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Phytochemical Compounds and Antibacterial Inhibition Activity of Gel of *Aloe vera* L. var. chinensis Haw. Berg. of Vietnam

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In present study, eight compounds were isolated from the *Aloe vera* L. var. chinensis Haw. Berg. grown in Vietnam from the methanolic extract. The isolated compounds were elucidated for their structures on the basis of NMR spectroscopic data and found to aloe emodin, emodin, 2-hydroxy-1-methoxy-anthraquinone, 1,6-dihydroxy-2-methylanthraquinone, kaempferol 3-O-α-L-arabinofuranoside, kaempferol 3-O-gentibioside, β-amyrin and ursolic acid. Antibacterial activity of *Aloe vera* gel extract showed strong effects on Gram-positive bacteria than Gram-negative stains, except *Escherichia coli*. At low concentration 50 µg/mL, the crude extract did not showed the inhibition. Four of the five Gram-positive bacteria were inhibited at 100 µg/mL of methanol extract. At 400 µg/mL, the maximum zone of inhibition was observed against *Bacilluss pumilus* (17 mm), *Escherichia coli* (15 mm), *Sarcina lurtea* (13 mm) and *Vibrio cholera* (12 mm).

Keywords: *Aloe vera* L. var. *chinensis* Haw. Berg., Phytochemical compounds, Antibacterial activity.

INTRODUCTION

Aloe vera L. var. *chinensis* Haw. Berg. (syn. *Aloe vera* L. Burm. F.) of the family Xanthorrhoeaceae has traditionally been used for healing in natural medicine under the name Lo hoi in Vietnam [1,2]. The phytochemical investigations of fresh plant extract of *Aloe vera* L. were carried several techniques and it is found that different Aloe species contains fatty acids, monosaccharaides, polysaccharides, sterols, lignin, saponins chromones and anthraquinones [3-8].

The Aloe plants has been reported in using to treat wound-healing [9,10], antibacterial and antimicrobial [11,12], detoxification, flushing out toxins and wastes from body [13,14]. *Aloe vera* gel extract also showed the other biological activities such as reducing blood glucose in diabetic patients, decreasing blood lipid levels [15-17]. Currently in Vietnam, there is not reports on the chemical composition of *A. vera* L. var. *chinensis* Haw. Berg. Therefore, herein, the chemical investigation of the methanolic extracts of *Aloe vera* L. var. *chinensis* Haw. Berg. gel grown in Vietnam and the bioactivity activities of the isolated compounds have also been evaluated.

EXPERIMENTAL

The plant samples were grown and harvested in September 2019 in Nam Dinh province, Vietnam. The plant material was identified by Dr. Trieu Anh Trung, Faculty of Biology, Hanoi National University of Education. The leaves were washed under running water to remove soil and inorganic solid, rinsed with distilled water and left for drying at room temperature. The leaves was cut into small pieces and removed the outer rind, the inner gel sample was used for experiments.

Crude extract preparation: Dried *A. vera* leaves was again dried in the oven at 50 ºC and then soaked in methanol three times, each time for 7 days at room temperature. The combined extracts were concentrated using rotary evaporator (Büchi, Rotavapor R215) to give the crude extracts 452 g.

Separation of extract fractions: The crude extract (80 g) was isolated using silica gel (63-100 mm, Merck) column chromatography, gradient elution with *n*-hexane/acetone 50:1, 30:1, 20:1, 8:1, 4:1, 2:1, 1:1 to give ten fractions.

Fraction 1 (10.39 g) was separated with *n*-hexane/acetone 9:1 to provide five fractions (1.1 and 1.5). Fraction 1.1 (3.90

g) was separated by column chromatography on silica gel with a gradient of *n*-hexane/ethyl acetate 19:1, 15:1, 10:1, 5:1, 1:1 and Sephadex LH-20 with dichloromethane/ MeOH 10:1 affording **AVG 1** (11 mg) and **AVG 2** (7 mg). Fraction 1.2 (2.12 g) was further purified with silica gel with gradient system of *n*-hexane/MeOH to give six fractions (1.2.1 to 1.2.6). Fraction 1.2.2 (410 mg) and 1.2.3 (652 mg) were washed with *n*-hexane/ethyl acetate 8:2 to give purified compounds **AVG 3** (11 mg) and **AVG 4** (15 mg).

Fraction 3 (4.145 g) was eluted with $CHCl_3/CH_3OH/H_2O$ 15:7:1 using silica gel column chromatography to give 4 fractions (3.1 to 3.4). RP 18 CC with acetonitrile/CH_3OH/ H_2O 2:2:1 and MeOH/H_2O 2:1 were a reversed phase to separate fraction 3.2 (150 mg) and fraction 3.4 (100 mg) to afford **AVG 5** (11 mg) and **AVG 6** (15 mg).

Fraction 5 (8.137 g) was subjected to silica gel column eluted with *n*-hexane/EtOAc 9:1, 4:1, 2:1, 1:1 to receive 4 fractions (5.1 to 5.4). Fraction 5.1 (0.8 g) was applied to silica gel mini column chromatography and washed with *n*-hexane to give **AVG 7** (9 mg). Fraction 5.2 (1.2 g) was further purified by silica gel column chromatography with CH_2Cl_2/acetone/ H_2O 1:1.5:0.05 and 2) silica gel mini column chromatography CH_2Cl_2/ methanol/ H_2O 17:1:0.03 to give **AVG 8** (5 mg).

The isolated compounds were characterized by ^1H & ^{13}C NMR analysis and compared with reported literature data.

Aloe emodin (AVG 1): Orange needles, ^1H NMR (DMSO-d_6, 500 MHz) δ ppm: 7.82 (1H, t, *J* = 7.5 Hz, H-6), 7.74 (1H, dd, *J* = 1.0, 7.5 Hz, H-5), 7.72 (1H, d, *J* = 1.5 Hz, H-4), 7.40 (1H, dd, *J* = 1.0, 8.0 Hz, H-7), 7.31 (1H, d, *J* = 1.0 Hz, H-2), 11.95 (2H, s), 4.64 (2H, d, *J* = 6.0 Hz, H-11) and 5.58 (1H, t, *J* = 6.0 Hz, 11-OH). ^{13}C NMR (DMSO-d_6, 125 MHz) δ ppm: 161.6 (C-1), 120.7 (C-2), 153.7 (C-3), 117.1 (C-4), 133.3 (C-4a), 119.3 (C-5), 137.3 (C-6), 124.4 (C-7), 161.3 (C-8), 115.9 (C-8a), 191.6 (C-9), 114.5 (C-9a), 181.5 (C-10), 133.1 (C-10a), 62.0 (C-11).

Emodin (AVG 2): Orange needles, ^1H NMR (DMSO-d_6, 500 MHz) δ ppm: 7.51 (1H, d, *J* = 1.5 Hz, H-2), 7.18 (1H, t, *J* = 1.5 Hz, H-4), 7.13 (1H, d, *J* = 2.5 Hz, H-5), 6.6 (1H, d, *J* = 2.0 Hz, H-7), 2.42 (3H, s, H-11), 12.02 (1H, s, 1-OH), 12.09 (1H, s, 8-OH). ^{13}C NMR (DMSO-d_6, 125 MHz) δ ppm: 161.4 (C-1), 124.1 (C-2), 148.2 (C-3), 120.4 (C-4), 135.1 (C-4a), 108.8 (C-5), 164.4 (C-6), 107.9 (C-7), 165.6 (C-8), 113.4 (C-8a), 189.6 (C-9), 108.9 (C-9a), 181.4 (C-10), 132.8 (C-10a).

2-Hydroxy-1-methoxyanthraquinone (AVG 3): Orange powder, ^1H NMR ($CDCl_3$, 500 MHz) δ ppm: 7.36 (1H, d, *J* = 9.0 Hz, H-3), 8.14 (1H, d, *J* = 9.0 Hz, H-4), 8.27 (2H, m, H-5, 8), 7.74 (2H, m, H-6, 7), 4.04 (s, OMe), 6.69 (s, OH). ^{13}C NMR ($CDCl_3$, 125 MHz) δ ppm: 146.6 (C-1), 155.6 (C-2), 120.3 (C-3), 125.8 (C-4), 127.6 (C-4a), 127.1 (C-5), 133.9 (C-6,7), 126.9 (C-8), 134.5 (C-8a), 182.7 (C-9), 125.7 (C-9a), 182.1 (C-10), 133.0 (C-10a), 62.3 (C-11).

1,6-Dihydroxy-2-methylanthraquinone (AVG 4): Orange powder, ^1H NMR ($CDCl_3$, 500 MHz) δ ppm: 7.55 (1H, d, *J* = 7.5 Hz, H-3), 7.61 (1H, d, *J* = 7.5 Hz, H-4), 7.44 (1H, d, *J* = 2.5 Hz, H-5), 7.21 (1H, dd, *J* = 2.5, 8.5 Hz, H-7), 8.08 (1H, d, *J* = 8.5 Hz, H-8), 2.27 (3H, s, 2-Me). ^{13}C NMR ($CDCl_3$, 125 MHz) δ ppm: 156.7 (C-1), 141.2 (C-2), 128.0 (C-3), 125.8 (C-4), 111.7 (C-4a), 112.6 (C-5), 163.6 (C-6), 121.9 (C-7), 130.1 (C-8), 125.9 (C-8a), 186.7 (C-9), 111.9 (C-9a), 187.0 (C-10), 136.1 (C-10a).

Kaempferol 3-*O*-α-L-arabinofuranoside (AVG 5): Yellow powder, $[\alpha_D^{25}]$ = -112.8º, ^1H NMR (DMSO-d_6, 500 MHz) δ ppm: 8.02 (2H, d, *J* = 9.0 Hz, H-2′,6′), 6.90 (2H, d, *J* = 9.0 Hz, H-3′,5′), 6.45 (1H, d, *J* = 2.0 Hz, H-8), 6.21 (1H, d, *J* = 2.0 Hz, H-6), 5.63 (1H, s, H-1″), 4.15 (1H, brs, H-2″), 3.73 (1H, brs, H-3″), 3.55 (1H, q, *J* = 5.5 Hz, H-4″), 3.28 (2H, m, 2H-5″), 12.61 (1H, s, 5-OH). ^{13}C NMR (DMSO-d_6, 125 MHz) δ ppm: 156.3 (C-2), 133.4 (C-3), 177.6 (C-4), 161.2 (C-5), 98.6 (C-6), 164.2 (C-7), 93.6 (C-8), 156.7 (C-9), 104.0 (C-10), 120.7 (C-1′), 130.7 (C-2′,6′), 115.3 (C-3′,5′), 159.9 (C-4′), 108.0 (C-1″), 82.1 (C-2″), 77.1 (C-3″), 86.3 (C-4″), 60.8 (C-5″).

Kaempferol 3-*O*-gentibioside (AVG 6): Yellow powder, ^1H NMR (DMSO-d_6, 500 MHz) δ ppm: 6.18 (1H, d, *J* = 2.0 Hz, H-6), 6.40 (1H, d, *J* = 2.0 Hz, H-8), 8.02 (2H, d, *J* = 7.0 Hz, H-2′,6′), 6.88 (2H, d, *J* = 7.0 Hz, H-3′,5′), 5.36 (1H, d, *J* = 7.5 Hz, H-1″), 2.83 (1H, m, H-2″), 2.99 (1H, m, H-3″), 2.96 (1H, m, H-4″), 2.85 (1H, m, H-5″), 3.60, 3.86 (2H, m, d, *J* = 10 Hz, 2H-6″), 4.04 (1H, d, *J* = 8.0 Hz, H-1‴), 2.84 (1H, m, H-2‴), 2.94 (1H, t, *J* = 8.0 Hz, H-3‴), 3.00 (1H, t, *J* = 8.0 Hz, H-4‴), 2.84 (1H, m, H-5‴), 3.36, 3.51 (2H, m, 2H-6‴), 12.61 (1H, s, 5-OH). ^{13}C NMR (DMSO-d_6, 125 MHz) δ ppm: 156.5 (C-2), 133.3 (C-3), 177.3 (C-4), 161.2 (C-5), 98.8 (C-6), 164.6 (C-7), 93.8 (C-8), 152.2 (C-9), 103.9 (C-10), 120.9 (C-1′), 130.9 (C-2′, 6′), 115.1 (C-3′), 159.9 (C-4′), 115.3 (C-5′), 130.9 (C-6′), 101.1 (C-1″), 74.1 (C-2″), 76.3 (C-3″), 69.8 (C-4″), 76.5 (C-5″). 68.0 (C-6″), 103.1 (C-1‴), 73.4 (C-2‴), 76.4 (C-3‴), 69.7 (C-4‴), 76.5 (C-5‴), 60.8 (C-6‴).

α-Amyrin (AVG 7): White powder, ^1H NMR ($CDCl_3$, 500 MHz) δ ppm: 5.18 (1H, t, *J* = 3.5 Hz, H-12), 0.99 (3H, s, H-23), 0.79 (3H, s, H-24), 0.93 (3H, s, H-25), 0.97 (3H, s, H-26), 1.13 (3H, s, H-27), 0.83 (3H, s, H-28), 0.87 (3H, s, H-29), 0.87 (3H, s, H-30), 3.21 (1H, dd, *J* = 11.0 Hz, 4.0 Hz, H-3), 1.93 and 1.01 (2H, m, 2H-1), 1.60 (2H, m, 2H-2), 0.73 (1H, m, H-5), 1.42 (2H, m, 2H-6), 1.12 (2H, m, 2H-7), 1.55 (1H, m, H-9), 1.87 (2H, m, 2H-11). ^{13}C NMR ($CDCl_3$, 125 MHz) δ ppm: 38.6 (C-1), 27.0 (C-2), 79.1 (C-3), 38.8 (C-4), 52.2 (C-5), 18.4 (C-6), 32.7 (C-7), 39.8 (C-8), 47.8 (C-9), 37.0 (C-10), 23.6 (C-11), 121.8 (C-12), 145.2 (C-13), 41.8 (C-14), 26.2 (C-15), 27.3 (C-16), 32.5 (C-17), 47.3 (C-18), 46.9 (C-19), 31.1 (C-20), 34.8 (C-21), 37.2 (C-22), 28.1 (C-23), 15.5 (C-24), 15.6 (C-25), 16.8 (C-26), 26.0 (C-27), 28.4 (C-28), 33.3 (C-29), 23.7 (C-30).

Ursolic acid (AVG 8): White crystalline solid, ^1H NMR (CD_3OD, 500 MHz) δ ppm: 0.98 (3H, s, H-23), 0.78 (3H, s, H-24), 0.92 (3H, s, H-25), 0.82 (3H, s, H-26), 1.09 (3H, s, H-27), 0.86 (3H, d, *J* = 6.5 Hz, H-29), 0.94 (3H, d, *J* = 6.5 Hz, H-30), 5.24 (1H, brs, H-12), 3.12 (1H, dd, *J* = 6.5 Hz, 9.5 Hz, H-3). ^{13}C NMR (CD_3OD, 125 MHz) δ ppm: 38.8 (C-1), 27.1 (C-2), 79.0 (C-3), 38.7 (C-4), 55.3 (C-5), 18.4 (C-6), 33.2 (C-7), 39.6 (C-8), 47.7 (C-9), 37.0 (C-10), 23.4 (C-11), 125.5 (C-12), 138.4 (C-13), 42.2 (C-14), 28.2 (C-15), 24.4 (C-16), 48.0 (C-17), 53.0 (C-18), 39.2 (C-19), 39.0 (C-20), 30.8 (C-21), 37.0 (C-22), 27.8 (C-23), 15.7 (C-24), 15.0 (C-25), 17.0 (C-26), 23.6 (C-27), 181.0 (C-28), 17.1 (C-29), 21.2 (C-30).

Antimicrobial bioassay: In this study, *Bacillus pumilus, Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Salmonella paratyphi*, *Sarcina lurtea*, *Shigella* spp., *Staphylococcus aureus* and *Vibrio cholera* were used for antimicrobial examination. The bacterial strains were cultured into nutrient agar medium ($1 \times 10^6 - 1 \times 10^8$ CFU/mL) for 24 h at 37 ºC. Antibacterial activity of *A. vera* extract was investigated by using the disc-diffusion agar method [18]. The concentrations of extract were prepared with range of 50- 400 mg/mL in MeOH. Sterile paper discs with 6 mm diameter were soaked in the isolated compounds solutions and put into the sterile petri plates containing agar and bacteria. The plates were incubated at 37 ºC for 24 h. Methanol was used for negative control and the antibacterial activity by the *Aloe vera* gel extract was assessed by measuring the zone inhibition. The assay was repeated twice and recorded the results.

RESULTS AND DISCUSSION

This study aimed to isolate and identify the chemical constitutents present in *Aloe vera* L. *var. chinensis* Haw. Berg. grown in Vietnam. Using the column chromatography method, eight compounds were separated from the methanolic extract of *Aloe vera* (**AVG 1**-**8**). The structure of isolated compounds were elucidated by using spectroscopic analysis and compared their respective spectral data with reported literature values: aloe emodin (**AVG 1**), emodin (**AVG 2**), 2-hydroxy-1-methoxy-anthraquinone (**AVG 3**), 1,6-dihydroxy-2-methyl-anthraquinone (**AVG 4**), kaempferol 3-*O*-α-L-arabinofuranoside (**AVG 5**), kaempferol 3-*O*-gentibioside (**AVG 6**), α-amyrin (**AVG 7**) and ursolic acid (**AVG 8**) [19-24]. The extract components analyzed in Vietnamese *Aloe* and the other *Aloe* species are presented in Table-1.

Kaempferol 3-*O*-α-L-arabinofuranoside (**AVG 5**) and kaempferol 3-*O*-gentibioside (**AVG 6**) exhibited an antioxidant activity *in vitro* and *in vivo*. A double bond at C2-C3, an oxo group at C4 and hydroxyl groups at C3, C5 and C4′ structural maybe related to the antioxidant activity. Compound **AVG 5** was expressed to inhibitory activity against the inflammation response, cancer growth, acute lung injury [24-26]. Aloe emodin (**AVG 1**) and emodin (**AVG 2**) were most commonly found in *A. vera* using UPLC-MS analysis and contained about 26.29% and 65.30%, respectively [27].

2-Hydroxy-1-methoxyanthraquinone (**AVG 3**) and 1,6-dihydroxy-2-methylanthraquinone (**AVG 4**) were also extracted from the local Vietnamese *Aloe vera* L. *var. chinensis* Haw. Berg, are found in wide range of species, also in fungi and some animals, but have not been reported from the other local *Aloe* species so that these two constituents could be useful for distinguishing *Aloe vera* L. *var. chinensis* Haw. Berg. in Vietnam with the other *Aloe* species. In addition, anthraquinones exhibit remarkable bioactive properties like anticancer, antitumor, antiarhritic, antidiabetic, antibacterial [8,23,27], which were possibly related to *Aloe vera* L. capacity in disease treatment, showed Vietnamese folk medicinial values.

Antioxidant activity: *in vitro* antibacterial activity of *Aloe vera* gel extracts collected from Vietnam were evaluated based on sizes of inhibition zones. The methanolic extracts did not show any antibacterial activity at 50 mg/mL, but all other its three concentrations exhibited various degree of inhibitory effects preventing growth of the selected bacterial pathogens (Table-2).

TABLE-1
COMPONENTS IN THIS STUDY AND THE OTHER *Aloe* SPECIES

Compound	Source	Ref.
Aloe emodin	*A. barbadensis, A. excels, A. ferox*	[19,30,31]
Emodin	*A. barbadensis,*	[27,32,33]
2-Hydroxy-1-methoxy-anthraquinone	–	–
1,6-Dihydroxy-2-methyl-anthraquinone	–	–
Kaempferol 3-O-α-L-arabinofuranoside	*A. arborescens, A. grandidentata*	[30,34]
Kaempferol 3-O-gentibioside	*A. arborescens, A. perfoliata*	[30,34]
β-Amyrin	*A. barbadensis*	[35]
Ursolic acid	*A. barbadensis*	[8,30]

TABLE-2
in vitro ANTIBACTERIAL ACTIVITY OF DIFFERENT CONCENTRATIONS OF METHANOL *Aloe vera* GEL EXTRACT

Bacterial strain	Type of bacterial strain	Diameter of inhibition zone (mm)			
		Conc. of *Aloe vera* gel extract (µg/mL)			
		50	100	200	400
Bacillus pumilus	Gram-positive	6	8	10	17
Bacillus subtilis	Gram-positive	6	7	9	10
Escherichia coli	Gram-negative	6	8	13	15
Pseudomonas aeruginosa	Gram-negative	6	6	6	6
Salmonella typhi	Gram-negative	6	6	6	6
Salmonella paratyphi	Gram-negative	6	6	6	6
Sarcina lurtea	Gram-positive	6	8	10	13
Shigella spp.	Gram-negative	6	6	7	7
Staphylococcus aureus	Gram-positive	6	6	6	6
Vibrio cholera	Gram-positive	6	8	9	12

Present study showed that the methanolic extract of *Aloe vera* display strong activity on Gram-positive bacteria than Gram negative strains, except *E. coli*. This result has confirmed several previous research [28]. Dimethyl sulfoxide (DMSO) crude extracts of *Aloe vera* showed its antibacterial and antifungal activity against all the tested bacteria and fungi. For the DMSO crude extract, the maximum diameter of inhibition zone is 13 mm for *E. coli*, 12 mm for *P. vulgaris* and 10 mm for *B. subtilis*, 11 mm for *C. albicians* and 9 mm for *Penicillium* sp.; however, Johnson *et al*. [28] showed a significant inhibition effect of *Aloe vera* gel on *S. aureus* (10.5 mm) in comparison to present study (6 mm for all tested concentration).

Antivirus activity: *Aloe vera* L. var. *chinensis* Haw. Berg. extract also confirmed its antivirus activity. The extract inhibited Herpes simplex virus type 1 (HSV-1) growth in vero cell line at 0.2-5% concentration without any observed toxicity. Higher concentrations of *Aloe vera* gel (1, 2 and 5%) had significantly more antiviral activity than lower concentrations (0.2% and 0.5%) [29].

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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