Phytochemical and Biological Investigation of Aloe Grandidentata Salm-Dyck

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Abstract

The crude alcoholic extract of the leaves of Aloe grandidentata Salm.-Deck showed significant antimicrobial activity (200 mg/ml), potent anti-inflammatory and chronic antihyperglycemic (100 mg/kg b.wt.) compared to standard positive drugs. Phytochemical studies of the potent extract revealed the isolation and characterization of seven compounds; two new compounds: 1,1’8,8’-tetrahydroxy-3-acetyl-3-methyl-6,6-bianthracene-9, 9,10,10-tetraone (2) and 1,6,8-trihydroxy-7-methoxy-3-methyl antraquinone (3), five known compounds, β-sitosterol (1), emodin (4), chrysophanol (5), physcion (6) and β-sitosterol-3-O-β–D-glucoside (7). This is the first report of the isolation of emodin and β-sitosterol-3-O-β–D-glucoside from genus Aloe and physicin from family Liliaceae. All structures of the isolated compounds were determined using several spectroscopic techniques; UV, IR, MS, NMR (1H NMR and 13C NMR) and by comparison with literature data.

Keywords: Aloe grandidentata; Anthraquinones; Emodin; Physcion; Anti-inflammatory; Antihyperglycemic; Antimicrobial

Introduction

Aloe (Liliaceae) is a large genus of 400 species native to Africa, Madagascar, and Arabia [1]. Aloe has a wide range of medicinal application such as laxative effect, wound healing effect, reduces blood sugar in diabetes, soothes burns, eases intestinal problems, reduces arthritic swelling, ulcer curative effect, stimulates immune response against cancer etc. [2]. Studied pharmacological effects of Aloe as in vitro or in animals include antimicrobial [3], anti-inflammatory and anti-arthritic activity [4,5] and hypoglycemic effects [6-8]. Several constituents were isolated from different Aloe species; sterols, lignin, saponins, anthrones, their dimmers, chromones, flavones, C-glycosides of anthrone and chromones [9] and glycoproteins and polysaccharides [9,10]. Aloe grandidentata is a green fleshy plant reaches up to 30 cm height, flourishes in Egypt and flowers in January till June; the subterranean part consists of rhizome and adventitious roots [11]. Nothing was found about chemical constituents and biological effect of the plant.

The protocol of the study was approved by the Research Ethics Committee in the Faculty of Pharmacy, Cairo University, Cairo, Egypt.

Materials and Methods

General experimental procedures: IR, Schimadzu IR-435, PU-9712 infrared spectrophotometer; UV, Schimadzu UV 1650 PC; 1H-NMR (300 MHz) and 13C-NMR (75 MHz), Jeol Ex-300 MHZ and Bruker AC – 300 spectrometer; MS, Varian Mat 711, Finnning mass SQS 7000 Mass spectrometer, 70 eV; CC, silica gel 60 (Merck, 230–400 mesh) and Sephadex LH-20 (Sigma); TLC, Pre-coated silica gel plates (Merck, Kieselgel 60 F254, 0.25 mm).

Microorganisms: Bacillus subtilis, Staphylococcus aureus, klebsiellapneumoniae, Escherichia coli, Pseudomonas aeruginosa and Candida albicans were obtained from Microbiology Department, Faculty of Pharmacy, Al Azhar University, Cairo, Egypt were used.

Animals: Adult male albino rats of Sprague Dawely Strain weighing (100-150 g) were obtained from the animal house colony at the National Research Center (Dokki, Giza, Egypt) and kept on standard laboratory diet and under hygienic conditions.

Drugs: Carrageenan (Sigma Co., USA), for induction of inflammation; indomethacin (Indomethacin), Egyptian Int. Pharmaceutical Industries Co.; (EIPICO, under license of Merck & Co. INC-RAHAWY N.J., USA), as standard anti-inflammatory; Alloxan (Sigma Co., USA), for induction of diabetes; metformin (Cidophage)®, European Int. Pharmaceutical Industries Co.; (EIPICO, under license of Merck & Co. INC-RAHAWY N.J., USA), as standard anti-inflammatory; Alloxan (Sigma Co., USA), for induction of diabetes; metformin (Cidophage)®, Ciprofloxacin antibiotic (Hoechst), standard antibacterial and Nystatin (Sucrill), standard antifungal.

Collection and extraction of plant material: Aloe grandidentata leaf was collected during the summer at flowering stage from EL Orman Garden and the Experimental and Research Station of Faculty of Pharmacy, Cairo University, Giza, Egypt. It was identified and authenticated by Dr. Wafaa Amer, Professor of Plant Taxonomy, Botany Department, Faculty of Science, Cairo University, Cairo, Egypt and a voucher specimen has been deposited in Pharmacognosy Department, College of Pharmacy, Cairo University, Egypt.

The powdered, air dried leaves (570 g) was exhaustively extracted by percolation in 95% ethanol. The extract was evaporated in vacuo to yield 79 g of crude alcohol extract (A). Crude alcohol extract was suspended in water and fractionated with petroleum ether, chloroform, ethyl acetate and n-butanol saturated with water. Each fraction was dried over anhydrous sodium sulphate and evaporated to dryness to

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yield fraction B (petroleum ether, 18.6 g), fraction C (chloroform, 9.1 g), fraction D (ethyl acetate, 4.3 g) and fraction E (n-butanol 6.1 g).

**Chromatographic separation of plant fractions**: Fraction B (15 g) was applied to flash chromatography using silica gel (200–400 mesh) column, (50 cm x 5 cm, 300 g). The column was eluted using increasing concentrations of n-hexane, chloroform, ethyl acetate, and methanol through increasing polarity by 10% to end up with five pooled subfractions. Subfraction II (540 mg, n-hexane: CHCl₃ 8:2) was subjected to further chromatographic separation on a sephadex LH20 column, eluted with methanol: β-sitosterol-1 (1) 56 mg was isolated, while further rechromatographic separation afforded 31.4 mg of 1,1’,8,8’-tetrahydroxy-3-acetyl-3-methyl-5,5’bianthracene-9,9,10,10-tetraone (2).

Subfraction III (100 mg, n-hexane: CHCl₃ 4:6) was further chromatographed on silica gel column, eluted with n-hexane-ethyl acetate (7:3 v/v) to afford 16.7 mg of 1,6,8-trihydroxy-7-methoxy-3-methyl anthraquinone (3).

Fraction C (8 g) was subjected to flash chromatography using silica gel (200-400 mesh) column, (50 cm x 3.5 cm, 200 g). The column was eluted using n-hexane, chloroform, ethyl acetate, and methanol through increasing polarity by 5% to yield three pooled subfractions (I-III). Subfraction I (361 mg, n-hexane: CHCl₃ 8:2) was subjected to chromatographic separation on a sephadex LH20 column, eluted with methanol: β-sitosterol-1 (5) 56 mg was isolated, while further rechromatographic separation afforded 31.4 mg of 1,1’,8,8’-tetrahydroxy-3-acetyl-3-methyl-5,5’bianthracene-9,9,10,10-tetraone (2).

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**Antihyperglycemic activity**: The alcohol extract of A. grandidentata was tested for its anti-hyperglycemic activity over long period (2 months). The blood glucose level was monitored after 4 and 2 months after drug administration, the rats were sacrificed; both hind paws excised and weighed separately. The percentage of oedema (inflammation) was calculated according to the following equation:

\[
\text{X100} \times \frac{\text{Weight of right paw} - \text{Weight of left paw}}{\text{Weight of left paw}} \times 100
\]

**Antiproliferative activity**: The alcohol extract of A. grandidentata was tested for its anti-proliferative activity over long period (2 months). The blood glucose level was monitored after 4 and 2 months after drug administration, the rats were sacrificed; both hind paws excised and weighed separately. The percentage of oedema (inflammation) was calculated according to the following equation:

\[
\text{X100} \times \frac{\text{Weight of right paw} - \text{Weight of left paw}}{\text{Weight of left paw}} \times 100
\]
The location of the biaryl bond followed from the absence of signal be 1,1',8,8'-tetrahydroxy-3'-acetyl-3-methyl-5,5'-bianthracene of the available spectral evidences the structure was established to polyhydroxybianthraquinoid isolated for the first time. On the basis high molecular weight supported that compound 2 was a new Gt. Percentage of change in blood glucose level was calculated from the following equation:

\[
\text{% of change} = \left( \frac{G_G - G_t}{G_G} \right) \times 100
\]

At the end of the experiments, all dead animals were getting rid by frozen till incineration.

**Results**

The structure (Figure 1A) of the known compounds; β-sitosterol (1) and β-sitosterol-3-O-β-D-glucoside (7) [16], emodin (4), chrysophanol (5) and physcion (6) [17,18] were determined by comparison of their physical and spectroscopic data (UV, 1H NMR, 13C NMR and MS) with those reported in literature. Physcion (6) is isolated for the first time in family Liliaceae. Meanwhile, Emodin (4) and β-sitosterol-3-O-β-D-glucoside (7) are isolated for the first time from the genus Aloe. β-sitosterol (1) and Chrysophanol (5) were previously reported from other Aloe species [19] but they are isolated for the first time from the leaves of A. grandidentata.

The structure (Figure 1B) of the new compounds; 1,1',8,8'-tetrahydroxy-3'-acetyl-3-methyl-5,5'-bianthracene-9,10,10-tetraene (2) and 1,6,8-trihydroxy-7-methoxy-3-methyl anthraquinone (3) were determined by their physical and chemical characters and spectroscopic data (UV, 1H NMR, 13C NMR and MS).

In addition the λ<sub>MeOH</sub> max at 260, 390 and 440 nm besides the high molecular weight supported that compound 2 was a new polyhydroxybianthraquinoid isolated for the first time. On the basis of the available spectral evidences the structure was established to be 1,1',8,8'-tetrahydroxy-3'-acetyl-3-methyl-5,5'-bianthracene-9,9',10,10'-tetraene.

**Table 1:** Results of antimicrobial screening of alcoholic extract of A. grandidentata.

<table>
<thead>
<tr>
<th>Micro-organisms</th>
<th>Alcohol extract of A. grandidentata (200 mg/ml)</th>
<th>Ciprofloxacin</th>
<th>Nystatin</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>R 22 ± 0.44 N.D</td>
<td>22 ± 0.44 N.D</td>
<td></td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>9 ± 0.53</td>
<td>22 ± 0.95 N.D</td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas</em></td>
<td>35 ± 0.78 N.D</td>
<td>35 ± 0.78 N.D</td>
<td></td>
</tr>
<tr>
<td><em>aeruginosa</em></td>
<td>11 ± 0.74</td>
<td>34 ± 0.83 N.D</td>
<td></td>
</tr>
<tr>
<td><em>klebsiellapnemonia</em></td>
<td>9 ± 0.37</td>
<td>19 ± 0.52 N.D</td>
<td></td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>8 ± 0.27</td>
<td>N.D</td>
<td>16 ± 0.62</td>
</tr>
</tbody>
</table>

R= no inhibition zone N.D=not done SE standard error

**Figure 1:** A: Structure of known compounds isolated from the leaf of A. grandidentata. B: Structure of new compounds isolated from the leaf of A. grandidentata.
that the alcoholic exerted 92.01% potency as that standard anti-inflammatory drug indomethacin. These results were in agreement with findings previously reported for several Aloe species [4,5,13].

This activity might be due to the presence of anthraquinones [23,24] \( \beta \)-sitosterol [25,26] and \( \beta \)-sitosterol-3-O-\( \beta \)-D-glucoside[26] which have been reported to possess anti-inflammatory activities.

The observed data (Table 3) revealed that \textit{A. grandidentata} Salm. – Dyck by dose at 100 mg/kg, reduced the blood glucose level by (45.03%) and (56.24%) after treatment for 4 weeks and 8 weeks respectively. This study represented the first report; for \textit{A. grandidentata} Salm. – Dyck to have a potent antidiabetic effect equivalent to 95.26% and 86.83% after 4 weeks and 8 weeks respectively comparing to the standard antidiabetic drug Metformin. These results were in agreement with findings previously reported for Aloe vera [6-8].

The hypoglycemic effect of alcoholic extract of \textit{A. grandidentata} may be attributed to its content of chrysophanol [27].

### Acknowledgement

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### References
