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Antiviral Activity of *Aloe hijazensis* against Some Haemagglutinating Viruses Infection and Its Phytoconstituents

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Evaluation of the antiviral activities of flowers, flower-peduncles, leaves, and roots of *Aloe hijazensis* against haemagglutinating viruses of avian paramyxovirus type-1 (APMV-1), avian influenza virus type A (AI-H5N1), Newcastle disease virus (NDV), and egg-drop syndrome virus (EDSV) in specific pathogen free (SPF) chicken embryos were carried out. Extract of the flowers and leaves showed relatively higher activity than the extracts of other plant parts. Thirteen compounds were isolated from both the flowers and flower-peduncles of *A. hijazensis*. The isolated compounds were classified into: five anthraquinones; ziganein, ziganein-5-methyl ether, aloesaponarin I, chrysophanol, aloe-emodin, one dihydroisocoumarin; feralolide, four flavonoids; homoplantaginin, isoorientin, luteolin 7-glucuronopyranoside, isovitexin, one phenolic acid; *p*-coumaric acid, the anthrone; barbaloin together with aloenin. Eleven compounds were attributed to the flowers and seven to the flower-peduncles. Homoplantaginin and luteolin 7-glucuronopyranoside are reported here for the first time from *Aloe* spp. To the best of our knowledge, this is the first report on the chemical composition and biological activity of those plant parts.

Key words: *Aloe hijazensis*, Haemagglutinating viruses, Phytochemical constituents

INTRODUCTION

Medicinal plants have been widely used to treat a variety of infectious and non-infectious ailments. According to one estimate, 25% of commonly used medicines contain compounds isolated from plants (Perera and Efferth, 2012). Several plants could offer a rich reserve for drug discovery of infectious diseases. Viruses have been resistant to therapy or prophylaxis longer than any other form of life. Currently, there are only a few drugs available for the cure of viral diseases, including acyclovir, which is modeled on a natural product parent. In order to combat viruses which have devastating effects on different living organisms, many research efforts have been devoted to the discovery of new antiviral natural products (Mbanga et al., 2010; Perera and Efferth, 2012). Screening studies have been carried out in order to find antiviral agents from natural sources, and the occurrence of antiviral activity in extracts of plants is frequent. It should be noted that a variety of medicinal plants have shown promise to treat a number of viral infections, and some of them possess broad-spectrum antiviral activity (Dao et al., 2011; Perera and Efferth, 2012).

One of the most common poultry disease problems in Saudi Arabia and Egypt is avian paramyxovirus (APMV) infection (Hines and Miller, 2012). Infection with APMV constitutes the most serious epizootic poultry disease throughout the world. Its infection occurs every year and frequently leaves no survivors in unvaccinated flocks, causing devastating epidemics and serious economic losses. Therefore, the disease is called 'the bomb' because it causes a huge number of victims in poultry flocks, as a bombshell would do (Hines and Miller, 2012). In addition, during the past ten years, a series of avian epidemics, a significant reemergence

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of highly pathogenic avian influenza (AI-H5N1), has been reported in several Asian, European and African countries including Egypt and Saudi Arabia (Dao et al., 2011). In attempts to control the disease, millions of birds have been destroyed. Anti-influenza agents from some plants, discovered as a result of chemical and pharmacological studies, were reported (Dao et al., 2011).

Newcastle disease virus (NDV) is an epizootic poultry disease and is a major cause of economic losses in the poultry market (Lee, 2009). Human infections with NDV have usually resulted from direct contact with the virus (Lee, 2009). The most frequently reported and best substantiated clinical signs in human infections have been eye infections.

Egg drop syndrome virus (EDSV), a haemagglutinating adenovirus, has become a major cause of lost egg production in breeder and layer flocks and severe economic losses throughout the world (Lee, 2009).

Antiviral agents of plant origin can have easy acceptability because they are both non-toxic and inexpensive (Perera and Efferth, 2012). In Kenya, Gambia, and Zimbabwe, the crushed leaves of *Aloe* species are given to infected chickens in drinking water as viral diseases remedy (Mbanga et al., 2010). The efficacy of the crude extract of *Aloe secundiflora* in chickens experimentally infected with Newcastle disease virus was reported (Waihenya et al., 2002). An *in vitro* study (Fahim and Wang, 1996) using zinc acetate and lyophilized *A. barbadensis* (7.5 and 10 percent) demonstrated an antiviral effect. The study concluded that it might be useful as a contraceptive, especially significant in preventing the transmission of human immunodeficiency virus (HIV). *Aloe vera* has even been approved in clinical trials as nutritional support for HIV patients (Singh et al., 2010). It apparently hinders the virus's ability to reproduce. *A. vera* showed antiviral activity against the herpes simplex virus (Zandi et al., 2007), and its gel and extract (0.5%) in hydrophilic cream were applied for the management of genital herpes in males (Syed et al., 1996). A pharmaceutical formula containing *A. vera* was used in treatment of cervical high risk human papillomavirus (HRHPV) infection (Iljazovic et al., 2006). Antiviral activity was shown in an *in vitro* study using a purified extract of *A. barbadensis* (Singh et al., 2010). The main activity against cytomegalovirus was shown 12-36 h after infection.

In our ongoing search for antiviral products from plant sources (Helmy et al., 2007; Abd-Alla et al., 2009a) by investigating the bioactive compounds of *Aloe hijazensis* (Abd-Alla et al., 2009b), one of 24 species of *Aloe* in Saudi Arabia (Colenette, 1986), the flowers and flower-peduncles were selected for the present study.

The present study was designed to evaluate the antiviral activities of different *A. hijazensis* parts against different haemagglutinating viruses in specific pathogen free (SPF) chicken embryos. On reviewing the appropriate literature, it was apparent that there are no previous reports about phytochemical or biological investigations of flowers and flower-peduncles of *A. hijazensis*. Therefore, it was considered to be of interest to carry out the present study.

MATERIALS AND METHODS

General

The NMR spectra were recorded at 300 and 500 (^{1}H) and 75 and 125 (^{13}C) MHz on Varian Mercury 300 (Varian) and JEOL GX-500 (JEOL) NMR spectrometers, respectively; δ values are reported as ppm relative to TMS in the convenient solvent. EI-MS spectra were recorded on a Finnigan MAT 95 spectrometer (70 eV) (Finnigan MAT) with perfluorkerosine as the reference substance for EIHRMS. High-resolution mass spectra (HRMS) were recorded by ESI MS on an Apex IV 7 Tesla (Bruker Daltonics). UV-VIS spectra were recorded on a Perkin-Elmer UV/VIS spectrometer (Perkin-Elmer). For column chromatography, Sephadex LH-20 (Pharmacia), microcrystalline cellulose and RP-8 (Merck) and polyamide S (Fluka) were used. For paper chromatography, Whatman No. 1 paper sheets (Whatman Ltd.) were used. Pre-coated silica gel 60 F_{254} plates for thin layer chromatography (TLC) and silica gel 60 for column chromatography (E. Merc) were used. The pure compounds were visualized by spraying with KOH (10% in ethanol); Naturstoff (NA/PE): (a) 1% diphenyl boryloxyethanolamine in ethanol, (b) 5% polyethylene glycol 400 in methanol; $AlCl_3$ (1% in ethanol); *p*-anisaldehyde-sulphuric acid (1% anisaldehyde in glacial acetic acid + 2% concentrated sulphuric acid). Solvent systems S_1 [*n*-BuOH-HOAc-H_2O (4:1:5, v/v/v, top layer)], S_2 (15% aqueous HOAc) were used for analytical grade.

Plant materials

The flowers and flower-peduncles of *A. hijazensis* were collected from Abha Jabal Sawdah in July 2008 and were identified by Dr. Farag Abd-Allah Elghamdi, Department of Botany, King Abdul-Aziz University, Jeddah, Saudi Arabia. Voucher specimens (no. N012411) were deposited in the herbarium of King Abdul-Aziz University.

Viruses

Avian paramyxovirus type-1 virus (APMV-1) of 10^3/

mL was obtained from commercial vaccines. Highly pathogenic avian influenza AI (A/chicken/CLEVB 2009, H5N1) at titer of 10^6 EID_{50}/mL (embryo infective dose), previously isolated and identified in our Central Laboratory for Evaluation of Veterinary Biologics (CLEVB), was used. Egg drop syndrome virus (EDSV) of 10^3/mL was obtained from the Viral Strain Bank of CLEVB, Quality Control of Specific Pathogen Free (SPF) Department, Cairo, Egypt. Newcastle disease virus (NDV), strain Lasota of 10^8 AID_{50}/mL, was obtained from the Newcastle Disease Virus Department of Veterinary Serum and Vaccine Research Institute.

Embryonated specific pathogen free (SPF) eggs

One day old SPF embryonated chicken eggs (ECE_s) were obtained from Nile SPF eggs, Koom Oshiem, Fayoum, Egypt. The eggs were incubated in an isolated incubator at the Quality Control of SPF Department, CLEVB, Cairo, Egypt.

Chicken erythrocytes

Freshly collected chicken erythrocytes (1% and 10%) prepared in saline solution after several washes were used in the haemagglutination assay (HA).

Antiviral activity

Evaluation of *A. hijazensis* as inhibitory agents against virus replication in SPF chicken embryos was carried out in groups of 9-to-11-day old SPF ECE_s inoculated with 5000 embryo infective dose fifty (EID_{50})/ 0.2 mL/egg of APMV-1; immediately followed different concentration (4-800 μg/ 0.2 mL/egg) of each part of *A. hijazensis* extract, separately. The previously mentioned method was done with H5N1, NDV, and EDSV, separately. Virus infectivity control for each virus and ethanolic extract of each plant part embryonic toxicity controls were conducted, separately. The eggs were inoculated via the chorioallantoic cavity. Tested eggs were incubated for 3-4 d at 37ºC and 80% humidity. Also, the therapeutic indexes (TI) of extracts were expressed as toxic concentration fifty/inhibitory concentration fifty (CC_{50}/IC_{50}). The CC_{50} were calculated according to Reed and Muench (1938). APMV-1, H5N1, NDV, and EDSV infectivity (EID_{50}) in embryonating chicken embryos was detected by haemagglutinating activity (HA) of the allantoic fluids of the inoculated eggs as measured by micro technique of the HA test (Takatsy, 1955).

Extraction and isolation

The air-dried powdered flowers (1.9 kg) and flower-peduncles (2.3 kg) were individually extracted with 80% aqueous methanol at room temperature. The methanol was evaporated *in vacuo* at 50ºC to dryness. The residue (68.5 and 72.2 g, respectively) was separately defatted with petroleum ether (60-80ºC). The defatted residue obtained (55.4 and 65.8 g) was separately suspended in water, and ether, ethyl acetate and *n*-butanol successively extracted from it and were saved for isolation and identification of major constituents.

Flowers of *A. hijazensis*

The ether soluble fraction (7.5 g) was applied to silica gel (Si) column chromatography (CC). Gradient elution was performed using petroleum ether-EtOAc (100:0-0:100) and concentrated to give three fractions. The first fraction gave ziganein (11 mg, **1**) after application to Si CC and elution with petroleum ether-EtOAc. The second fraction was applied to Si CC and elution with petroleum ether-benzene mixtures afforded chrysophanol (20 mg, **3**) and aloesaponarin I (13 mg, **5**). From the third fraction, aloe-emodin (36 mg, **4**) was obtained after applying on Si CC and elution by *n*-hexane-EtOAc, followed by Sephadex LH-20 (MeOH). The ethyl acetate soluble fraction (9.2 g) was applied to polyamide CC using a step gradient of H_2O-MeOH (100:0-0:100) to give the main two fractions. The first fraction was applied to Sephadex LH-20 (EtOH) and it gave rise to two sub fractions, (i) and (ii). Sub fraction (i) was applied to Sephadex LH-20 (MeOH-H_2O, 9:1, v/v) twice to afford *p*-coumaric acid (9 mg, **6**). Sub fraction (ii) was chromatographed over Sephadex LH-20 (MeOH) and followed by a RP-8 column (MeOH-H_2O, 8:2, v/v) to afford feralolide (12 mg, **7**). The second fraction was applied on a Sephadex LH-20 using *n*-butanol-*iso*-propanol-water (BIW, 4:1:5, v/v/v, top layer) as an eluent and finally purified on Sephadex LH-20 (EtOH), affording homoplantaginin (16 mg, **8**) and isoorientin (23 mg, **9**). The dried *n*-butanol soluble fraction (8.5 g) was chromatographic over Si and eluted with CH_2Cl_2-MeOH (7:3, v/v) to give two fractions. The first fraction was applied to the cellulose column (*n*-butanol saturated with water) to give luteolin 7-glucuronopyranoside (17 mg, **10**) as well as aloenin (22 mg, **12**) after it was purified with Sephadex LH-20 (MeOH). Barbaloin (38 mg, **13**) was obtained after applying the second fraction to the RP-8 column (MeOH-H_2O, 7:3, v/v), followed by Sephadex LH-20 (MeOH).

Flower-peduncles of *A. hijazensis*

The ether soluble fraction (5.5 g) was applied by the same method as described before with flowers to afford three fractions. Ziganein (12 mg, **1**) was obtained from the first fraction. The second fraction was re-chromatographed by Si CC using a petroleum ether-EtOAc gradient to give ziganein 5-methyl ether (11 mg, **2**).

By the same method, the third fraction delivered chrysophanol (13 mg, **3**) along with aloe-emodin (16 mg, **4**) after it was chromatographed on Sephadex LH-20 (MeOH). The ethyl acetate soluble fraction (11.2 g) afforded two fractions. The first was subjected to Sephadex LH-20 (BIW, 4:1:5, v/v/v, top layer) to give two compounds; isoorientin (14 mg, **9**) and isovitexin (19 mg, **11**) while the second fraction was chromatographed (EtOAc-MeOH-H_2O, 100:16.5:13.5, v/v/v) using Si CC to obtain barbaloin (10 mg, **13**).

Isolated compounds

Homoplantaginin (6-methoxy apigenin-7-*O*-β-D-glucopyranoside) (8)

R_f values: 0.48 (S_1), 0.36 (S_2); UV (deep purple), UV/NH_3 (yellow), $FeCl_3$ (green), NA/PE (bright greenish yellow); UV spectral data: λ_{max}, nm, MeOH: 218, 276, 334; (+NaOMe): 235, 268, 390; (+NaOAc): 229, 272, 330, 398 (sh); (NaOAc+H_3BO_3): 229, 272, 330; (+$AlCl_3$): 228 (sh), 280, 300, 360; (+$AlCl_3$-HCl): 228 (sh), 280, 300, 360. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 7.99 (2H, d, J = 8.4 Hz, H-2′/6′), 7.02 (1 H, s, H-3), 7.05 (2H, d, J = 8.4 Hz, H-3′/5′), 6.84 (1 H, s, H-8), 5.11 (1H, d, J = 7 Hz, H-1″), 3.83 (3 H, s, OCH_3), 3.8-3.3 (m, remaining sugar protons). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 182.8 (C-4), 165.0 (C-2), 162.4 (C-4′), 157.2 (C-7/9), 152.8 (C-5), 133.3 (C-6), 129.2 (C-2′/6′), 121.6 (C-1′), 116.8 (C-3′/5′), 106.3 (C-10), 103.3 (C-3), 101.0 (C-1″), 95.2 (C-8), 77.9 (C-5″), 77.4 (C-3″), 73.9 (C-2″), 70.4 (C-4″), 61.7 (C-6″), 61.1 (OCH_3).

Luteolin 6-*C*-β-D-glucopyranoside (isoorientin) (9)

R_f values: 0.41 (S_1), 0.25 (S_2), UV (deep purple), UV/NH_3 (yellow), $FeCl_3$ (green), NA/PE (orange); UV spectral data: λ_{max}, nm, MeOH: 273, 347; (+NaOMe): 277, 336 (sh), 408; (+NaOAc): 278, 368; (NaOAc+H_3BO_3): 273, 360; (+$AlCl_3$): 271, 302 (sh), 359 (sh), 418; (+$AlCl_3$/HCl): 272, 300 (sh), 354, 389; HRESI-MS, 447.09544 (M^+); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 13.57 (1 H, s, 5-OH), 7.42 (1 H, dd, J = 8.2, 1.9 Hz, H-6′), 7.36 (1 H, d, J = 1.9 Hz, H-2′), 6.84 (1 H, d, J = 8.4 Hz, H-5′), 6.60 (1 H, s, H-3), 6.44 (1 H, s, H-8), 4.54 (1 H, d, J = 9.2 Hz, H-1″), 4.02 (t like , J = 9 Hz, H-2″), 3.90-3.10 (5H, m, H-3″, 4″, 5″, 6″); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 182.2 (C-4), 163.8 (C-2), 163.4 (C-7), 161.2 (C-5), 156.8 (C-9), 150.7 (C-4′), 146.4 (C-3′), 121.6 (C-1′), 119.4 (C-6′), 116.6 (C-5′), 113.6 (C-2′), 109.5 (C-6), 103.4 (C-10), 103.0 (C-3), 94.2 (C-8), 82.1 (C-5″), 79.5 (C-3″), 73.6 (C-1″), 71.1 (C-2″), 70.6 (C-4″), 62.0 (C-6″).

Luteolin 7-*O*-β-D-glucuronopyranoside (10)

R_f values: 0.28 (S_1), 0.25 (S_2), UV (deep purple), UV/NH_3 (yellow), $FeCl_3$ (green), NA/PE (orange); UV spectral data: λ_{max}, nm, MeOH: 212, 250, 261, 347; (+NaOMe): 220, 261, 284; (+NaOAc): 222, 261, 350; (NaOAc+H_3BO_3): 227, 260, 289 (sh), 362; (+$AlCl_3$): 219, 271, 293 (sh), 387; (+$AlCl_3$/HCl): 216, 269, 291 (sh), 394; ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 7.46 (2 H, brs, H-2′/6′), 6.94 (1 H, d, J = 8.6 Hz, H-5′), 6.85 (1 H, brs, H-8), 6.77 (1 H, s, H-3), 6.46 (1 H, brs, H-6), 5.20 (1 H, d, J = 6.8 Hz, H-1″), 3.7-3.1 (m, remaining sugar protons); ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm 182.6 (C-4), 171.7 (C-6″), 165.2 (C-2), 163.3 (C-7), 161.7 (C-5), 157.6 (C-9), 150.5 (C-4′), 146.4 (C-3′), 122.0 (C-1′), 119.9 (C-6′), 116.8 (C-5′), 114.0 (C-2′), 106.1 (C-10), 103.6 (C-3), 100.2 (C-1″), 100.1 (C-6), 95.4 (C-8), 76.5 (C-5″), 75.6 (C-3″), 73.6 (C-4″), 72.2 (C-2″).

RESULTS AND DISCUSSION

Antiviral activity

Haemagglutination viruses are viruses capable of agglutinating the red blood cells of a variety of animals. They directly agglutinate erythrocytes by binding to specific receptor sites on the surface of the erythrocyte, and this characteristic can be used in detection of the virus. Inhibition of haemagglutination caused by these viruses is the detection of their activity's inhibition. The plant parts of leaves and flowers showed remarked antiviral activity compared to the other parts of roots and flower-peduncles. In chicken embryos, the activity of plant parts as determined by haemagglutinating activity in allantoic fluids (Table I) indicated 6, 6, 8 and 6 μg/0.2 mL/egg for the leaves, roots, flower-peduncles, and flowers, respectively. The ethanol extract of leaves and flowers of *A. hijazensis* respectively were fully reduced the infectivity of 5000 EID_{50} of APMV-1 virus (Table I). In each case of the tested virus; APMV-1, H5N1, NDV, and Adenovirus; egg drug syndrome virus (EDSV), the toxicity assays in chicken embryos indicated that at concentration of 700 and 800 μg/egg for flowers and leaves, respectively were showed by 100% of the inoculated embryos without death on the 3rd - 4th days after inoculation. The recorded therapeutic indices (TI) were 116 and 133, respectively in APMV-1 infection while it was 100% in each of avian H5N1 and NDV virus infection for both flowers and leaves. The recorded therapeutic indices were 114 and 116, respectively in avian Adenovirus infection. Contrasting this study's results (Table II), embryonated eggs inoculated with a mixture of the APMV-1 with the ethanol extract of each plant part separately did not retard the virus replication, and pooled allantoic fluid from each group of eggs receiving varying concentration of each extract with the same concentration

Table I. Antiviral Activity of ethanol extracts of different *Aloe hijazensis* parts against different viruses' replication in SPF chicken embryos and their embryonic-toxicity

Plant Part	CC_{50}	IC_{50}				TI			
		APMV-1	AI-H5N1	NDV	EDSV	APMV-1	AI-H5N1	NDV	EDSV
Flowers	>700	≤ 6	≤ 7	≤ 5	≤ 6	116	100	140	116
Flower-peduncles	>600	≤ 8	≤ 9	≤ 8	≤ 8	75	66.6	75	75
Leaves	>800	≤ 6	≤ 8	≤ 5	≤ 7	133	100	160	114
Roots	>500	≤ 6	≤ 8	≤ 8	≤ 7	83	62.5	62.5	71

CC_{50}: Toxic concentration fifty; IC_{50}: Inhibitory concentration fifty; TI: Therapeutic index; APMV-1: avian paramyxovirus type-1; AI-H5N1: avian influenza virus type A (H5N1); NDV: Newcastle disease virus; EDSV: egg-drop syndrome virus; group III adenovirus (AdV).

of virus showed 1:8 to 1:32 HA titer at 5 μg/mL for the leaves while the HA titer was 1:16 to 1:32 HA titer at the 5 μg/mL for the flowers. However, the present study conclusively revealed the pronounced inhibitory effect of ethanol extracts of leaves and flowers against haemagglutinating viruses' infections. The activity may be related to the bioactive constituents present in plant parts. The leaves and roots of *A. hijazensis* are rich sources of biologically active compounds such as anthraquinones, chromones, anthrones, and flavonoids (Abd-Alla et al., 2009b). The antiviral action may be due to direct and indirect effects (Hamman, 2008). Indirect effect is due to stimulation of the immune system. Direct effect is due to anthraquinones. The anthraquinone barbaloin inactivates various enveloped viruses such as influenza (Sydiskis et al., 1991). Many anthraquinones, such as emodin and barbaloin, have shown antiviral and/or virucidal effects (Alves et al., 2004). Antiviral activity of anthrone and anthraquinone derivatives against ganciclovir-sensitive and -resistant strains of human cytomegalovirus (CMV) were reported (Barnard et al., 1992). Saoo et al. (1996) suggested that a major mechanism of inhibition of CMV infection by *Aloe* extract is through interference with DNA synthesis. Anthraquinones isolated from the exudate of *A. vera* have shown wide antimicrobial activity. The antimicrobial activity of emodin was proposed to be mediated through inhibition of solute transport in membranes (Hamman, 2008). Aloe-emodin is an interferon-inducing agent with antiviral activity against Japanese encephalitis virus and enterovirus (Lin et al., 2008) and also showed anti-SARS coronavirus 3C-like protease effect when tested (Lin et al., 2005). It dose-dependently inhibited cleavage activity of the $3CL^{pro}$, in which the IC_{50} was 366 μM. The antiviral activity of 1,8-dihydroxy-3-methylanthraquinone against poliovirus was reported (Semple et al., 2001).

The structure-activity relationship of flavonoids of luteolin and apigenin derivatives as antiviral agents were reported (Liu et al., 2008). In the present study, thirteen compounds (Fig. 1) were isolated, from which eleven compounds were attributed to the flowers and

Table II. Haemagglutination activity of APMV-1 incubated alone or with separate different *Aloe hijazensis* parts in embryonating chicken embryos (ECE)

HA-titers		Virus dilution				
		10^{-7}	10^{-8}	10^{-9}	10^{-10}	10^{-11}
Allantoic fluids	1	4096*	4096*	1024	32	0
	2	4096*	4096*	512	32	0
	3	4096*	1024	512	16	0
	4	4096*	512	256	8	0
	5	-	-	256	4	0
	Inf./Total	4/4	4/4	5/5	5/5	0/5
	Ext. conc./egg	4	5	6	7	8
Leaves	1	8	8	0	0	0
	2	16	16	0	0	0
	3	32	16	0	0	0
	4	32	0	0	0	0
	5	0	0	0	0	0
	Inf./Total	4/5	3/5	0/5	0/5	0/5
Roots	1	8	16	16	8	0
	2	8	16	16	16	0
	3	16	32	16	8	0
	4	32	32	16	0	0
	5	32	0	0	0	0
	Inf./Total	5/5	4/5	4/5	3/5	0/5
Flower-peduncles	1	16	16	8	8	0
	2	32	32	16	16	0
	3	32	32	16	16	0
	4	64	64	0	0	0
	5	64	0	0	0	0
	Inf./Total	5/5	4/5	3/5	3/5	0/5
Flowers	1	16	16	0	0	0
	2	32	32	0	0	0
	3	32	32	0	0	0
	4	0	0	0	0	0
	5	0	0	0	0	0
	Inf./Total	3/5	3/5	0/5	0/5	0/5

seven to the plant flower-peduncles. They can be classified into: five anthraquinones: ziganein (**1**), ziganein-5-methyl ether (**2**), chrysophanol (**3**), aloe-emodin (**4**), aloesaponarin I (**5**); one phenolic acid: *p*-coumaric acid (**6**); one dihydro isocoumarin: feralolide (**7**); and four flavonoids: homoplantaginin (**8**), isoorientin (**9**), luteolin 7-glucuronopyranoside (**10**), and isovitexin (**11**). Aloenin (**12**) was also obtained together with the anthrone; barbaloin (**13**).

Structural elucidation of isolated compounds

Structures of ziganein (**1**) (Schripsema and Dagnino, 1996), ziganein-5-methyl ether (**2**) (Jung et al., 1984), chrysophanol (**3**) (Abd El-Fattah et al., 1994), aloe-emodin (**4**) (Abd El-Fattah et al., 1994), aloesaponarin I (**5**) (Cameron et al., 1981), *p*-coumaric acid (**6**) (Chaudhuri and Thakur, 1986), feralolide (**7**) (Speranza et al., 1993; Abd-Alla et al., 2009b), isovitexin (**11**) (Agrawal and Bansal, 1989), aloenin (**12**) and barbaloin (**13**) (Holzapfel et al., 1997) were elucidated using studies of their NMR, MS spectra and comparison with authentic samples.

Depending on chromatographic properties and UV spectral data, compound (**8**) was expected to be a 6- or 8-hydroxy flavone, and the absence of bathochromic shift in band II on addition NaOAc indicated 7-OH substitution. The compound gave glucose and apigenin by acid hydrolysis. ^{1}H-NMR spectrum showed the glycosidation at 7-OH as indicated by downfield of H-8, which appeared at δ 6.84 as a singlet with another singlet at δ 3.83 for the group of OCH_3 was referred to methoxylation at C-6. This methoxylation was confirmed by an upfield shift with about -8 ppm of C-7 (δ 157.2 ppm) and C-5 (δ 152.8 ppm) in the ^{13}C NMR spectrum. The *O*-glycosidation at C-7 was confirmed by the downfield shift of C-8 (δ 94.2 ppm). The compound can be identified as 6-methoxy apigenin-7-*O*-β-D-glucopyranoside (homoplantaginin) (Harborne and Mabry, 1982).

Upon complete hydrolysis of compound (**9**), it remains without any change (CoPC) to support the evidence of its *C*-glycoside. Depending on its chromatographic properties and UV spectral data, it was suspected to be a mono-*C*-glycosyl derivative (Harborne and Mabry, 1982). Negative HRESI-MS spectrum showed a molecular ion peak at 447.09544 corresponding to the molecular weight and molecular formula of mono-C-hexosyl-luteolin structure. ^{1}H-NMR spectrum, B-ring protons were assigned as an ABX-spin coupling system of the three types of protons H-2', H-6' and H-5'. At ~δ 6.6 ppm, H-3 was assigned as a singlet. The absence of H-6 resonance in the spectrum and appearance of H-8 as a singlet at ~δ 6.4 led us to the placement of the C-hexoside moiety at C-6. This evidence was further supported by the diagnostic anomeric proton signal at δ 4.54 ppm of the characteristic *J* value (9.2 Hz) for the β-*C*-glycosyl moiety. ^{13}C-NMR spectrum of compound (**9**) revealed the presence of 15 carbon resonances, characteristic for a luteolin moiety (Agrawal and Bansal, 1989). The presence of a β-*C*-glycopyranoside was proved from its characteristic six C-resonances in the range of about 82-60 ppm, particularly that of C-1" (anomeric carbon) at δ 73.6 ppm. The location of this glucopyranoside moiety on C-6 was suggested from the downfield shift of its resonance to 109.5 (±10 ppm) and the slight

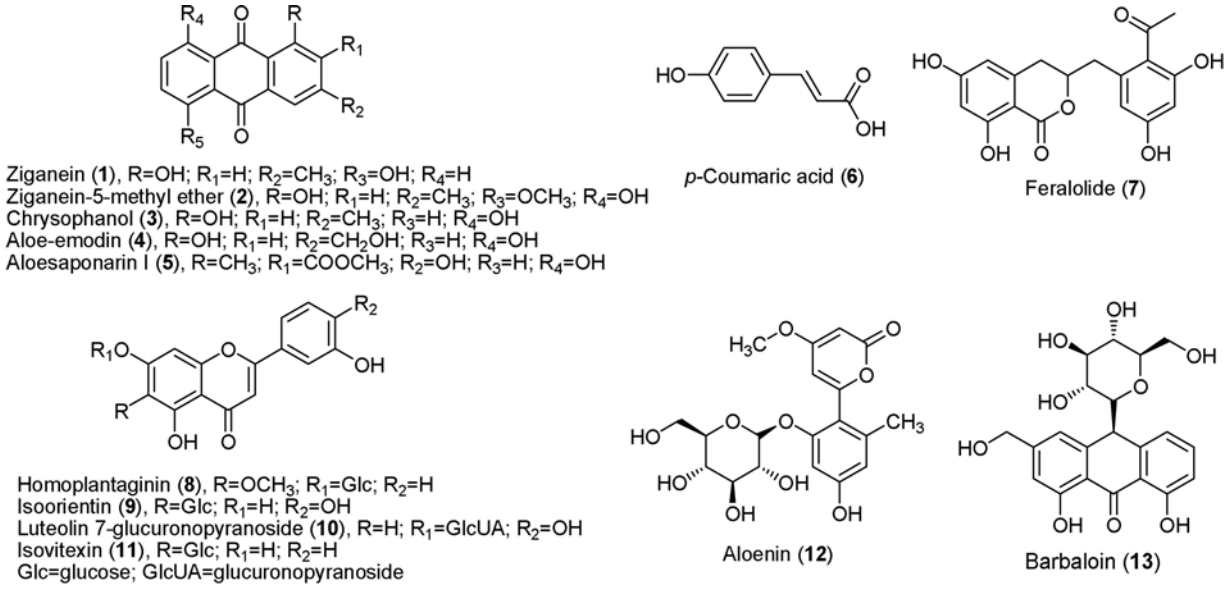

Fig. 1. Chemical structures of the isolated compounds from flowers and flower-peduncles of *Aloe hijazensis*.

upfield shifts of both C-5 and C-7 signals (Harborne and Mabry, 1982; Agrawal and Bansal, 1989). The compound was identified as luteolin 6-*C*-β-D-glucopyranoside (isoorientin).

Depending on the chromatographic properties and UV spectral data of compound (**10**), it was suggested to be luteolin *O*-glycoside (Harborne and Mabry, 1982). The absence of a bathochromic shift in addition to NaOAc referred to 7-OH substitution. Upon acid hydrolysis, it gave D-glucuronic acid and luteolin. ^{1}H-NMR spectrum showed downfield shifts by both H-8 and H-6 of about δ 0.2 ppm that indicated 7-*O*-glycosidation (Agrawal and Bansal, 1989) at δ 6.85 and 6.46 ppm, respectively. The anomeric protons at δ 5.20 (J = 6.8 Hz) indicated the structure of a sugar moiety. ^{13}C-NMR spectrum showed the characteristic 15 carbon resonances of a luteolin moiety (Agrawal and Bansal, 1989). The upfield shift of C-7 and downfield shifts of C-6 and C-8 were observed (Harborne and Mabry, 1982). The resonance of C-6" at δ 171.7 ppm was a confirmation of the uronic acid of a sugar moiety. The difference of 0.9 ppm between the C-3" and C-5" δ values was characteristic of a glucuronoide moiety. The compound may be identified as luteolin 7-*O*-β-D-glucuronopyranoside. Compounds (**8**) and (**10**) are reported here for the first time from the *Aloe* species.

Interestingly, *in vivo* studies should be taken to demonstrate the applicable values of plant parts of *A. hijazensis* for the prophylactic treatment of avian haemagglutinating viruses' infections. The result was in agreement with the previous studies and demonstrated the antiviral efficacy of the crude extract of *A. secundiflora* in chickens experimentally infected with Newcastle disease virus (Waihenya et al., 2002).

REFERENCES

Abdel-Fattah, H., Gohar, A., el-Dahmy, S., and Hubaishi, A., Phytochemical investigation of *Rumex luminiastrum*. *Acta Pharm. Hung*., 64, 83-85 (1994).

Abd-Alla, H. I., Moharram, F. A., Gaara, A. H., and El-Safty, M. M., Phytoconstituents of *Jatropha curcas* L. leaves and their immunomodulatory activity on humoral and cell-mediated immune response in chicks. *Z. Naturforsch. C*, 64, 495-501 (2009a).

Abd-Alla, H. I., Shaaban, M., Shaaban, K. A., Abu-Gabal, N. S., Shalaby, N. M., and Laatsch, H., New bioactive compounds from *Aloe hijazensis*. *Nat. Prod. Res*., 23, 1035-1049 (2009b).

Agrawal, P. K. and Bansal, M. C., Flavonoid glycosides. In Agrawal, P. K. (Ed.), Studies in Organic Chemistry 39**,** ^{13}C-NMR of Flavonoids. Elsevier Science, New York, USA, pp. 283-364, (1989).

Alves, D. S., Pérez-Fons, L., Estepa, A., and Micol, V., Membrane-related effects underlying the biological activity of the anthraquinones emodin and barbaloin. *Biochem. Pharmacol*., 68, 549-561 (2004).

Barnard, D. L., Huffman, J. H., Morris, J. L., Wood, S. G., Hughes, B. G., and Sidwell, R. W., Evaluation of the antiviral activity of anthraquinones, anthrones and anthraquinone derivatives against human cytomegalovirus. *Antiviral. Res*., 17, 63-77 (1992).

Cameron, D. W., Deutscher, D. J., Feutrill, G. I., and Griffiths, P. G., Chemistry of the *Coccoidea*. VIII. Synthesis of the ancient dyestuff kermesic acid and of related anthraquinones. *Aust. J. Chem*., 34, 2401-2421 (1981).

Chaudhuri, P. K. and Thakur, R. S., An acylated flavone apigenin 7-*O*-β-D-(4-*cis*-*p*-coumaroyl)glucoside from *Echinops echinatus*. *Phytochemistry*, 25, 1770-1771 (1986).

Colenette, S., An illustrated guide to the flowers of Saudi Arabia. *Brittoni*a, 38, 430-432 (1986).

Dao, T. T., Nguyen, P. H., Lee, H. S., Kim, E., Park, J., Lim, S. I., and Oh, W. K., Chalcones as novel influenza A (H1N1) neuraminidase inhibitors from Glycyrrhiza inflata. *Bioorg. Med. Chem. Lett*., 21, 294-298 (2011).

Fahim, M. S. and Wang, M., Zinc acetate and lyophilized *aloe barbadensis* as vaginal contraceptive. *Contraception*, 53, 231-236 (1996).

Hamman, J. H., Composition and applications of *Aloe vera* leaf gel. *Molecules*, 13, 1599-1616 (2008).

Harborne, J. B. and Mabry, T. J., The Flavonoids: Advances in Researches, Chapter 2: Chapman and Hall Ltd, London, pp. 14-134, (1982).

Helmy, W. A., Abd-Alla, H. I., Amer, H., and El-Safty, M. M., Chemical composition and *'in vitro'* antiviral activity of *Azadirachta indica* A. juss (Neem) leaves and fruits against Newcastle disease virus and infectious bursal disease virus. *Aust. J. Basic Appl. Sci.*, 1, 801-812 (2007).

Hines, N. L. and Miller, C. L., Avian paramyxovirus serotype-1: a review of disease distribution, clinical symptoms, and laboratory diagnostics. *Vet. Med. Int*., 2012, 17 (2012).

Holzapfel, C. W., Wessels, P. L., Wyk, B.-E. V., Marais, W., and Portwig, M., Chromone and aloin derivatives from *Aloe broomii*, *A. Africana* and *A. speciosa*. *Phytochemistry*, 45, 97-102 (1997).

Iljazovic, E., Zulcic-Nakic, V., Latifagic, A., Sahimpasic, A., Omeragic, F., and Avdic, S., 245 ORAL Efficacy in treatment of cervical HRHPV infection by combination of interferon, *Aloe vera* and propolis gel associated with different cervical lesion. *Eur. J. Surg. Oncol.*, 32, S73 (2006).

Jung, M. E., Lowe, J. A., III, Lyster, M. A., Node, M., Pfluger, R. W., and Brown, R. W., Regiospecific synthesis of mono- and bicyclic 6-alkoxy-2-pyrones and their use in the preparation of substituted aromatics, anthraquinones, and tetracyclic intermediates for 11-deoxyanthracycline synthesis. *Tetrahedron*, 40, 4751-4766 (1984).

Lee, J. Y., The identification of unknown poultry viruses through established methods. M. S. Thesis, University of the Free State, Bloemfontein, South Africa, pp. 6-10, (2009).

Lin, C. W., Tsai, F. J., Tsai, C. H., Lai, C. C., Wan, L., Ho, T. Y., Hsieh, C. C., and Chao, P. D., Anti-SARS coronavirus

3C-like protease effects of *Isatis indigotica* root and plant-derived phenolic compounds. *Antiviral. Res.*, 68, 36-42 (2005).

Lin, C. W., Wu, C. F., Hsiao, N. W., Chang, C. Y., Li, S. W., Wan, L., Lin, Y. J., and Lin, W. Y., Aloe-emodin is an interferon-inducing agent with antiviral activity against Japanese encephalitis virus and enterovirus 71. *Int. J. Antimicrob. Agents*, 32, 355-359 (2008).

Liu, A. L., Wang, H. D., Lee, S. M., Wang, Y. T., and Du, G. H., Structure-activity relationship of flavonoids as influenza virus neuraminidase inhibitors and their *in vitro* antiviral activities. *Bioorg. Med. Chem.*, 16, 7141-7147 (2008).

Mbanga, J., Mangoma, N., and Saidi, B., An evaluation of the antimicrobial activities of *Aloe barbadensis, A. chabaudii* and *A. arborescens* leaf extracts used in folklore veterinary medicine in Zimbabwe. *J. Anim. Vet. Adv.*, 9, 2918-2923 (2010).

Perera, C. and Efferth, T., Antiviral medicinal herbs and phytochemicals. *J. Pharmacogn.*, 3, 45-48 (2012).

Reed, L. J. and Muench, H., A simple method of estimating 50 percent endpoint. *Am. J. Hyg.*, 27, 493-497 (1938).

Saoo, K., Miki, H., and Ohmori, M., Antiviral activity of *Aloe* extracts against cytomegalovirus. *Phytother. Res.*, 10, 348-350 (1996).

Schripsema, J. and Dagnino, D., Elucidation of the substitution pattern of 9,10-anthraquinones through the chemical shifts of *peri*-hydroxyl protons. *Phytochemistry*, 42, 177-184 (1996).

Semple, S. J., Pyke, S. M., Reynolds, G. D., and Flower, R. L., *In vitro* antiviral activity of the anthraquinone chrysophanic acid against poliovirus. *Antiviral. Res.*, 49, 169-178 (2001).

Singh, S., Sharma, P. K., Kumar, N., and Dudhe, R., Biological activities of *Aloe vera. Int. J. Pharm. Technol.,* 2, 259-580 (2010).

Speranza, G., Manitto, P., Cassara, P., and Monti, D., Feralolide, a dihydroisocoumarin from Cape *Aloe. Phytochemistry*, 33, 175-178 (1993).

Sydiskis, R. J., Owen, D. G., Lohr, J. L., Rosler, K. H., and Blomster, R. N., Inactivation of enveloped viruses by anthraquinones extracted from plants. *Antimicrob. Agents Chemother.*, 35, 2463-2466 (1991).

Syed, T. A., Cheema, K. M., Ahmad, S. A., and Unit, A. H., Jr., *Aloe vera* extract 0.5% in hydrophilic cream versus *Aloe vera* gel for the management of genital herpes in males. A placebo-controlled, double-blind, comparative study. *J. Eur. Acad. Dermatol. Venereol.*, 7, 294-295 (1996).

Takatsy, G., The use of spiral loops in serological and virological micromethods. *Acta Microbiol. Acad. Sci. Hung.*, 3, 191-202 (1955).

Waihenya, R. K., Mtambo, M. M., and Nkwengulila, G., Evaluation of the efficacy of the crude extract of *Aloe secundiflora* in chickens experimentally infected with Newcastle disease virus. *J. Ethnopharmacol.*, 79, 299-304 (2002).

Zandi, K., Zadeh, M. A., Sartavi, K., and Rastian, Z., Antiviral activity of *Aloe vera* against herpes simplex virus type 2: An *in vitro* study. *Afr. J. Biotechnol.*, 6, 1770-1773 (2007).