Pak. j. sci. ind. res. Ser. B: biol. sci. 2015 **58** (3) 147-154

In-vitro Phytochemical and Antibacterial Activity of *Abies cilicica* subsp. *cilicica*

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(received March 4, 2015; revised June 18, 2015; accepted July 27, 2015)

Abstract. Leaf and flowering cones methanolic, ethanolic and acetonic extracts of *Abies cilicica* subsp. *cilicica* plant were screened for phytochemical and inhibitory effect against 8 bacterial isolates. Qualitative phytochemical assay revealed that, flowering cones acetone extract exhibited the most of bioactive compounds compared to the leaf extracts with all examined solvents. Antibacterial activity of *A. cilicica* subsp. *cilicica* was determined by measuring the zone inhibition diameter (ZIs), activity index (A.I) and minimum inhibitory concentrations (MICs) against 8 bacteria (S*taphylococcus aureus, Listeria monocytogenes, Bacillus cereus, Salmonella typhimurium, Escherichia coli*: O157, *Acinetobacter baumannii, Brucella abortus* and *Pseudomonas aeruginosa*) isolates. Overall, acetonic flowering cones extracts were more potent against all tested isolates compared to the leaf ones. The lowest MICs value was recorded to be 0.42, 0.52 and 1.04 µg/mL for acetone, methanol and ethanol flowering cones, respectively, against the same pathogen *A. baumannii*. Based upon the current investigation, *A. cilicica* spp. *cilicica* could be considered as a potential endemic source against bacterial isolates.

Keywords: *Abies cilicica*, antibacterial activity, phytochemical assay, flowering cones

Introduction

Abies cilicica subsp. *cilicica* is an endemic subspecies to the mountains adjacent to the north-eastern Mediterranean coast. It occurs in Syria, Lebanon and Turkey. In Syria, it occurs at Slenfch (Lattakia) and forms mixed forests with *Ostrya carpinifolia, Carpinus orientalis, Sorbus torminalis, Fraxinus ornus* and *Cerasus mahleb* (Browicz, 1982). It is known as Cilician fir as an associated name. It is evident that this subspecies becomes one of the near threatened in the world. It worth noting that this subspecies is threatened in Syria and Lebanon (Knees and Gardner, 2013).

In Syria it grows in nature reserve located at 1500 m altitudes and occupies an area of 1350 hectares of a series of Syrian coastal mountains on eastern and western summit of the Prophet Mata (the highest peak in the Syrian coastal mountains 1562 m). It has been declared natural reserve since 1996, but the start of its implementation was delayed until 2002. It has been protected by the financing of the Global Environment Facility.

A. cilicica (Ant. and Kotschy) subsp. *cilicica* (Cilician fir) belongs to the Pinaceae (Abietaceae) family. *Abies* genus involved 10 species and divided into 2 sub-species: subsp. *cilicica* (Buds not rosinous; young shoots hairy) and subsp. *isaurica* (Buds reinous; young shoots glabrous). *A. cilicica* subsp. *cilicica* is native to Mediterranean region of Turkey (Dayisoylu *et al*., 2009; Davis, 1967).

It has been demonstrated that, the essential oil cones *A. cilicia* subsp. *cilicica* has antimicrobial activity due to the effective compounds found, mainly, limonene, α-pinene, β-pinene, and myrcene (Dayisoylu *et al*., 2009). Whereas, Alma *et al*. (2003) reported the antimicrobial activity of leaves essential oil of Syrian oreganum (*Origanum syriacum* L.). The later investigation revealed that, γ-terpinene, carvacrol, *p*-cymene and β-caryophyllene were the major compounds present in the *O. syriacum* L. leaf oil.

Recently, Patel *et al*. (2014) reported biological activity of *A. pindrow* leaves extracts and found that, leaf methanolic extract exhibited antioxidant effect due to presence of phenol and flavonoids. While, bronchoprotective activity was also attributed to the presence of terpenoids and flavonoids in leaf benze, acetone and ethanol extracts.

Few investigations focused on antibacterial and antifungal activities of *Abies* spp. extracts (Dayisoylu *et al*., 2009; Lee and Hong, 2009; Kizil *et al*., 2002; Diğrak *et al*., 1999; Bağci and Diğrak, 1997). Vishnoi

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et al. (2007) reported the antibacterial and antifungal activity of leaf of *A. webbiana* extract, while, Benli *et al*. (2008) described the antimicrobial activity of six endemic plant species from Turkey, *A. nordmanniana* subsp. *bornmuelleriana* is one of them. More recently, Patel *et al*. (2014) reported different pharmacological activities (anti-inflammatory, antioxidant, antiulcerogenic, antidiabetic and anxiolytic) activity of *A. pindrow* leaves extracts.

The study of biologically active compounds of plants has always been very interesting to researchers looking for novel sources of practical alternative against diseases (Alfatemi *et al*., 2015; Sharifi-Rad *et al*., 2015; 2014a; 2013), Pharmacological investigations in *Abies* spp. have been mainly focused on cones and resins essential oil activity. However, *Abies* spp. antibacterial inhibitory effect has not yet been examined in detail. Thereby, the current study was aimed to assess their potential use against different gram-positive and negative bacterial pathogens. A comparative assessment of its methanol, acetone and ethanol leaves and flowering cones extracts were studied.

Materials and Methods

Plant materials. Samples (leaf and flowering cones) were harvested from their natural habitats along the Syrian coastal mountains (Slenfch-Lattakia) at 1500 m altitudes with 1400 mm annual rainfall. Sampling was carried out in autumn 2013 (35°36 21 N longitude and 36°11 18 E latitude) and fractions plants were shade dried for one week, powdered by special electric mill and stored separately in polyethylene bags until needed for analysis.

Microorganisms and growth conditions. The pure clinical isolates of *Staphylococcus aureus, Listeria monocytogenes, Bacillus cereus, Salmonella typhimurium, Escherichia coli*: O157, *Acinetobacter baumannii, Brucella abortus* and *Pseudomonas aeruginosa* were collected from the Microbiology and Immunology Division, Department of Molecular Biology and Biotechnology of Atomic Energy Commission of Syria (AECS) in Damascus-Syria. These bacteria present considerable interest in clinical laboratories.

The cultures were maintained at 37 °C on 2YT agar (peptone, 16 g/L; yeast extract, 10 g/L; NaCl, 5 g/L; agar, 13 g/L [Difco, BD, Spars, MD]); and incubated for 24-48 h. Prior to antimicrobial sensitivity test, 0.2 mL of overnight culture of each organism was dispensed into 20 mL of sterile Mueller Hinton Broth (Hi-media Laboratory Pvt. Ltd., Mumbai, India) and then incubated for about 18-24 h to standardize the cultures to approximately 10^6 CFU/mL (Al Mariri *et al*., 2013). The bacteria were suspended in a sterile phosphate-buffered saline (PBS). Bacterial abundance in PBS was monitored by recording the optical density (OD) at 590 nm. The exact counts were assessed retrospectively by viable counts on 2YT agar plates.

Extraction of plant material. Crude extracts of *A. cilicica* leaf (LE) and flowering cones (FE) were phytochemicals screened using methanol, ethanol and acetone solvents as previously reported in many investigations (Gupta *et al*., 2011; Abdullahi *et al*., 2010; Rajesh *et al*., 2010).

For crude extracts preparation 500 g of shadedried pulverised plant material were subjected to extraction in a Soxhlet apparatus successively with solvent 10 times the volume of plant extract. The extraction was conducted until no more coloured matter was extracted. Solvent from each extracted mixture was evaporated to dryness using a rotary evaporator under reduced pressure at 40 °C. All dried extracts were then kept in tightly fitting stopper bottles and stored in 4 °C. The concentration of extract was considered 100 mg/mL.

Phytochemical screening of extracts. Qualitative assay for the presence of secondary plant metabolites was carried out on the methanolic, ethanolic and acetonic leaf and flowering cones of *A. cilicica* for the presence of phytochemicals *e.g*. alkaloids, flavonoids, saponins, terpenoids, tannins and steroids compounds using the standard procedures previously described by Sharifi-Rad *et al*. (2014b), Aida *et al*. (2001), Harborne (1991) and Trease and Evans (1989).

Determination of alkaloids content. The alkaloids content was determined as follows: 0.5 g of the sample was accurately weighed and defatted with 5% ethyl ether for 15 min. The defatted sample was extracted for 20 min with 5.0 mL of 2 M HCl on a steam bath. The resulting mixture was centrifuged for 10 min at 3000 rpm to remove supernatant. One mL of the filtrate was treated with a few drops of Mayer's reagent and a second 1.0 mL portion was treated similarly with Dragendorff's reagent. The observed coloured precipitates in the test tubes for either of these reagents, was taken as evidence for the presence of alkaloids.

Determination of flavonoids content. For flavonoids test, 1.0 mL of 10% lead acetate was added to 1.0 mL

of the extract contained in a test tube. The formation of a yellow precipitate was taken as positive for flavonoids.

Determination of saponins content. The ability of saponins to produce frothing in aqueous solution was used as a screening test for the sample. 0.5 g of dried extract was shaken with water in a test tube, frothing (which persists on warming), was taken as evidence for the presence of saponins.

Determination of tannins content. For tannins test, 5.0 g of dried extract was stirred with 10.0 mL of distilled water. This was filtered and ferric chloride reagent (Ferric chloride hexahydrate: 7.50 g, concentrated hydrochloric acid: 1.0 mL, water to 100 mL) was added to the filtrate. The formation of green precipitate was an indication of the presence of tannins.

Determination of steroids and terpenoids content. Chloroform extract (0.5 mL) of the dried extracts was evaporated to dryness on a water bath and heated with 3 mL of concentrated sulphuric acid for 10 min on a water bath, shaken well and allowed to stand. Appearance of red colour in the lower layer indicated the presence of steroids. Formation of reddish brown colour of interface after addition of concentrated sulphuric acid to the side carefully (without shaking) indicated the presence of terpenoids.

Antibacterial activity assay. *The disc-diffusion assay.* The disc-diffusion method was adopted to test the antibacterial activity as reported by earlier scientists (Derwich *et al*., 2010; Benli *et al*., 2008; Diğrak *et al*., 1999). Ciprofloxacin was used as a standard drug to compare the results of experimental plant. Filter paper discs (Whatman No.1) of 6 mm diameter were prepared and sterilised. The discs impregnated with 100 µL of extract dilutions (100 mg/mL) and reconstituted in minimum amount of methanol were applied over each of the culture plates previously seeded with the 10^6 CFU/mL cultures of bacteria. Bacterial cultures were then incubated at 37 °C for 18 h, while the paper discs impregnated with 20 µL of a solution of 10 mg/mL of ciprofloxacin were used as standard antimicrobials for comparison. Negative control was prepared using methanol (final concentration of the solvent in the highest concentration of plant extract was tested). Antimicrobial activity was determined by measurement of zone of inhibition (in mm) around each paper disc. For each extract, duplicate trials were conducted against each organism.

Activity index. Activity index (A.I) of *A. cilicica* subsp. *cilicica* plant extracts was calculated as previously reported by Gopalakrishnan *et al*. (2012) using the following formulae:

Activity index (A.I) = Inhibition zone of sample/ Inhibition zone of standard.

Commercial antibiotics ciprofloxacin (100 mg/mL) was used as standard for antibacterial activity.

Determination of minimum inhibitory concentrations (MICs). Microdilution broth susceptibility test was assessed according to Ríos-Dueñas *et al*. (2011). Three replicates of serial dilutions of extract (50 mg/mL) were prepared in LB broth medium in 96-well microtiter plates, using a range of concentrations for methanol and aqueous extracts of both *A. cilicica* leaf (LE) and flowering cones (FE) from 0.166 to 40 µL per well. One hundred microlitres of freshly grown bacteria standardised 10^6 CFU/mL in LB broth were added to each well. Positive control was achieved with the same conditions but without extract, negative control was also made with the same conditions but without adding the bacteria. The plate was incubated with shaking for 24 h at 37 °C. The lowest concentration that completely inhibited visual growth was recorded and interpreted as the MICs.

Statistical analysis. All statistical tests were performed in triplicates and values are presented as mean±SD. Data analyses were evaluated by two-ways analysis of variance (ANOVA). All analyses were conducted with version 5.0 GraphPad Prism. P values of 0.05 or less were considered statistically significant.

Results and Discussion

Phytochemical analysis. Phytochemical test of LE and FE *A. cilicica* was studied in order to support their observed biological assay against tested bacteria isolates. Flavonoids and terpenoids were present in the three tested solvents (methanol, acetone and ethanol) (Table 1). While, tannins were presented in leaf extract in opposite manner to flowering cones. Saponins were present in leaf extract for the three examined solvents; whereas, they present only in acetone flowering cones and absent in methanol and ethanol of the same plant part (Table 1).

Bağci *et al*. (1999) investigated the essential oil composition of two shoots *A. cilicica* subsp. *cilicic*a and *isaurica* in Turkey using GC-MS analysis. The later

Table 1. Phytochemical test of leaf and flowering cones *A. cilicica* subsp. *cilicica* extracts

Chemical components	LE			FE		
	Methanol	Acetone	Ethanol	Methanol	Acetone	Ethanol
Alkaloids	+	-	-	-	+	-
Flavonoids	+	+	+	+	+	+
Saponins	+	+	+	-	+	-
Terpenoids	+	+	+	+	+	+
Tannins	-	+	-	+	-	+
Steroids	-	-	-	+	+	-

LE = leaf extract; FE = flowering cones extract; + = present; - = absent

study revealed that oil of subsp. *cilicica*, was characterised by 57 compounds in which car-3-ene (14.2%), caryophyllene oxide (8.6%) and β-caryophyllene (7.8%) were the major constituents. While, for subsp. *isaurica*, 23 compounds were characterised in their oil, where, β-pinene (29%), α-pinene (10%), eremophilene (9.3%) and β-caryophyllene (8.8%) were the major compounds. Dönmez *et al*. (2012) and Kilic *et al*. (2010) reported that *Abies* species cones exhibited more phenolic compounds compared to other coniferous growing naturally in Turkey.

Phytochemical content and *in-vitro* antioxidant activities of *A. pindrow* LE dichloromethane, methanol and acetone extracts were investigated by Gupta *et al*. (2011). The study revealed that, acetone extract exhibited the highest total phenolic, flavonoid and flavonol content followed by methalonic then dichloromethane extract.

Recently, Uçar and Uçar (2014) reported the geographical distribution impact on the chemical compounds of needle oils of *A. bornmuelleriana* in Turkey using GC-MS/FID analysis. The later investigation revealed that α-pinene and camphene% compounds among 38 chemicals compounds, increased in Turkey west-east direction.

Zone of inhibitions (ZIs) and activity index (A.I) assay. Susceptibility of examined bacteria isolates to LE and FE *A. cilicica* extracts has been presented in Table 2. In this regards, ZIs values ranged between 14 mm with ethanol LE against *P. aeruginosa* and 24 mm with methanol FE against *S. typhimurium* isolate.

Table 2 shows that *S. typhimurium* was the most sensitive isolate with ZIs value of 20 and 24 mm with methanol LE and FE, 19 and 22 mm with ethanol LE and FE; while, it was recorded to be 20 and 23 mm with acetone LE and FE extracts, respectively. This observation suggests that, among the three tested solvents, acetone and methanol extracts were more potent than ethanol. Otherwise, FE extracts exhibited highest inhibitory effect against tested bacteria compared to the LE one.

Variance analysis showed that the effect of different extracts from the same plant parts using different solvents was significantly ($p<0.05$) different. In this respect, it was more significant ($p<0.001$) vs acetone for *B. cereus*; vs ethanol for *B. abortus* and also vs acetone for *P. aeruginosa* (Table 2). Moreover, our data revealed significant ($p<0.05$) differences regarding the effect of the same solvent using extracts from different plant parts. In this regards, these differences were more significant ($p<0.001$) vs FE for *S. typhimurium* and *A. baumannii*, vs FE for *A. baumannii* and also vs FE for *L. monocytogeneses* and *A. baumannii* (Table 2).

Previously, Bağci and Diðrak (1997) studied the antimicrobial effects of 4 fir essential oil against 12 microorganisms. The later study revealed that all extracts had antimicrobial activity against examined bacteria at different ratios. Moreover, not all tested extracts had inhibitory effect against *E. coli, S. aureus* and *S. cerevisiae* isolates.

Moreover, Diğrak *et al*. (1999) reported the antimicrobial activity of 5 trees grown in Turkey. In the later study, chlorophorm, acetone and methanol (leaves, resins, bark, cones and fruits) were examined against 14 microorganisms. The later investigation showed that, the growth of *E. coli* was not affected by different plant parts extracts except by chlorophorm and acetone *A. cilicica* LE with ZIs of 16 and 18 mm, respectively. While, Benli *et al*. (2008) reported that the ZIs value of LE *A. nordmanniana* extract was found to be 14 mm for *B. subtilis* RSHI pathogen.

Hemaiswarya *et al*. (2009) reported that, the mean ZIs of *Ficus religiosa, Thespesia populnea* and *Hibiscus tiliaceus* leaves aqueous, methanol and chloroform extracts against 9 microorganisms ranged between 10 mm (*S. aureus*) and 21 mm (*P. aeruginosa* and *S. typhimurium*). While, Derwich *et al*. (2010) reported the antibacterial activity of the leaves oil of *Cedrus atlantica* against 7 bacteria isolates. The later study revealed that *E. coli, P. aeroginosa* and *S. aureus* were the most sensitive tested pathogens as showed by the highest ZIs of 25, 21 and 22 mm, respectively. Whereas, *K. pneumoniae, S. intermedius* and *Enterococcus faecalis*, were found to be more sensitive among bacteria with ZIs of 12, 19 and 11 mm, respectively. While,

moderate inhibitory effect was observed against *Bacillus sphericus* with ZIs of 6 mm.

Table 2 shows that, the tested bacteria responded to *A. cilicica* extracts in different manner according to the examined plant fraction, tested solvent and bacteria isolate. In this regards, the highest ZIs value was recorded with methanol FE against *S. typhimurium* isolate (24 mm) (Table 2), whereas, the lowest ZIs one was pronounced with ethanol leaf against *P. aeruginosa* (14 mm).

Activity index (A.I) was also estimated and Table 3 shows that, ethanol LE exhibited the lowest A.I values against all tested isolates with A.I of 0.6 against *S. typhimurium*, While, the highest antibacterial effect value was recorded for acetone and methanol FE with 1.2 against *P. aeruginosa* and *B. abortus* (Table 3). Similar finding (A.I = 1.2) was also observed in the case of methanol FE against *B. abortus* (Table 3).

From the data presented in Table 3, variance analyses showed that the effect of different extracts from the same plant parts using different solvents; and the effect of the same solvent using extracts from different plant parts, were significantly ($p<0.05$) different (Table 3). Significant differences regarding A.I was followed the same tendency as for ZI.

Table 2. Antibacterial activity of the leaf and flowering cones *A. cilicica* subsp. *cilicica* extracts using disc-diffusion method

Microorganisms	Zone of inhibition (mm)						Control
	Methanol		Ethanol		Acetone		
	LE	FE	LE	FE	LE	FE	
S. aureus	17 ± 0.09^{a}	20 ± 0.25	16 ± 0.11^{c}	19 ± 0.20	18 ± 0.2^{g}	21 ± 0.17	24 ± 0.12
L. monocytogeneses	17 ± 0.14	19 ± 0.14	15 ± 0.07	$17 \pm 0.12^{++++}$	16 ± 0.15^{h}	20 ± 0.14	19 ± 0.14
B. cereus	$15 \pm 0.07^{***}$	17 ± 0.1	$17 \pm 0.12^{*}$	$18 \pm 0.13^{++++}$	19 ± 0.17	20 ± 0.09	21 ± 0.12
S. typhimurium	20 ± 0.22^{b}	$24 \pm 0.26^{+}$	19 ± 0.15^{d}	22 ± 0.21	20 ± 0.14^{f}	23 ± 0.16	34 ± 0.1
E. coli O:157	$18 \pm 0.15^{*a}$	21 ± 0.17	$17 \pm 0.07^{*c}$	$19 \pm 0.15^{++++}$	19 ± 0.15^{f}	22 ± 0.21	27 ± 0.02
A. baumannii	$19 \pm 0.08^{****b}$	$23 \pm 0.27^{++}$	16 ± 0.14^{e}	20 ± 0.17	17 ± 0.16^{h}	22 ± 0.15	25 ± 0.18
B. abortus	17 ± 0.16^{a}	$20 \pm 0.14^{+++}$	15 ± 0.16	$17 \pm 0.22^{++++}$	17 ± 0.21^{f}	20 ± 0.27	17 ± 0.2
P. aeruginosa	$15 \pm 0.12^{**}$	17 ± 0.12	$14 \pm 0.09^{**}$	$15 \pm 0.17^{++++++}$	17 ± 0.14	19 ± 0.23	15 ± 0.14

Table 3. Activity index (A.I) of the leaf and flowering cones *A. cilicica* sp. *cilicica* extracts

Microorganisms	Activity index (A.I)					
	Methanol		Ethanol		Acetone	
	LE	FE	LE	FE	LE	FE
S. aureus	0.7 ± 0.0^{a}	0.8 ± 0.01	0.7 ± 0.00^{c}	0.8 ± 0.01	0.7 ± 0.01^{g}	0.9 ± 0.01
L. monocytogeneses	0.9 ± 0.01	1.0 ± 0.01	0.8 ± 0.00	$0.9 \pm 0.01^{++++}$	0.8 ± 0.01^{h}	1.0 ± 0.01
B. cereus	$0.7 \pm 0.00^{***}$	0.8 ± 0.00	$0.8 \pm 0.01^{*}$	$0.8 \pm 0.01^{++++}$	0.9 ± 0.01	0.9 ± 0.00
S. typhimurium	0.6 ± 0.01^{b}	$0.7 \pm 0.01^{+}$	0.6 ± 0.00^{d}	0.6 ± 0.01	0.6 ± 0.00^{f}	0.7 ± 0.00
E. coli O:157	$0.7 \pm 0.01^{*a}$	0.8 ± 0.01	$0.6 \pm 0.00^{*c}$	$0.7 \pm 0.01^{++++}$	0.7 ± 0.01^{f}	0.8 ± 0.01
A. baumannii	$0.8 \pm 0.00^{****b}$	$0.9 \pm 0.01^{++}$	0.6 ± 0.01^{e}	0.8 ± 0.01	0.7 ± 0.01^{h}	0.9 ± 0.01
B. abortus	1.0 ± 0.01^{a}	$1.2 \pm 0.01^{+++}$	0.9 ± 0.01	$1.0 \pm 0.01^{++++}$	1.0 ± 0.01^{f}	1.2 ± 0.02
P. aeruginosa	$1.0 \pm 0.01^{**}$	1.1 ± 0.01	$0.9 \pm 0.01^{**}$	$1.0 \pm 0.01^{++++++}$	1.1 ± 0.01	1.2 ± 0.02

LE = leaf extract; FE = flowering cones extract.

(1) Comparing the effect of different extracts from the same plant parts using different solvents: * = $p<0.05$ vs acetone for *B. cereus* and *E. coli* O:157; ** = $p<0.01$ vs acetone for *P. aeruginosa*; *** = $p<0.001$ vs acetone for *B. cereus*; **** = $p<0.01$ vs ethanol for *A. baumannii*; $^{+}$ = $p<0.05$ vs ethanol for *S. typhimurium*; $^{++}$ = $p<0.01$ vs ethanol for *A. baumannii*; $^{+++}$ = $p<0.001$ vs ethanol for *B. abortus*; $^{++++}$ = $p<0.01$ vs acetone for *L. monocytogeneses*, *B. cereus*, *E. coli* O:157 and *B. abortus*; $^{++++++}$ = $p<0.001$ vs acetone for *P. aeruginosa*.

(2) Comparing the effect of the same solvent using extracts against different plant parts: ^{a} = $P<0.01$vs FE for *S. aureus*, *E. coli* O:157 and *B. abortus*; ^{b} = $p<0.001$vs FE for *S. typhimurium* and *A. baumannii*; ^{c} = $p<0.05$ vs FE for *S. aureus* and *E. coli* O:157; ^{d} = $p<0.01$ vs FE for *S. typhimurium*; ^{e} = $p<0.001$ vs FE for *A. baumannii*; ^{f} = $p<0.05$ vs FE for *E. coli* O:157, *B. abortus* and *S. typhimurium*; ^{g} = $p<0.01$ vs FE for *S. aureus*; ^{h} = $p<0.001$ vs FE for *L. monocytogeneses* and *A. baumannii*.

Other investigation, however reported the antibacterial effect of essential oil extracts for *A. holophylla* and *A. kornean*. The later investigation indicated that *A. kornean* oil was more potent as antibacterial and antifungal properties than *A. holophylla* (Lee and Hong, 2009).

Minimum inhibitory concentrations (MICs) test. Susceptibility and MICs was investigated against 8 bacterial isolates using standard antibiotics ciprofloxacin (100 mg/L) as a reference.

The tube dilution method was applied to determinate the MICs. Calculated MICs values were illustrated in Table 4. MICs was determined; these values were varied mainly according to the plant part (Table 4). It was worth noting that, the best MICs value was recorded to be 0.42 and 0.52 µg/mL for acetone and methanol FE against *A. baumannii* followed by *E. coli* O:157 (0.63 µg/mL) and *S. typhimurium* (0.83 µg/mL) with FE acetone extract (Table 4).

Our results showed no significant differences regarding the effect of different extracts from the same plant parts using different solvents, and the effect of the same solvent using extracts from different plant parts on MICs; except $P<0.05$ *vs* FE for *L. monocytogeneses* (Table 4).

Phytochemical test revealed the presence of steroids and alkaloids in acetonic FE and their absence in the other extracts, could explain the difference in their biological activity. These chemical components differ in their solubility degree according to examined solvent. These secondary metabolites have been successfully identified in plant extracts and investigated on its anti-microbial inhibitory against some bacterial pathogens (Gupta *et al*., 2011; Adeshina *et al*., 2010; Ebana *et al*., 1991). These compounds had different inhibitory mechanisms against microorganisms and acted in different manners. Thereby, the synergic effect of all bioactive compounds presented in FE acetonic makes it as the most potent extract among the different examined crude extracts.

Dayisoylu *et al*. (2009) reported the antimicrobial effect of *A. cilicica*. The later study mentioned that, the highest MICs value (3.5 µg/mL) was determined against *P. aeruginosa* and *K. pneumonia*. Our observation was in accordance of the previous finding, where MICs value was found to be 3.33 and 4.17 µg/mL for methanol FE and LE, respectively, against *P. aeruginosa*.

Lee and Hong (2009) reported the antibacterial effect of essential oil extracts for *A. holophylla* and *A. kornean*. The previous study showed the great inhibitory effect extracts against different tested bacteria in range of 2.2-8.8 µg/disc by the agar disc-diffusion methods with MICs value of 5.5-21.8 mg/mL by the microdilution methods.

Previously, Vishnoi *et al*. (2007) reported that, the methanol LE of different species of *Abies* spp. exhibited wide spectrum antimicrobial activity. While, Benli *et al*. (2008) reported the antimicrobial activity of *A. nordmanniana* subsp. *bornmuelleriana* endemic plant species from Turkey. The later investigation revealed that, the MICs of LE *A. nordmanniana* extract was 314 mg/mL against *B. subtilis* with minimum bacteriocidal concentration (MBC) value of 4.91 mg/mL.

Lee and Hong (2009) reported the antibacterial effect of essential oil extracts for *A. holophylla* and *A. kornean*.

Table 4. Minimum inhibition concentrations (MICs) values of the leaf and flowering cones *A. cilicica* sp. *cilicica* extracts against the pathogenic bacterial isolates

Microorganisms	Minimum inhibition concentrations (MICs) (µg/mL)					
	Methanol		Ethanol		Acetone	
	LE	FE	LE	FE	LE	FE
S. aureus	6.67 ± 2.89	4.17 ± 1.44	8.33 ± 2.89	5.00 ± 0.00	5.83 ± 3.82	3.33 ± 1.44
L. monocytogeneses	6.67 ± 2.89	3.33 ± 1.44	8.33 ± 2.89	5.00 ± 0.00	8.33 ± 2.89*	3.33 ± 1.44
B. cereus	8.33 ± 2.89	3.33 ± 1.44	6.67 ± 2.89	3.33 ± 1.44	6.67 ± 2.89	3.33 ± 1.44
S. typhimurium	2.92 ± 1.91	1.04 ± 0.36	2.08 ± 0.72	1.45 ± 0.96	1.67 ± 0.72	0.83 ± 0.36
E. coli O:157	2.92 ± 1.91	0.83 ± 0.36	3.33 ± 1.44	1.04 ± 0.36	2.08 ± 0.72	0.63 ± 0.00
A. baumannii	2.08 ± 0.72	0.52 ± 0.18	2.92 ± 1.91	1.04 ± 0.36	2.08 ± 0.72	0.42 ± 0.18
B. abortus	2.08 ± 0.72	1.67 ± 0.72	2.5 ± 0.00	2.08 ± 0.72	2.92 ± 1.91	1.04 ± 0.36
P. aeruginosa	4.17 ± 1.44	3.33 ± 1.44	5.83 ± 3.82	6.67 ± 2.89	4.17 ± 1.44	4.17 ± 1.44

LE = leaf extract; FE = flowering cones extract; * = $p<0.05$ vs FE for *L. monocytogeneses*.

The later investigation revealed that *Abies* extracts oils was highly potent against bacteria with MICs value of 5.5-21.8 mg/mL using the microdilution methods. Whereas, Derwich *et al*. (2010) investigated inhibitory effect of the leaves oil of *C. atlantica* against 7 bacteria pathogens. The previous study showed that, this extract was active against *E. coli, P. aeroginosa, S. aureus* and *S. intermedius* with MICs value of 0.25, 0.98 0.68 mg/mL and 1.25 mg/mL, respectively. While, it exhibited more potent activity against *K. pneumoniae, E. faecalis* and *B. sphericus* with MICs value of 1.45, 1.31 and 1.62 mg/mL, respectively.

Recently, Patel *et al*. (2014) described the biological activity of *A. pindrow* LE. They reported that ethanol LE exhibited anti-inflammatory, antidiabetic and anxiolytic activity, antioxidant effect with LE methanolic extract while, antiulcerogenic activity was detected with petroleum ether, benzene and chloroform extracts.

Conclusion

Phytochemical test and inhibitory effect of the crude ethanol, methanol and acetone of LE and FE of *A. cilicica* subsp. *cilicica* were evaluated against 8 bacteria isolates. It was noticed that, FE acetone extract exhibited the most of bioactive compounds compared to the LE with all examined solvents. It is worth noting that the *A. cilicica* antibacterial activity against both gram positive and negative bacteria was in the order of acetone < methanol < ethanol. Overall, FE were more potent against all tested isolates compared to the LE ones. On the other hands, ethanol extract had the lowest antibacterial activity by showing the smallest ZIs values. Overall, *A. baumannii* isolate could be considered as the most sensitive pathogen to *A. cilicica* extracts by showing the lowest MICs values of 0.42, 0.52 and 1.04 µg/mL for acetone, methanol and ethanol FE, respectively.

Acknowledgement

We thank I. Othman (Director General of AECS) and N. Mir Ali (Head of Molecular Biology and Biotechnology Department in AECS) for their support, and also the Plant Biotechnology Group for technical assistance. Indeed, thanks to Mr. M. Safi for his help in statistical analysis performance.

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