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 -5,5-bianthracene -9, 9,10,10'-tetrone (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–D-glucoside from genus Aloe and physcion from family Liliaceae. All structures of the isolated compounds were determined using several spectroscopic techniques; UV, IR, 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 activity of A. grandidentata, so the present study was planned to investigate both 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, Finnigan mass SSQ 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-RAAWY N.I., USA), as standard anti-inflammatory; Alloxan (Sigma Co., USA), for induction of diabetes; metformin (Cidophage), Chemical Industries Development Co. (CID CO.), Giza, Egypt, as antidiabetic; Kits for measuring blood glucose levels Bio-Merieux Co., France; Ciprofloxacin antibiotic (Hoechst), standard antibacterial and Nystatin (Squibb), 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
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-3-O-β–D-glucoside (7).

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, eluting with methanol to afford 39 mg of emodin (4). Subfractions II and III (200 mg, n-hexane: CHCl3 6:4 and 687 mg, CHCl3; ethyl acetate 1:1, respectively) were separately, subjected to repeated chromatographic separation on a sephadex LH20 column, to yield 10.8 mg of chrysophanol (5) and 60.2 mg of gypenosides, from subfractions II and III, respectively.

Fraction D (4 g) was similarly, subjected to silica gel column chromatography, subfraction 42 (187 mg, ethyl acetate: CH3OH 60.2 mg of physcion(6), from subfractions II and III, respectively. They were separately, subjected to repeated chromatographic separation on a sephadex LH20 column, eluting with 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-3-O-β–D-glucoside (7).

**Results and Discussion:**

1. **Phytochemical Analysis:**
   - **Identification of Compounds:**
     - Identification of compounds was based on their physical characteristics (melting point, IR, and NMR spectra) and comparison with authentic standards from literature.
     - The compounds were identified as 9',10,10'-tetraone (2).

2. **Antimicrobial Activity:**
   - The antimicrobial activity was tested using agar disc diffusion method [12]. A suspension of the tested microorganisms (0.1 ml of 108 cells per ml) was spread on solid media plates. Aliquots of 15 μg of the alcohol extract (fraction A) dissolved in dimethyl sulfoxide (DMSO, Merck, Germany) were applied on sterile paper discs (6 mm diameter). Ciprofloxacin and nystatin were used as standard antibacterial and antifungal agents, respectively, as positive controls, and DMSO without the extracts was used as a negative control. The discs were deposited on the surface of inoculated agar plates. These plates were held at 4°C for 2 h, followed by incubation at 37°C for 24 h for bacteria, or at 30°C for 48 h for yeasts. The diameters of the inhibitory zones were measured in millimeters. All tests were performed in triplicate.

3. **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 8 weeks from zero time. Thirty male albino rats of the Sprague Dawley strain (130 – 140 g) were injected intraperitoneal with anloxicon (150 mg/kg body weight) to induce diabetes mellitus [14]. Animals were divided into 3 groups; Hyperglycemia was assessed after 72 hours by measuring blood glucose [15] and after 1 and 2 months intervals from treatment. First group: diabetic rats that served as positive control, second group; received 20 mg/kg b. wt. of the reference drug Indomethacin. One hour later after drug administration, the rats were sacrificed; both hind paws excised with care to avoid damage of the palmar artery and veins. The right hind paw and 0.1ml saline in the left hind paw. Four hours after drug administration, all the animals received a subplantar injection of 0.1ml of 1% carrageenan solution in saline in the right hind paw and 0.1ml saline in the left hind paw. Four hours 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{Antihypertensive activity:} \quad \text{X100} \% \text{ Oedema} = \frac{\text{Weight of right paw} - \text{Weight of left paw}}{\text{Weight of left paw}} \times 100
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diabetic rats that received 100 mg/kg b. wt. of the ethanol extract (A), third group; diabetic rats that received 150 mg/kg b. wt. of Metformin as reference standard drug. At the end of each study period, blood samples were collected from the retro orbital venous plexus through the eye canthus of anaesthetized rats after an overnight fast. Serum was isolated by centrifugation and the blood glucose level was measured [15]. Blood glucose level was measured at zero G₀ and after treatment Gₓ. Percentage of change in blood glucose level was calculated from the following equation:

\[
\% \text{ of change} = \left( \frac{G_y - G_0}{G_0} \right) \times 100
\]

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).

Compound 2 (Figure 1B) was obtained as orange crystals soluble in CHCl₃, gave positive tests for anthraquinones. A molecular formula of C₁₆H₁₂O₆ was determined for compound 2 on the basis of molecular ion peak at m/z 300 (M+CO-CH₃). The 1H-NMR spectra showed four hydrogen bonded phenol proton signals appeared at δ 11.92, 11.94, 12.03 and 12.49. It also revealed signals for four aromatic protons resonating with o-coupling at δ 7.21 (d, J = 8.7, H-7'); δ 7.31(d, J = 7.8, H-7); δ 7.51(d, J = 8.7, H-6') and δ 7.88 (d, J = 7.8, H-6) and four singlets resonating with o-coupling at δ 7.21 (d, J = 8.7, H-7'); δ 7.31(d, J = 7.8, H-7); δ 7.51(d, J = 8.7, H-6') and δ 7.88 (d, J = 7.8, H-6) and four singlets.

The assay revealed that oral administration of 100 mg/kg b. wt. alcoholic extract reduced paw edema by 59.7%. The results showed Table 1:

| Table 1: Results of antimicrobial screening of alcohol extract of A. grandidentata. |
| --- |
| Diameter of inhibition zone ± SE (mm) |
| Micro-organisms | Alcohol extract of A. grandidentata(200 mg/ml) | Ciprofloxacin | Nystatin |
| Staphylococcus aureus | R | 22 ± 0.44 | N.D |
| Bacillus subtilis | 9 ± 0.53 | 22 ± 0.95 | N.D |
| Pseudomonas | R | 35 ± 0.78 | N.D |
| aeruginosa | 11 ± 0.74 | 34 ± 0.83 | N.D |
| klebsiella aeruginosa | 9 ± 0.37 | 19 ± 0.52 | N.D |
| Candida albicans | 8 ± 0.27 | N.D | 16 ± 0.62 |

R= no inhibition zone N.D=not done SE standard error
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 [4,5,11,24].

This activity might be due to the presence of anti-inflammatory compounds detected in Aloe vera gel and leaf. African Journal of Biotechnology 4: 1413-1414.

Potency calculated as compared to the standard anti-inflammatory drug Indomethacin

* Significantly different from control group at p <0.01

% of change calculated as regard to the control group. S.E.=standard error

Table 2: Results of acute anti-inflammatory effect of the alcoholic extract of A. grandidentata Salm.– Dyck, in male albino rats (n=6).

| Group                          | Mean ± S.E. | % of change | % of Potency |
|-------------------------------|-------------|-------------|--------------|
| Control (1 ml saline)         | 61.8±1.3    | -           | -            |
| Alcohol extract (100 mg/kg b. wt.) | 24.9±1.1*   | 59.7         | 92.01        |
| Indomethacin (20 mg/kg b. wt.) | 21.7±0.9*   | 64.88       | 100          |

Table 3: Effect of alcoholic extract of A. grandidentata Salm.–Dyckon diabetic male albino rats (n=10).

| Group                        | Mean ± S.E. | % of change | % of Potency |
|------------------------------|-------------|-------------|--------------|
| Zero                         |             |             |              |
| -ve control                  | 261.2±9.8   | -           | -            |
| Alcohol extract (100 mg/kg b. wt.) | 256.9±10.5  | 45.03       | 95.26        |
| Metformin (150 mg/kg b. wt.)  | 258.9±8.4   | 47.27       | 100          |

*P<0.01 vs control group or statistically significant difference from zero time at p <0.01

% of change calculated as regard to the control group. S.E.=standard error

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