

Phytochemical and Biological Investigation of *Aloe Grandidentata* Salm-Dyck

Taghreed A Ibrahim^{1,3*}, Nabaweya M EL Fiki¹, Ibrahim A Shehata^{1,2}, Amany A Sleem⁴ and Mouchira M Shoukry¹

¹Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Egypt

²Pharmacognosy Department, Faculty of Pharmacy, King Abd El Aziz University, Saudi Arabia

³Pharmacognosy Department, Faculty of Pharmacy, King Saud University, Saudi Arabia

⁴Pharmacology Department, National Research Center, Dokki, Giza, Egypt

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'bianthrane -9, 9,10,10-tetraone (2) and 1,6,8-trihydroxy-7-methoxy-3- methyl anthraquinone (3), five known compounds, β -sitosterol (1), emodin (4), chrysophanol (5), physicon (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 physicon 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; Physicon; 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-arthritis activity [4,5] and hypoglycemic effects [6-8]. Several constituents were isolated from different *Aloe* species; sterols, lignin, saponins, anthrones, their dimers, 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, Shimadzu IR-435, PU-9712 infrared spectrophotometer; UV, Shimadzu 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 Dawley 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), 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

***Corresponding author:** Taghreed Abdou Ibrahim Shehata, Associate Professor of Pharmacognosy, College of Pharmacy, King Saud University, Saudi Arabia, Tel: 00966556639972; Fax: 00966112062183; E-mail: tshehata@ksu.edu.sa

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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×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) 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 x3.5 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 physicon(6), 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 9:1) was rechromatographed on LH20 column to afford 15 mg of β-sitosterol-3-O-β-D-glucoside (7).

Compounds 1, 4-7 were detected through comparison of TLC behavior with authentic standard, melting point and mixed melting point. In addition to comparing their spectroscopic data with that reported in literatures. In addition, compounds 2,3 were investigated through their spectroscopic data.

1,1',8,8'-tetrahydroxy-3'-acetyl-3-methyl-5,5' bianthracene-9,9',10,10'-tetraone (compound 2)

MP: 190-192°C.

R_f: 0.47 (n-Hexane-EtOAc, 8:2).

IR (KBr): 3563, 3436, 2923, 2851, 1671, 1624, 1452, 1428,1374,1270,1202 cm⁻¹.

UV/Vis λ_{max} (MeOH) nm: 206,222, 260, 390, 440.

¹H NMR (300 MHz, CDCl₃): 11.92, 11.94, 12.03 and 12.49 (1H, s, H-1', H-1, H-8' and H-8), 7.04, 7.19, 7.52, and 7.63 (1H, s, H-2, H-2', H-4' and H-4), 7.21 (d, J = 8.7 Hz, H-7'); δ 7.31(d, J = 7.8 Hz, H-7); δ 7.51(d, J = 8.7 Hz, H-6') and δ 7.88 (d, J = 7.8 Hz, H-6), 2.42 (3H, s, H-3), 2.07 (3H, s, H-3').

¹³C NMR (75 MHz CDCl₃): 162.87 (C-8'), 162.74 (C-8) 162.03 (C-1), 159.64 (C-1'),149.37 (C-3), 149.15 (C-3'), 137.10(C-4a), 135.95 (C-4'a),134.01(C-6), 133.53 (C-6'), 130.82 (C-5'), 128.77(C-5), 125.83 (C-10a), 125.05 (C-10'a), 124.27 (C-2), 124.10 (C-2'), 121.66 (C-4), 121.32 (C-4'), 120.31 (C-9'a), 119.98 (C-9a), 115.72(C-7), 115.46(C-7'), 114.80 (C-8a) and 113.91(C-8'a).

MS (EI, 70 eV): m/z (%) = 507 [M-CO-CH₃]⁺ (8), 506 [M-CO-CH₃-1]⁺ (29), 489 [M-CO-CH₃-H₂O]⁺ (13), 253[M/2]⁺ (11).

1,6,8-trihydroxy-7-methoxy-3-methyl anthraquinone (compound 3)

MP: 236-238°C.

R_f: 0.8 (CHCl₃-MeOH, 9.5:0.5).

IR (KBr): 3418, 2920, 2838, 1720, 1622, 1516, 1454, 1265, 1201, 1161, 1032 cm⁻¹.

UV/Vis λ_{max} (MeOH) nm: 233, 250, 302, 312, 458.

¹H NMR (300 MHz, CDCl₃): 12.3,12.5 (1H, s, OH at H-1 and H-8), 2.51(3H,s Me-3), 4.45(1H, brs, OH at H-6), 3.80 (3H,s, OCH₃ at H-7), 6.83(1H, s, H-2), 7.72(1H, d, J = 3, H-4), 7.42 (1H, s H-5).

¹³C NMR (75 MHz DMSO-d₆): 22.29 (Me, C-3), 68.17 (OCH₃, C-7) and 159.92 (OH, C-6), 120.01 (C-8a), 121.35 (C-9a), 124.14 (C-4), 125.53 (C-2), 128.78 (C-10a), 130.84 (C-5), 137.14 (C-4a), 149.18 (C-3), 162.06 (C-1), 162.90 (C-8), 180.06 (C-10), 192.88 (C-9).

MS (EI, 70 eV): m/z (%) = 300 [M⁺] (96), 285 [M⁺-CH₃] (38), 271 [M⁺-CHO] (46), 257 [M⁺-CO-CH₃] (100).

Antimicrobial activity: The antimicrobial activity was tested using agar disc diffusion method [12]. A suspension of the tested microorganism (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.

Anti-inflammatory activity: This effect was determined according to the method described by Winter et al. [13]. Eighteen male albino rats, weighing (130-150g) were randomly divided into three groups, each of six animals, first group; received 1ml of saline serving as control, second group; received 100 mg/kg b. wt. of the ethanol extract (A), third group; received 20 mg/kg b. wt. of the reference drug Indomethacin. One hour later after drug administration, all the animals received a sub plantar 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:

$$X100\% \text{ Oedema} = \frac{\text{Weight of right paw} - \text{Weight of left paw}}{\text{Weight of left paw}} \times 100$$

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 alloxan (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 groups;

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 G_0 and after treatment G_t . Percentage of change in blood glucose level was calculated from the following equation:

$$\% \text{ of change} = (G_0 - G_t / G_0) \times 100$$

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 physicon (6) [17,18] were determined by comparison of their physical and spectroscopic data (UV, ^1H NMR, ^{13}C NMR and MS) with those reported in literature. Physicon (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 -9,9',10,10'-tetraone (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, ^{13}C NMR and MS).

Compound 2 (Figure 1B) was obtained as orange crystals soluble in CHCl_3 , gave positive tests for anthraquinones. A molecular formula of $\text{C}_{31}\text{H}_{18}\text{O}_{10}$ was determined for compound 2 on the basis of molecular ion peak at m/z 507 (M- CO- CH_3). 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 at 7.04, 7.19, 7.52 and 7.63 (1H, s, H-2, H-2', H-4' and H-4). A singlet of three protons appeared at δ 2.42 designated for methyl group at position 3' and also another one at δ 2.07 for an acetyl group at position 3'. The location of the biaryl bond followed from the absence of signal arising from H-5 and H-5' [20]. ^{13}C -NMR spectrum revealed 31 signals that could be assigned to individual Carbon atoms on the following basis; the carbonyl carbons C-9, C-9', C-10' and C-10 resonated at δ 192.82, 192.31, 182.29 and 181.84 ppm respectively. The acetyl carbon resonated at δ 167.70 for the carbonyl C [OCO CH_3] and at δ 21.38 for the methyl C [OCO CH_3]. The methyl group was found at δ 22.27 ppm. These data coincided with ^1H -NMR data.

In addition the $\lambda_{\text{max}}^{\text{MeOH}}$ 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'-tetraone.

Compound 3 (Figure 1B) was obtained as orange crystals with m.p. 236- 238°C, soluble in chloroform and gives positive tests for anthraquinone. A molecular formula of $\text{C}_{16}\text{H}_{12}\text{O}_6$ was determined for compound 3 on the basis of the observed molecular ion peak at

m/z 300. The ^1H - NMR spectra of compound 3 displayed a signal for CH_3 appeared at 2.51 (3H, s, CH_3 -3), the hydroxyl group appeared at 4.45 (1H, brs, H-6) this elucidation is comparable to that of emodin but it differs by the presence of a sharp signal appeared at 3.80 (3H,s) denoting the presence of methoxy (OCH_3) group typical for aromatic methoxy group at position 7. Those signals were beside the aromatic proton signal which appeared at 6.83(1H, s,H-2). The meta coupling protons is confirmed by the presence of a signal at 7.72 (1H, d, J = 3, H-4) and a signal at δ 7.42 (1H, s H-5). In addition to a characteristic methoxy group signal at δ 68.17 and methyl at 22.29, the ^{13}C NMR spectra of compound 3 displayed 14 carbon signals. The chemical shifts of these 14 resonance signals suggested the presence of two aromatic rings and two doubly conjugated carbons (δ 180.06, 192.88). Accordingly, the structure of this new compound (3) was determined as 1,6,8-trihydroxy-7-methoxy-3- methylanthraquinone.

The results of antimicrobial activity of the alcoholic extract of *A. grandidentata* (200 mg/ml) are shown in Table 1. It showed mild antibacterial activities against G +ve bacteria, G -ve bacteria and fungi with the strongest effect being detected against *Pseudomonas aeruginosa*. These results were in agreement with observations previously reported for several Aloe species [3]. The antimicrobial activities of the alcoholic extract of *A. grandidentata* might be attributed to its contents of anthraquinones [21] or β -sitosterol [22].

The results of experiments examining the effects of the alcoholic extract of *A. grandidentata* leaf on carrageenan-induced paw edema are presented in Table 2.

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

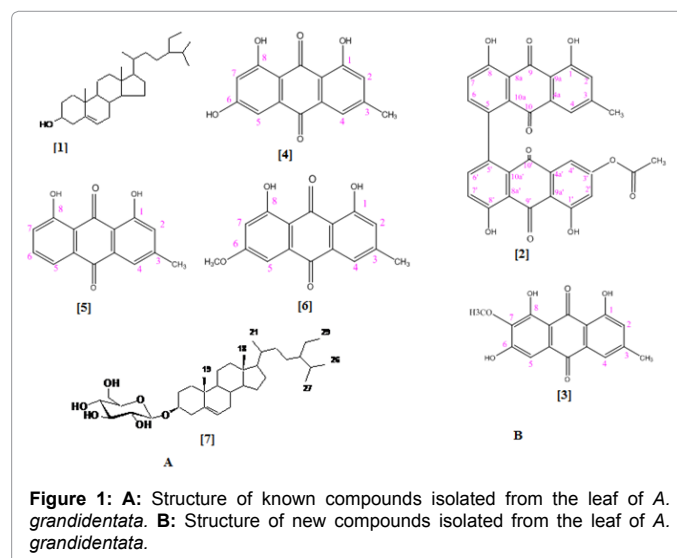


Figure 1: A: Structure of known compounds isolated from the leaf of *A. grandidentata*. B: Structure of new compounds isolated from the leaf of *A. grandidentata*.

Micro-organisms	Diameter of inhibition zone \pm SE (mm)		
	Alcohol extract of <i>A. grandidentata</i> (200 mg/ml)	Ciprofloxacin	Nystatin
<i>Staphylococcus aureus</i>	R	22 \pm 0.44	N.D
<i>Bacillus subtilis</i>	9 \pm 0.53	22 \pm 0.95	N.D
<i>Pseudomonas aeruginosa</i>	R	35 \pm 0.78	N.D
<i>klebsiellapneumonia</i>	11 \pm 0.74	34 \pm 0.83	N.D
<i>Candida albicans</i>	9 \pm 0.37	19 \pm 0.52	N.D
	8 \pm 0.27	N.D	16 \pm 0.62

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

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

Group	% oedema		
	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

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

* Significantly different from control group at p <0.01

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

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

Group	Zero			4 weeks		8 weeks	
	Mean ± S.E	Mean ± S.E	% of change	% of potency	Mean ± S.E	% of change	% of potency
-ve control	261.2 ± 9.8	263.4 ± 10.3	-	-	265.7 ± 11.9	-	-
Alcohol extract (100 mg/kg b. wt.)	256.9 ± 10.5	141.2 ± 6.7*	45.03	95.26	112.4 ± 5.1*	56.24	86.83
Metformin (150 mg/kg b. wt.)	258.9 ± 8.4	136.5 ± 6.1*	47.27	100	91.2 ± 4.6*	64.77	100

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

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

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

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] β-sitosterol [25,26] and β-sitosterol3-O-β-D-glucoside[26] which have been reported to possess anti-inflammatory activities.

The observed data (Table 3) revealed that *A. grandidentata* Salm. – Dyck at dose 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 *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 *A. grandidentata* may be attributed to its content of chrysophanol [27].

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