In vitro evaluation of cytotoxic and antimicrobial potentials of the Saudi traditional plant *Alhagi graecorum* boiss

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Abstract: Various species of *Alhagi* are known for a wide diversity of ethnopharmacological uses. This study explored the *in vitro* antimicrobial and cytotoxic potentials of the crude ethanolic extract and isolated fractions from the traditional Saudi plant *Alhagi graecorum*. The antimicrobial activity was evaluated against 19 pathogenic microbial strains using the broth micro dilution assay. On the other hand, the cytotoxicity was assessed using the crystal violet staining method on MCF-7, HepG-2, HCT-116, A-549, and HEp-2human cell lines. Results showed that the highest antimicrobial activity was shown by the ethyl acetate extract, followed by the dichloromethane and total alcohol extracts. The aqueous extract had no effect on any of the tested microorganisms at 20mg/ml. In addition, the ethyl acetate extract displayed remarkable cytotoxic activity on all five human cell lines with IC_{50} values ranging from 2.7 to 8.23µg/mL, followed by the dichloromethane extract with IC_{50} values between 9.4 and 19.7µg/mL using doxorubicin as a reference drug.

Keywords: Cytotoxicity, antimicrobial, *Alhagi graecorum*, crystal violet assay.

INTRODUCTION

The growing incidence of resistance to antibiotics is a serious health problem in contemporary medicine. In April 2014, the World Health Organization (WHO) reported that this issue threatens every region of the world, potentially affecting anyone, anywhere, and at any age (WHO, 2014). WHO also reported that cancer will remain the main cause of morbidity and mortality over the next few decades in all regions around the world (World health statistics, 2014). Therefore, the challenges of discovering potent natural antimicrobial agents, as well as natural and safe drugs to treat cancer, are enormous and of great necessity. These facts fortified the investigation of the therapeutic potential of the crude extract and various fractions obtained from *Alhagi graecorum*, a traditionally used medicinal plant in Saudi Arabia.

The genus *Alhagi* belongs to the family Fabaceae and is comprised of about nine species (Singh *et al*., 1999). The Latin name is derived from the Arabic name *Alhag*, meaning the old wise man (Boulos, 2000). Variety of *Alhagi* species are customarily used in folk medicine as remedies to treat rheumatism, bronchitis, ulcers, liver disorders and jaundice, urinary tract diseases, asthma and gallbladder problems (Atta and Mouneir, 2004; Alqasoumi *et al*., 2008; Naseri and Mard, 2007; Amiri *et al*., 2014). Recent *in vivo* and *in vitro* biological activity studies on *Alhagi* species revealed their antibacterial, antifungal, antioxidant, antiproliferative, hepatoprotective spasmolytic, and ureter-relaxing effects (Atta and Mouneir, 2004; Marashdah and Farraj 2010; Marashdah and Al-Hazimi, 2010; Shaker *et al*., 2010; Abu-Taleb *et al*., 2011; Awaad *et al*., 2011; Rahman *et al*., 2011; Saatchi *et al*., 2014).

The investigated species, *A. graecorum* Boiss, known locally as al-akool, is a shrubby, evergreen, branched, perennial herb with rigid spiny twigs. This well-known medicinal plant is indigenous to Saudi Arabia and widely distributed in the Nile region, Mediterranean Basin, eastern and western deserts, Red Sea coast, and Sinai (Awmack and Lock, 2002). The plant is used as a laxative and is known to treat bilharzias, hemorrhoids, and various types of gastrointestinal ailments (Al-Douri and Al-Essa, 2010; Zain *et al*., 2012). Alkaloids, phenolics, steroids, triterpenes, resins, and tannins have been reported from *A. graecorum*. In addition, different types of flavonoid aglycones and glycosides have been found in the plant (Elsayed *et al*., 1993).

Our literature search revealed no previous investigations concerning the cytotoxicity and chemo prevention potential of this plant. The only published study exploring its antibacterial properties was limited to testing the total methanolic extract against a few bacterial strains (Tarawneh *et al*., 2010). Therefore, the reported antibacterial (Abu-Taleb *et al*., 2011; Laghari *et al*., 2012; 2014; Rahman *et al*., 2011) and anticancer activities (Behzad *et al*., 2014; Loizzo *et al*., 2014; Sapko and Kunaeva, 1999) of different species of *Alhagi* and the lack of research on the above-mentioned species motivated us to start this investigation. This study evaluated the antimicrobial efficacy of *A. graecorum* ethanolic extract and different organic solvents fractions taken from the aerial portions of the plant on clinical isolates (14 bacterial and 5 fungal species). The extracts were also evaluated against five human cancer cell lines.

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Table 1: Antimicrobial activities (zone of Inhibition, mm) and MICs of *A. graecorum* fractions (A–D) against selected clinical pathogens

Tested microorganisms Fungi	Test sample	Zone of Inhibition (mm) ±SD	MIC μgmL^{-1}
Aspergillus fumiagatus (ATCC 90906)	A	16.3±0.44	31.25
	B	NA	NA
	C	17.6±0.58	15.63
	D	18.3±0.33	7.81
	AMB	23.7±0.1	0.24
Geotricum candidum (RCMB 05097)	A	19.1±0.37	3.9
	B	NA	NA
	C	20.3±0.38	1.95
	D	20.4±0.34	1.95
	AMB	28.7±0.2	0.015
Candida albicans (ATCC 10231)	A	NA	NA
	B	NA	NA
	C	NA	NA
	D	NA	NA
	AMB	25.4±0.1	0.06
Absidia corymbifera (RCMB 02564)	A	NA	NA
	B	NA	NA
	C	19.33±0.58	3.9
	D	22.33±0.58	0.98
	AMB	23.7±0.10	0.98
Trichophyton mentagrophytes (RCMB 0925)	A	NA	NA
	B	NA	NA
	C	18.33±0.58	7.81
	D	20.0±0.0	3.9
	AMB	25.4±0.16	0.49
Gram positive bacteria			
Streptococcus pneumoniae (RCMB 010010)	A	16.2±0.15	31.25
	B	16.0±0.44	31.25
	C	18.9±0.44	3.9
	D	20.3±0.43	1.95
	AMP	27.4±0.18	0.12
Streptococcus pyogenes (RCMB 010015)	A	NA	NA
	B	NA	NA
	C	20.0±0.01	3.9
	D	21.0±0.0	1.95
	AMP	26.4±0.34	0.49
Staphylococcus aureus (RCMB 010027)	A	NA	NA
	B	NA	NA
	C	17.33±1.15	31.25
	D	19.0±1.0	7.81
	AMP	28.9±0.14	0.49
Staphylococcus epidermidis (RCMB 010024)	A	NA	NA
	B	NA	NA
	C	16.33±0.48	31.25
	D	20.67±0.58	1.95
	AMP	25.4±0.18	0.49
Clostridium perfringens (RCMB 010034)	A	NA	NA
	B	NA	NA
	C	12.67±0.58	125
	D	19.67±0.58	3.9
	AMP	18.4±0.34	7.81

Table 1 continued…

Tested microorganisms Fungi	Test sample	Zone of Inhibition (mm) ±SD	MIC μgmL^{-1}
Listeria innocua (RCMB 010052)	A	NA	NA
	B	NA	NA
	C	17.0±1.0	31.25
	D	18.0±0.0	7.81
	AMP	18.3±0.34	7.81
Bacillis subtilis (RCMB 010067)	A	12.8±0.42	125
	B	NA	NA
	C	20.3±0.58	1.95
	D	21.4±0.53	0.98
	Amp	32.4±0.10	0.007
Gram negative bacteria			
Pseudomonas aeruginosa (RCMB 010043)	A	NA	NA
	B	NA	NA
	C	NA	NA
	D	NA	NA
	GEN	17.3±0.15	15.63
Escherichia coli (RCMB 010052)	A	16.9±0.25	15.63
	B	14.9±0.46	62.5
	C	18.9±0.63	3.9
	D	17.4±0.53	15.63
	GEN	22.3±0.18	3.9
Neisseria gonorrhoeae (RCMB 010076)	A	NA	NA
	B	NA	NA
	C	NA	NA
	D	18.66±0.58	7.81
	GEN	19.9±0.18	1.95
Proteous vulgaris (RCMB 010085)	A	NA	NA
	B	NA	NA
	C	NA	NA
	D	18.0±0.0	15.63
	GEN	23.4±0.3	0.98
Klebsiella pneumoniae (RCMB 0010093)	A	17.0±0.0	31.25
	B	NA	NA
	C	19.0±1.0	7.81
	D	22.66±0.58	0.98
	GEN	26.3±0.15	0.49
Shigella flexneri (RCMB 0100542)	A	15.33±0.58	62.5
	B	NA	NA
	C	17.33±1.15	31.25
	D	22.0±0.0	0.98
	GEN	24.8±0.24	0.49
Salmonella enteritidis (RCMB 010084)	A	19.67±0.58	3.9
	B	NA	NA
	C	17.0±0.0	31.25
	D	19.0±1.0	3.9
	GEN	25.3±0.18	0.49

NA: no activity, AMB: amphotericin B, AMP: ampicillin, GEN: gentamicin, data are expressed in the form of mean ± S.D.

MATERIALS AND METHODS

Plant materials

The aerial parts of *A. graecorum* were obtained in March 2013 from Wadi Hanifa in the Nejd region of central Saudi Arabia. The plant was, kindly, identified by a plant taxonomist in College of Pharmacy, King Saud University. A voucher specimen (#16081) was deposited at the Department of Pharmacognosy, College of Pharmacy, King Saud University.

Table 2: *In vitro* cytotoxic activities of *A. graecorum* fractions (A-D) on MCF-7 cell line

Tumor cell line MCF-7		Mean of surviving fraction± S.D.[#]		Doxorubicin	
Sample concentration (µg/mL)	A	B	C	D	
50	41.8±3.8[a]	61.5±3.5[a]	8.3±1.7[a]	5.3±1.7[a]	3.2±0.3
25	56.9±3.2[a]	78.8±2.0[a]	14.7±1.4[a]	10.8±2.1[b]	7.2±0.6
12.5	75.2±3.4[a]	90.3±1.3[a]	26.4±3.0[a]	18.1±1.3[a]	11.7±1.0
6.25	87.1±3.1[a]	96.0±0.7[a]	73.9±3.1[a]	29.4±3.2[a]	18.7±1.0
3.125	94.1±2.9[a]	99.1±0.2[a]	88.4±1.4[a]	45.7±3.5[a]	22.9±1.3
1.56	98.1±1.0[a]	100[a]	94.1±1.0[a]	63.3±2.8[a]	32.5±2.00
*IC_{50}µg/mL	36.4	>50	9.4	2.7	0.44

Table 3: *In vitro* cytotoxic activities of *A. graecorum* fractions (A-D) on Hep G-2cell line

Tumor cell line HepG-2	Mean of surviving fraction± S.D.[#]				Doxorubicin
Sample concentration (µg/mL)	A	B	C	D	
50	38.8±3.0[a]	74.8±1.7[a]	12.6±1.2[a]	7.4±1.0[a]	6.8±0.4
25	54.4±2.5[a]	88.1±3.2[a]	28.2±2.7[a]	19.9±3.4[a]	8.7±0.5
12.5	74.0±6.6[a]	94.9±0.65[a]	49.3±3.5[a]	30.5±2.7[a]	14.8±1.2
6.25	86.0±4.8[a]	98.4±1.1[a]	78.3±2.4[a]	46.3±2.2[a]	16.2±1.3
3.125	93.4±3.5[a]	100[a]	91.6±0.7[a]	60.2±2.0[a]	25.1±1.5
1.56	97.2±2.3[a]	100[a]	98.4±0.7[a]	75.1±3.1[a]	35.4±1.4
*IC_{50}µg/mL	32	>50	12.3	5.4	0.47

Table 4: *In vitro* cytotoxic activities of *A. graecorum* fractions (A-D) on HCT-116 cell line

Tumor cell line HCT-116	Mean of surviving fraction± S.D.[#]				Doxorubicin
Sample concentration (µg/mL)	A	B	C	D	
50	44.9±3.6[a]	66.5±2.9[a]	20.2±2.8[a]	7.4±0.4[a]	5.4±0.8
25	58.7±4.1[a]	83.1±2.1[a]	38.3±3.5[a]	12.7±1.4[a]	8.7±0.9
12.5	72.6±5.6[a]	91.8±2.1[a]	47.9±3.1[a]	22.9±3.5[a]	15.3±1.6
6.25	84.5±3.9[a]	96.7±1.6[a]	61.5±2.3[a]	62.6±6.7[a]	18.2±2.1
3.125	92.6±2.3[a]	99.1±1.1[a]	75.1±7.9[a]	83.6±2.3[a]	23.7±1.6
1.56	97.4±1.2[a]	100±0.00[a]	90.5±2.5[a]	91.3±2.5[a]	33.1±1.8
*IC_{50}µg/mL	40.7	>50	11.5	8.23	0.46

[#]Mean of surviving fraction ± standard deviation: Mean of three assays. [a]$p<0.001$ compared to the reference drug. *IC_{50}: concentration of extract required to reduce cell survival by 50%

Preparation of extracts

The finely-ground dried plant material (250g) was extracted with 85% ethanol by cold maceration until exhaustion. The ethanol extract was dried in a rotary evaporator to give a dark residue (A, 20g), 15 grams of which were mixed with water and subsequently extracted with *n*-hexane, dichloromethane, and ethyl acetate. All fractions were evaporated till dryness to yield fractions B (*n*-hexane, 2g), C (dichloromethane, 3.5g), D (ethyl acetate, 2.5g) and E (remaining aqueous mother liquor, 6.0g).

Microbial strains

To assess the microbial activity, five fungi, seven Gram-positive bacteria, and seven Gram-negative bacteria were selected. The fungi included *Aspergillus fumigatus* (ATCC 90906), *Geotrichum candidum* (RCMB 05097), *Candida albicans* (ATCC 10231), *Absidia corymbifera* (RCMB 02564) and *Trichophyton mentagrophytes* (RCMB 0925). The Gram-positive bacteria included *Streptococcus pneumoniae* (RCMB 010010), *Streptococcus pyogenes* (RCMB 010015), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (RCMB 010024), *Clostridium perfringens* (RCMB 010034), *Listeria innocua* (RCMB 010052) and *Bacillus subtilis* (RCMB 010067). The Gram-negative bacteria were *Pseudomonas aeruginosa* (RCMB 010043), *Escherichia coli* (RCMB 010052), *Neisseria gonorrhoeae* (RCMB 010076), *Proteus vulgaris (RCMB 010085)*, *Klebsiella pneumoniae* (RCMB 0010093), *Shigella flexneri* (RCMB 0100542) and *Salmonella enteritidis* (RCMB 010084). The different strains of microbes were acquired from the American Type Culture Collection (ATCC).

Determination of antimicrobial activity

Antimicrobial tests were carried out by agar well diffusion in accordance with the National Committee for

Clinical Laboratory Standards (NCCLS) criteria. Bacterial and fungal suspensions were prepared at 0.5 McFarland standard turbulence in a volume of 100µL and were cultivated on Mueller-Hinton agar and Sabouraud dextrose media punched with 6-mm diameter wells for the bacteria and fungi, respectively. Then 100µl of 10% *A. graecorum* extracts were added to the wells, while 10% DMSO was used as the negative control. Ampicillin, gentamicin, and amphotericin B (30µg/mL) were used as standard agents against the Gram-positive bacteria, Gram-negative bacteria, and fungi, respectively. The antimicrobial activity was estimated, after incubation of the plates at 37°C for 18 to 24 h, by calculating the diameter of inhibition zones (nm). Each test was done three times and the mean of the results was determined. The extraction solvents were used as negative controls (NCCLS, 2002; 2004).

Determination of the minimum inhibitory concentration

The broth micro dilution method (NCCLS, 2002; 2004) was used to estimate the minimum inhibitory concentration (MIC). Bacteria were multiplied in Mueller-Hinton broth while fungi were grown in Sabouraud broth. Then 20µL of 10^6cells/mL were inoculated in tubes with broth media supplemented with different concentrations (two-fold serial dilutions 1000-0.0015µL) of the plant fractions dissolved in DMSO. The tubes were incubated at 37°C for 24h for bacteria and 3-7 days at 25°C for fungi, optical density in the spectrophotometer (620 nm) was used to measure the MIC of each sample and uninoculated broth media served as a negative control. All determinations were performed in triplicate.

Cytotoxicity assay

Cell culture

The mammalian cell lines MCF-7 cells (human breast cancer), HepG-2 (human liver cancer), HCT-116 (human colon cancer), A-549 (lung carcinoma) and HEp-2 (Human epithelial type 2 cancer) were obtained from the ATCC. The cells were proliferated in Dulbecco's modified Eagle's Medium (DMEM) complemented with fetal bovine serum (10%), L-glutamine, HEPES buffer (1%), and gentamicin (50µg/mL) (Sigma Chemical Co.). the cells were maintained at 37°C with 5% CO_2 and were subcultured two times a week.

Cytotoxicity evaluation

The cytotoxic activity was assessed by the crystal violet staining (CVS) method (Saotome *et al*., 1989; Itagaki *et al*., 1991) using a 96-well tissue culture micro plate seeded with cells at concentration of 1×10^4 cells in 100µL of medium per well. After 24h, different concentrations of *Alhagi* extracts (A-E), were added. Sequential set of dilutions of *Alhagi* extracts were added into a flat-bottomed 96-well microtiter plates and incubated with 5%CO_2 at 37°C. Three wells were used for each concentration of the test sample. The control cells were incubated without a test sample and with or without DMSO. Then, several concentrations of sample (50, 25, 12.5, 6.25, 3.125 and 1.56µg) were added. After 48 h, the media were removed and crystal violet solution (1%) was added to each well for 30 min. Then the stain was removed by rinsing the plates with distilled water. The quantitative evaluation (colorimetric estimation of fixed cells) was carried, by measuring the absorbance in an automatic Microplate reader (TECAN, Inc., San Jose, CA, USA) at 595 nm. The effect on cell growth was estimated by measuring the difference in absorbance percentage in the presence and absence of the tested extracts and presented in a dose-response curve. The concentration that inhibited cell growth by 50% (IC_{50}) was obtained. The standard antitumor drug used was doxorubicin.

STATISTICAL ANALYSIS

Data were expressed as means ± SD. The unpaired Student's t-test was conducted using the Graph PadIn Stat (ISI Software) computer program. Differences were considered significant at P values of less than 0.05.

RESULTS

Antimicrobial activity

The antimicrobial activity of *A. graecorum* ethanolic extract and its fractions was evaluated by determining their zone of inhibition and MIC values against seven Gram-positive bacteria, seven Gram-negative bacteria, and five fungi (table 1). *In vitro* antimicrobial screening demonstrated various degrees of growth inhibition for the organic solvent extracts. However, the aqueous fraction showed no growth inhibition for any of the tested microorganisms at 20mg/mL and therefore, was not included in table 1.

Among the fractions, the maximum antimicrobial activity was shown by the ethyl acetate extract (D, 17.4-22.66mm zone of inhibition diameter), followed by the dichloromethane extract (C, 12.67-20.3mm zone of inhibition diameter). The total alcohol extract was moderately active against only a few bacterial strains (A, 12.8-19.7 mm zone of inhibition diameter). The *n*-hexane fraction revealed moderate activity against the Gram-positive bacteria *S. pneumoniae* and the Gram-negative bacteria *E. coli* (B, 16.0 and 14.9mm diameter of zones of inhibition, respectively), but no activity against rest of the tested microorganisms. The MIC of the *A. graecorum* extracts ranged from 125-0.98µg/mL, with the ethyl acetate extract demonstrating the lowest values (0.98µg/mL) against *A. corymbifera*, *K. pneumoniae* and *S. flexneri*, followed by the dichloromethane extract against *G. candidum* with MIC value of 1.95µg/mL (table 1).

Cytotoxic activity

The cytotoxic activity was determined for the total ethanol, *n*-hexane, dichloromethane and ethyl acetate

Table 5: *In vitro* cytotoxic activities of *A. graecorum* fractions (A-D) on A-549 cell line

Tumor cell line A-549	Mean of surviving fraction± S.D.[#]				Doxorubicin
Sample concentration (µg/mL)	A	B	C	D	
50	45.6±3.3[a]	65.9±3.3[a]	16.9±4.1[a]	14.1±1.0[a]	4.7±0.3
25	74.8±3.6[a]	78.5±2.4[a]	39.5±2.4[a]	16.8±1.8[a]	8.6±0.8
12.5	86.7±3.2[a]	91.1±2.5[a]	64.4±2.9[a]	20.7±1.7[a]	14.7±0.9
6.25	94.4±2.3[a]	96.7±1.7[a]	80.8±1.3[a]	37.5±2.1[a]	22.2±1.4
3.125	98.2±1.5[a]	99.5±0.6[a]	94.3±1.0[a]	46.0±2.6[a]	33.4±4.0
1.56	99.7±0.8[a]	100.0±0.0[a]	99.1±0.4[a]	72.5±5.4[a]	41.9±1.3
*IC_{50}µg/mL	46.2	>50	19.7	2.88	0.85

Table 6: *In vitro* cytotoxic activities of *A. graecorum* fractions (A-D) on HEp-2 cell line

Tumor cell line HEp-2	Mean of surviving fraction± S.D.[#]				Doxorubicin
Sample concentration (µg/mL)	A	B	C	D	
50	34.9±2.4[a]	61.2±2.6[a]	10.2±2.9[a]	8.6±1.2[a]	4.7±0.6
25	45.9±1.9[a]	75.7±3.6[a]	17.4±1.3[a]	12.3±1.3[a]	9.3±0.7
12.5	61.5±1.9[a]	87.6±2.9[a]	53.9±2.1[a]	22.3±3.4[b]	15.7±0.7
6.25	77.1±3.1[a]	94.4±2.9[a]	76.9±2.0[a]	36.4±2.8[a]	24.3±3.0
3.125	88.8±3.6[a]	98.1±1.2[a]	91.2±1.2[a]	48.1±3.5[a]	33.5±2.4
1.56	94.6±2.2[a]	99.8±0.5[a]	98.8±0.4[a]	68.6±5.7[a]	44.9±2.4
*IC_{50}µg/mL	21.7	>50	13.8	2.98	1.13

[#]Mean of surviving fraction ± standard deviation: Mean of three assays. [a]$p<0.001$ and [b]$p<0.01$, compared to the reference drug. *IC_{50}: concentration of extract required to reduce cell survival by 50%

extracts (A–D) against MCF-7, HepG-2, HCT-116, A-549, and HEp-2carcinoma cell lines, using the CVS method employing doxorubicin as a reference drug. The IC_{50} was estimated for each cell line (tables 2-6). According to the results shown, all the extracts manifested a dose-dependent cytotoxic effect against the five cell lines. However, dichloromethane (C) and ethyl acetate (D) extracts showed the highest activities. The ethyl acetate extract exhibited remarkable cytotoxic activity against all the cell lines, with IC_{50} values ranging from 2.7 to 8.23µg/mL whereas the dichloromethane extract showed less selective cytotoxic activity (IC_{50}= 9.4-19.7) in comparison with doxorubicin, the reference drug, (IC_{50}=0.44-1.13µg/mL). The *n*-hexane extract was inactive against the tested cell lines (IC_{50}>50µg/mL) while the crude ethanolic extract showed moderate activity with IC_{50} ranging from 21.7 to 46.2µg/mL compared to doxorubicin.

DISCUSSION

The observed findings corroborate with the literature, as the aqueous extract of another *Alhagi* species, *Alhagi maurorum,* was also inactive and showed no inhibitory zones against the tested microorganisms (*E. coli* and *P. aeruginosa*) (Neamah, 2012). The ethyl acetate extract of *A. graecorum* showed strong inhibition against all the bacterial strains tested (MIC between 0.98 and 15.63µg/mL, table I). Among these, *A. corymbifera* showed the lowest MIC (0.98µg/mL), identical to the results for the standard drug amphotericin B. The ethyl acetate extract exhibited similar activity against Gram-positive bacteria *L. innocua*, with results identical to the standard antibacterial drug ampicillin (MIC 7.81µg/mL).

The observed activities could be attributed to the constituents present in the *A. graecorum* extracts. The reported occurrence of flavonoids, such as quercetin, isorhamnetin, and their glycosides, in high concentrations in several species of *Alhagi* (Amani *et al*., 2006), can justify the dominant antibacterial activity of tested extracts against various Gram-positive and Gram-negative bacteria (Omidiji and Ehimidu, 1990; Rigano *et al*., 2007). The activity is suggested to be through their ability to complex with bacterial cell walls as well as with extra cellular and soluble proteins (Cowan, 1999).

The organisms tested in this study are associated with various types of human infections that can be particularly dangerous in susceptible patients. *A. fumigatus*is, a major cause of invasive lung infections in immunosuppressed and granulocytopenic patients (Hohl and Feldmesser, 2007). Together with *Aspergillus*, *Candida* is the most frequently isolated pathogen in nosocomial urinary tract infections, with *Candida albicans* being the most recognizable causative of fungal bloodstream infections (Achkar and Fries, 2010). Moreover, *S. pneumoniae* is the main cause of septicemia in HIV-infected people and community-acquired pneumonia and meningitis in children and the elderly (Siemieniuk *et al*., 2011). The pronounced activity of both ethyl acetate and dichloromethane extracts against Gram-negative, Gram-

positive bacteria and fungi indicates that *A. graecorum* can be a source of potent antibiotics with broad-spectrum activity to treat the drug-resistant microorganisms prevalent among hospital environments and crowded populations.

On the other hand, the remarkable cytotoxic effect observed for both the dichloromethane and ethyl acetate extracts of *A. graecorum* could be due to the plant's bioactive constituents, such as the triterpene derivatives identified in many *Alhagi* species (Hamed *et al*., 2012) and reported to have prominent anticancer activity (Dinda *et al*., 2010). Isorhamnetin, isolated from *A. graecorum*, was shown to be a powerful antiproliferative agent with an IC_{50} of 72 µM as assessed by MTT assay (Jaramillo *et al*., 2010). In addition, lupeol and lupeol epoxide, identified in the root barks of *A. maurorum* (Laghari *et al*., 2011), were found responsible for the antiproliferative activity against MCF-7 and MDA-MB-231cancer cell lines (Behbahani, 2014).

CONCLUSION

A. graecorum carries powerful antimicrobial and remarkable cytotoxic activities against the tested microbial strains and cancer cell lines, respectively. Results revealed that both activities mainly reside in the ethyl acetate and dichloromethane fractions, which can be a potential source of antibiotics and anticancer compounds. This plant should be considered for further phytochemical investigation and pharmacological evaluation.

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