: 1H-1H correlation spectroscopy, ESI-MS: Electrospray ionization mass spectrometry, DMSO: dimethyl sulfoxide

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INTRODUCTION

Indoleamine 2, 3-dioxygenase (IDO) is an intracellular enzyme that catalyzes the transformation of L-tryptophan to N-formylkynurenine, which is the first and rate-controlling step in the kynurenine pathway.[1] The role of IDO in immunomodulation has been reported in previous studies, including preclinical studies of allograft tolerance, inflammation, and cancer.[2] Both animal and human studies have demonstrated that IDO-expressing cells function as immunosuppressors by increasing T-lymphocyte tolerance. These observations strongly suggest that IDO plays an important role in the regulation (suppression) of the adaptive immune response and thus provides an excellent therapeutic target for anticancer immunotherapy.[3]

Aloe is a short-stemmed succulent herb that belongs to the Asphodelaceae family. The seven genera of this family include approximately 650 species.[4] Of these, the 400 species of the genus Aloe typically grow in temperate and subtropical regions of Africa.[5-7] Based on morphological characteristics,[8] the genus Aloe has been divided into 20 subgroups, ranging from grass to tree aloes. In addition to its use in traditional medicines for the treatment of various diseases, Aloe is an ingredient in many cosmetics and health foods. A previous phytochemical study revealed that the main constituents of Aloe are phenolic compounds, including anthraquinones, chromones, and pyrones.[9] Aqueous Aloe exudates and several monomeric compounds isolated from Aloe exert anti-inflammatory, antioxidant, anticancer, and antidiabetic properties.[10-15] However, to our knowledge, the IDO inhibitory activity of the plant has not been reported. In the present study, 18 compounds isolated from aqueous dissolved Aloe exudates were evaluated for their IDO inhibitory activities. Our findings have medical and pharmacological applications.

MATERIALS AND METHODS

General experimental procedures

The NMR spectra were recorded using a JEOL ECA 600 spectrometer (H, 600 MHz and 13C, 150 MHz), with tetramethylsilane as an internal standard. Heteronuclear multiple quantum correlation, heteronuclear multiple bond correlation, and 1H-1H correlation spectroscopy spectra were recorded using a pulsed field gradient. The ESI-MS: Electrospray ionization mass spectrometry and DMSO: dimethyl sulfoxide, spectra

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Plant material
The dried exudates of *Aloe* were purchased from herbal company, Naemone Dah, Ulsan, Korea in April 2014, and identified by Prof. Young Ho Kim, College of Pharmacy, Chungnam National University. A voucher specimen (CNU14105) was deposited at the herbarium of the College of Pharmacy, Chungnam National University in Korea.

Extraction and isolation
The dried exudation of *Aloe* (200 g) were dissolved in H$_2$O three times under ultrasonication at ambient temperature and then filtered. The filtrate was concentrated and partitioned with CHCl$_3$, EtOAc, and n-BuOH to yield CHCl$_3$ (2.0 g), EtOAc (24.0 g), and n-BuOH (20.0 g) fractions. The EtOAc fraction (24.0 g) was subjected to silica gel (10 × 30 cm) column chromatography with a gradient of CHCl$_3$–MeOH–H$_2$O (1:0, 0.025:0.025:1, 0.05:0.05:1, 0.1:0.1:1, 0.2:0.2:1, 0.3:0.3:1, 0.4:0.4:1; 1.0 L for each step) to give seven fractions (Fr. E1–E7). Fraction E2 was further chromatographed on a reverse-phase (RP; 1.0 × 80 cm) column chromatography using a gradient of MeOH–H$_2$O (0.025:0.025:1, 0.05:0.05:1, 0.1:0.1:1, 0.2:0.2:1, 0.3:0.3:1, 0.4:0.4:1; 1.0 L for each step) to give six fractions (Fr. E2.1–E2.6). Compound 12 (15.0 mg) was isolated from fraction E2.5 using a silica gel (1.0 × 80 cm) column chromatography with CHCl$_3$–MeOH–H$_2$O (6.5:1:0.1; 800 mL).

IDO assay and determination of inhibition pattern of IDO inhibitors
IDO assays were performed mainly as described previously by Nakano et al.[26] Briefly, a compound serially diluted in DMSO was mixed with 1 μg of purified human IDO in an IDO assay buffer (50 mM potassium phosphate buffer, pH 6.5). Then L-(-)-ascorbic acid, methylene blue, and catalase and L-tryptophan were added to the enzyme–compound mixture to final concentrations of 50 mM, 20 mM, 10 mM, 100 μg/mL, and 200 μM, respectively. The enzyme reaction mixture was incubated at 37°C for 1 hr. After incubation, the reaction mixture was supplemented with 40 μL of 30% trichloroacetic acid and heated for 15 min at 65°C followed by centrifugation to remove the precipitate. The supernatant taken after centrifugation was mixed with an equal volume of Ehrlich’s reagent (2% p-dimethylaminobenzaldehyde in acetic acid) and incubated at RT. The intensity of the color developed, which represents the concentration of L-kynurenine produced during the enzyme reaction, was measured by reading the absorbance at 480 nm wavelength.

Inhibition patterns of IDO inhibitors were determined with Lineweaver-Burk plot, for which IDO assays were run at five different L-tryptophane concentrations and four different inhibitor concentrations (0, 25, 50, 100 μL), respectively. K$_i$ of IDO inhibitors were calculated from the y-intercept of Lineweaver-Burk plot, which is denoted by (1 + [I]/K$_i$)/V$_{max}$.[17,18]

Statistical analysis
All measurements were performed independently at least triplicate. Data were expressed as the mean ± standard deviation (SD). Statistical significance is determined by one-way analysis of variance followed by Dunnett’s multiple comparison test, P < 0.05.

RESULTS
In this study, 18 compounds (1-18) were isolated from the aqueous dissolved *Aloe* exudate. The structures of compounds 1-18 were identified based on spectroscopic data, chemical evidence, and comparisons with previously reports [Figure 1]. Their structures were elucidated as aloemodin (1),[19] aloin A (2),[20] aloin B (3),[21] desoxyaloin (4),[22] aloinoside B (5),[23] aloinoside C (6),[24] aloinoside D (7),[25] aloenin glycone (8),[26] feroxidin (9),[27] 7-hydroxy-5-(hydroxymethyl)-2-methylchromone (10),[28] 5-methylresorcinol (11),[29] aloine resin D (12),[30] 7-O-methylaloesinol (13),[31] aloesin G (14),[32] C-2’-deoxymaronolaoresin G (15),[33] 5-((S)-2’-oxo-4’-hydroxypropyl)-2-hydroxymethylchromone (16),[34] aloeveroside A (17),[35] and aloenin B (18).[36] The inhibitory activities of the 18 isolated compounds against IDO were evaluated. Menadione served as the positive control (IC$_{50}$; 3.71 ± 0.26 μM). The IDO inhibition of compounds 1–18 were tested at concentration of 100 μM. At this concentration, the effects of several compounds were minimal, although compounds 1–7 showed over 60% inhibition at 100 μM. The activities of these seven compounds were examined further at lower concentrations to determine their respective IC$_{50}$ values. Potent inhibitory activities with IC$_{50}$ values of 46.50 ± 1.51, 40.32 ± 0.80, 42.21 ± 1.51, 44.81 ± 1.32, 43.88 ± 1.95, 39.41 ± 1.94, and 53.93 ± 0.95 μM, were determined for compounds 1–7, respectively [Figure 2 and Table 1].
Figure 1: Structures of compounds 1–18 from Aloe.
Figure 2: IDO inhibitory effects of compounds 1–7, 12, and 17. * Relative to that obtained in the absence of IDO inhibitor were plotted. Concentrations (μM) of inhibitors used in the experiment are as shown.

Figure 3: The inhibition pattern of compounds 1–7. Michaelis-Menten plots with data obtained in the presence (25, 50, and 100 μM, respectively) or absence of compounds 1 (A), 2 (B), 3 (C), 4 (D), 5 (E), 6 (F), and 7 (G). Lineweaver-Burk plots [H], 2 (I), 3 (J), 4 (K), 5 (L), 6 (M), 7 (N)] generated after transformation of data used in (A–G).
An analysis of the structure-activity relationships of the isolated compounds showed that compounds 1-7 all have an anthraquinone core structure. It therefore seems likely that the anticyclic molarity is responsible at least in part for the observed inhibition of IDO.

DISCUSSION

To more precisely determine the modes of inhibition of compounds 1-7, enzyme kinetics studies employing Lineweaver-Burk (double reciprocal) plots were performed. As shown in Figure 3, regardless of the compound used, Lineweaver-Burk plots generated in the absence (0 µM) or presence of three different concentrations (25, 50, and 100 µM) of the respective compounds yielded almost parallel line. These results suggest that compounds 1-7 should be uncompetitive inhibitors [Figure 3]. Based on the notion that compounds 1-7 are uncompetitive inhibitors, their inhibition constants (K) were also calculated using the same plots (Table 1).

In recent years, several studies have reported IDO inhibition by various synthetic and microbial secondary products. However, studies on IDO inhibitors derived from plants are limited. To our knowledge, this is the first report detailing the IDO inhibitory activities of anthraquinone derivatives from Aloe. Our finding suggest that aqueous dissolved Aloe extract can be used as a source of novel natural IDO inhibitors and merit testing as therapeutic agents in the treatments of cancer and immunopathologic diseases such as autoimmune, inflammatory, and allergic disorders. Moreover, the anthraquinone compounds identified in this study may be exploitable as templates for the synthesis of more potent and potentially clinical IDO inhibitors.

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Table 1: The IDO inhibitory activities of compounds 1–18

| Compounds | 100 µM (%) | IC50 (µM) | Type (K) |
|-----------|------------|-----------|----------|
| 1         | 67.40 ± 2.53 | 46.50 ± 1.51 | Uncompetitive (85.26 ± 2.59) |
| 2         | 66.59 ± 0.51 | 40.32 ± 0.80 | Uncompetitive (68.56 ± 6.27) |
| 3         | 67.68 ± 0.82 | 42.21 ± 1.51 | Uncompetitive (70.21 ± 7.46) |
| 4         | 61.64 ± 1.67 | 48.81 ± 1.32 | Uncompetitive (80.27 ± 5.14) |
| 5         | 74.58 ± 1.17 | 43.88 ± 1.95 | Uncompetitive (77.32 ± 0.87) |
| 6         | 67.80 ± 1.38 | 39.41 ± 1.94 | Uncompetitive (65.32 ± 3.13) |
| 7         | 63.03 ± 1.84 | 53.93 ± 0.95 | Uncompetitive (135.45 ± 4.68) |
| 8         | 6.36 ± 4.72  | N.T.  | N.T.  |
| 9         | 11.16 ± 0.35 | N.T.  | N.T.  |
| 10        | 5.86 ± 3.08  | N.T.  | N.T.  |
| 11        | 10.15 ± 1.75 | N.T.  | N.T.  |
| 12        | 49.32 ± 0.27 | 99.52 ± 2.75 | N.T.  |
| 13        | 26.73 ± 0.98 | N.T.  | N.T.  |
| 14        | 22.82 ± 0.48 | N.T.  | N.T.  |
| 15        | 28.78 ± 1.25 | N.T.  | N.T.  |
| 16        | 9.66 ± 4.70  | N.T.  | N.T.  |
| 17        | 52.35 ± 0.48 | 83.81 ± 1.33 | N.T.  |
| 18        | 20.56 ± 0.15 | N.T.  | N.T.  |
| Menadione*| -           | 3.71 ± 1.26 | N.T.  |

*Positive control; N.T. = Not tested; IC50 values are means ± SDs (n = 3).

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Conflicts of interest

There are no conflicts of interest.

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