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Leaf and branch extracts of Eriobotrya japonica exert antibacterial activity against ESBL-producing Escherichia coli and Klebsiella pneumoniae

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Abstract

In this study, **t**he antimicrobial activity of leaves and branches of Eriobotrya japonica, a Lebanese endegenious plant, against Extended Spectrum Beta Lactamase -producing Escherichia coli and Klebsiella pneumoniae was determined and the specific plant fraction responsible for this antimicrobial activity were identified. The plants were extracted with ethanol to yield the crude extract which was further subfractionated by different solvents to obtain the petroleum ether, the dichloromethane, the ethyl acetate and the aqueous fractions. The Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) were determined using broth microdilution. Both inhibitory and bactericidal effects of Eriobotrya japonica on Escherichia coli and Klebsiella pneumoniae were mainly observed with the crude extract of the plant, the ethyl acetate, the Dichloromethane**,** and the aqueous fractions. The antibacterial effect of the Petroleum ether fraction was limited with the leaf extract; however, it was acceptable with the branch extract. The lowest MIC_{90} was observed with ethyl acetate fraction for both leaf and branch extracts with Escherichia coli and Klebsiella pneumoniae. The concentrations at which most of strains were inhibited ranged between 40 µg/µl and 80 µg/µl. MICs and MBCs effects were detected within 1 dilution. This study constitutes a good example for the screening of antimicrobial activities of plants on highly resistant organisms of clinical importance; however, toxicity of these extracts needs more investigation.

Keywords: Eriobotrya japonica, Extended Spectrum Beta Lacatamase, Minimum Inhibitory Concentration, medicinal herbs.

Introduction

The appearance of resistant pathogens paved the way to the occurrence of infections that are only treated by a limited number of antimicrobial agents. Bacterial resistance to antimicrobial agents is a medical problem with public health, socio-economic and even political implications. The change in the resistance patterns will continue to menace the developed and developing countries. The world is nowadays witnessing an emergence of several multi-drug resistant organisms rendering the treatment options more and more limited. Resistance in Gram negative bacteria presents a major challenge for the antimicrobial therapy and significantly narrows the treatment options of human infections [1]. Extended Spectrum Beta-Lactamase (ESBL) producing bacteria are spread worldwide. Their prevalence in Lebanon is increasing through the

years and their incidence depends on the region and environment [2, 3]. In view of the increase in ESBL resistance, and the negligible development of antibiotics in the past few years, there is an urgent need for new antibacterial compounds in order to fight the emergence of these new resistant pathogens.

Plants have been used as remedies and treatments of diseases; there is evidence that they were used in ethnomedicine 60,000 years ago in Iraq [4,5]. The Middle Eastern Mediterranean region is rich in plant species; there are about 2,600 species of which many are considered to have medicinal effects. However, there is relatively limited research on medicinal plants in this region [5]. Plant derived antimicrobials represent a vast untapped source for medicines and further exploration of plant antimicrobials needs to occur [5]. In consequence, plants are starting to be considered as the base of modern medicine and antibiotic production [6]. We had previously studied the antibacterial activity of Rosmarinus officinalis, Origanum majorana, and Trigonella foenum-graecum and found important effect on highly resistant bacteria [7]

The antimicrobial activity of a plant is highly related to secondary substances that are synthetized and produced by these plants. Secondary metabolites are substances of low molecular weight, which were not products of the primary metabolic pathway of the producing organism and at first thought to be with no advantage to the plant. Nowadays it is believed that they have vital functions. They may act as messenger molecules under specific circumstances (e.g. against the aggression) or natural pressures in order to protect the producer organism. They also give plants their pigment and odors [8].

Eriobotrya japonica,
A member of the Rosaceae family, is an Asian plant with fruits known as loquat. Its leaves have been used in folk medicine for treatment of chronic bronchitis, coughs, phlegm, high fever, and ulcers in Japan and other Asian countries. It contains terpenoids and flavonoids that have been found in the leaves and some of these compounds have been reported to be biologically active, exhibiting anti-inflammatory, anti-HIV, and hypoglycemic properties [9, 10]. Eriobotrya japonica was shown to have antibacterial activity, the water extract inhibited the growth some gram positive and gram negative bacteria [11]. The seeds extracts were also shown to suppress hyperglycemia and improve glucose tolerance in mice with type 2 diabetes [9].

The aim of this study was to determine the antimicrobial activity leaf and branch extracts of this plant against microorganisms with high level of acquired resistance to traditional antibiotics (Escherichia coli and Klebsiella pneumoniae producing Extended Spectrum Beta Lactamases) and to identify the specific fraction/s responsible for the antimicrobial activity.

Materials and Methods

Bacterial strains

Twenty strains of Escherichia coli and ten strains of Klebsiella pneumoniae were isolated at the clinical microbiology laboratory of the Saint George Hospital-University Medical Center, between December 2007 and May 2009. In addition to being ESBL producers, these isolates exhibited different profiles of resistance.

Selected Plants

The herbal sample consisted of leaves and branches of an indigenous Lebanese plant: Eriobotrya japonica**.** They were collected from different Lebanese areas and directly from nature. They were identified and characterized by a taxonomist. The name of the plant, time, place and date of collection were recorded.

Antimicrobial activity, ESBL and AmpC Detection.

The Antimicrobial Susceptibility Testing was performed as recommended by the Clinical and Laboratory Standards Institute [12,13]. The production of ESBL was detected phenotypically

using the double disk synergy method described previously [14]. The strain showing a key-hole effect between one or more of the third cephalosporin disks and the amoxicillin/clavulanic acid disk or showing a boost of the inhibition zone of one of the third generation cephalosporin disks toward the amoxicillin/clavulanic acid disk was considered as an ESBL producer. The antimicrobial agents that were tested were: ampicillin, piperacillin, imipenem, amoxicillin/clavulanic acid, piperacillin/tazobactam, cephalotin, cefoxitin, cefuroxime, ceftriaxone, ceftazidime, cefepime, gentamicin, ciprofloxacin, ofloxacin, tigecycline and trimethoprim/sulfamethoxazole. The breakpoints for the different antibacterial agents recommended by the CLSI were used. Since tigecycline has no CLSI breakpoints, the SFM guidelines were adopted for this antibiotic as alternative (Diameter < 19 mm for Resistance). Although resistance to cephamycins cannot be a confirmatory test for AmpC production and might be conferred sometimes by ESBLs, resistance to cephamycin was looked at as an indicator for AmpC production since this is true in the majority of the cases.

Preparation of Crude Extract. Fresh plants were dried in the shade at room temperature and ground in a coffee bean grinder. The dried plant material was weighed and then soaked in 80% ethanol for 7 days with continuous shaking in a shaker at room temperature. At day seven the plant material was filtered and the filtrate collected. This was repeated and the filtrates were combined and concentrated in a rotary evaporator to obtain the crude extract (fraction 1).

Fractionation Method.

The crude extract of each plant was further partitioned by extraction with different solvents in a 1:1 (v/v) ratio in order to sub-fractionate the plant components according to their polarity: petroleum ether (fraction 2), dichloromethane (fraction 3), and ethyl acetate (fraction 4). Extractions were repeated three times and fractions were combined. The remaining aqueous layer was collected as fraction number 5. Fractions 1 and 5 were dried using a freeze dryer, but fractions 2, 3 and 4 were dried under the hood to dryness due to the inconvenience of introducing vapor solvent into the freeze dryer. Controls were prepared for each fraction by drying the same amount of solvent and following the same subfractionation method without plant extract (solvent control).

Study of Antimicrobial Activity of the Plant Extracts

The plant powders were weighed and dissolved in sterile distilled water. The solutions were filtered through 0.22 µm sterile filter membranes and stored at 4 ˚C for further use. The concentration of the original solution of the plant extract/fraction corresponds to the concentration obtained after re-suspension of the dried plant extracts. This was used as the stock solution and the most concentrated one from which the MIC series were prepared.

MIC and MBC Determination

The Microdilution Broth Method was used for the determination of the MIC of plant extracts as recommended by the Clinical and Laboratory Standards Institute [12, 13]. Broth (100 µl) was dispensed in each well of a sterile microdilution tray. An appropriate volume of plant extract suspension was added to the first tube in each series (after removing the same volume of broth) in order to achieve the desired concentration after the addition of the bacterial inoculum. A standardized bacterial inoculum was prepared and adjusted to 0.5 McFarland and then diluted to 10^6 CFU/ml. Within 15 minutes, the wells were inoculated with 100µl of this inoculum resulting in a 1:2 concentration of the content of the well in plant extract and of the bacterial suspension ($5x10^5$ CFU/ml). A routine bacterial count was performed in duplicates to verify the bacterial concentration. Positive and negative control wells were used. The negative control well consisted of 200 µl of MHB, the positive well consisted of 200 µl MHB with a bacterial suspension but without a plant extract.

Table 1: Phenotypic profiles of susceptibility of Escherichia coli and Klebsiella pneumoniae Strains.

Strain	AM	AMC	PIP	TZP	CF	CXM	FOX	CTX	CRO	CAZ	CEF	IMP	GN	AN	SXT	OF	CIP	TGC	Group
Ec001SGH	R	R	R	S	R	R	S	R	R	R	R	S	S	S	S	S	S	S	ESBL+ QS
Ec002SGH	R	R	R	S	R	R	S	R	R	R	R	S	S	S	S	S	S	S	ESBL+ QS
Ec003SGH	R	R	R	S	R	R	R	R	R	R	R	S	R	S	R	R	R	S	ESBL+ QR AmpC +
Ec004SGH	R	R	R	S	R	R	R	R	R	R	R	S	R	S	R	R	R	S	ESBL+ QR AmpC +
Ec007SGH	R	R	R	S	R	R	S	R	R	R	R	S	S	S	R	R	R	S	ESBL+ QR
Ec010SGH	R	R	R	S	R	R	S	R	R	R	R	S	S	S	S	R	R	S	ESBL+ QR
Ec011SGH	R	R	R	S	R	R	S	R	R	R	R	S	R	S	R	R	R	S	ESBL+ QR
Ec012SGH	R	R	R	S	R	R	S	R	R	R	R	S	R	S	R	R	R	S	ESBL+ QR
Ec013SGH	R	R	R	S	R	R	S	R	R	R	R	S	R	S	R	S	S	S	ESBL+ QS
Ec016SGH	R	R	R	R	R	R	R	R	R	R	R	S	R	S	R	R	R	S	ESBL+ QR AmpC +
Ec017SGH	R	R	R	S	R	R	R	R	R	R	R	S	R	R	S	S	S	S	ESBL+ QS AmpC +
Ec018SGH	R	R	R	S	R	R	R	R	R	R	R	S	R	S	R	R	R	S	ESBL+ QR AmpC +
Ec019SGH	R	R	R	S	R	R	S	R	R	R	R	S	R	S	R	R	R	S	ESBL+ QR
EC020SGH	R	R	R	S	R	R	S	R	R	R	R	S	S	S	R	R	R	S	ESBL+ QR
EC021SGH	R	R	R	S	R	R	S	R	R	R	R	S	S	S	R	R	R	S	ESBL+ QR
Ec023SGH	R	R	R	S	R	R	S	R	R	R	R	S	S	S	R	R	R	S	ESBL+ QR
Ec026SGH	R	R	R	S	R	R	S	R	R	R	R	S	R	S	R	S	S	S	ESBL+ QS
Ec030SGH	R	R	R	S	R	R	S	R	R	R	R	S	R	S	R	R	R	S	ESBL+ QR
EC031SGH	R	R	R	S	R	R	S	R	R	R	R	S	R	S	R	R	R	S	ESBL+ QR
Ec032SGH	R	R	R	S	R	R	S	R	R	R	R	S	S	S	S	S	S	S	ESBL+ QS
Kp001SGH	R	R	R	S	R	R	S	R	R	R	R	S	R	S	R	S	S	S	ESBL+ QS
Kp002SGH	R	R	R	S	R	R	S	R	R	R	R	S	R	S	R	S	S	S	ESBL+ QS
Kp005SGH	R	R	R	S	R	R	S	R	R	R	R	S	R	S	R	S	S	S	ESBL+ QS
Kp006SGH	R	R	R	S	R	R	S	R	R	R	R	S	R	S	R	S	S	S	ESBL+ QS
Kp007SGH	R	R	R	R	R	R	S	R	R	R	R	S	R	S	R	R	R	I	ESBL+ QR
Kp008SGH	R	R	R	S	R	R	S	R	R	R	R	S	R	S	R	R	R	S	ESBL+ QR
Kp009SGH	R	R	R	R	R	R	S	R	R	R	R	S	R	S	R	R	R	S	ESBL+ QR
Kp010SGH	R	R	R	S	R	R	S	R	R	R	R	S	S	S	R	R	R	S	ESBL+ QR
Kp013SGH	R	R	R	S	R	R	S	R	R	R	R	S	S	S	R	S	S	S	ESBL+ QS
Kp016SGH	R	R	R	S	R	R	S	R	R	R	R	S	R	S	S	R	R	S	ESBL+ QR

Ec: Escherichia coli, Kp: Klebsiella pneumoniae, AM:ampicillin, AMC:amoxicillin/clavulanic acid, PIP:piperacillin, TZP:piperacillin/tazobactam, CF:cephalotin, CXMcefuroxime, FOX:cefoxitin, CTX:cefotaxime, CRO:ceftriaxone, CAZ:ceftazidime, CEF:cefepime, IMP:imipenem, GN:gentamicin, AN:amikacin, SXT:trimethoprim/sulfamethoxazol, OF:ofloxacin, CIP:ciprofloxacin, TGC:tigecycline QR: quinolone Resistant, QS: quinolone sensitive S: Sensitive, I: Intermediate, R: Resistant; AmpC +: naturally occurring Cephalsporinase; ESBL+: ESBL producer

The tray was incubated at 35 °C for 18-24 hours after which the MIC was recorded as the highest dilution of each plant extract that still retained an inhibitory effect resulting in no visible growth or in other terms absence of turbidity observed with the naked eye. The MBC was determined by sub-culturing samples from the tubes with concentrations above the MIC on new plates of MHA. The MBC corresponded to the lowest concentration of the extract associated with no bacterial culture.

All experiments were performed three independent times in duplicate form. The MIC_{90} is defined as the Minimum Inhibitory Concentration required to inhibit the growth of 90% of organisms, it was calculated as the percentile below which 90% of the individual MICs values fall. In view of the relatively small population of tested bacteria, it was not advantageous to calculate MIC_{50}.

Table 2: MICs and MBCs of the Different Fractions of Eriobotrya japonica leaves on Escherichia coli and Klebsiella pneumonia.

Bacterial strain	Crude (µg/µl)		Petroleum ether (µg/µl)		Dichloromethane (µg/µl)		Ethyl acetate (µg/µl)		Aqueous (µg/µl)	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Ec001SGH	80	80	80	80	X	X	X	X	40	40
Ec002SGH	80	80	ND	ND	ND	ND	2.5	10	80	ND
Ec003SGH	80	80	ND	ND	40	40	2.5	10	80	80
Ec004SGH	80	80	80	80	X	X	X	X	40	ND
Ec007SGH	80	ND	40	80	40	40	10	10	40	ND
Ec010SGH	80	80	80	80	40	40	2.5	10	40	40
Ec011SGH	80	80	40	80	80	ND	5	5	40	ND
Ec012SGH	40	40	40	80	20	80	40	40	40	80
Ec013SGH	40	80	80	ND	20	40	80	80	40	80
Ec016SGH	40	80	ND	ND	40	40	5	5	20	80
Ec017SGH	80	80	40	80	ND	ND	5	5	ND	ND
Ec018SGH	ND	ND	ND	ND	ND	ND	10	20	80	80
Ec019SGH	40	80	80	80	80	80	5	10	40	40
Ec020SGH	80	ND	ND	ND	ND	ND	20	80	40	ND
Ec021SGH	ND	ND	80	80	40	80	X	X	20	40
Ec023SGH	40	80	40	80	40	40	X	X	40	80
Ec026SGH	80	80	80	80	40	40	2.5	10	40	40
Ec030SGH	80	80	80	ND	40	40	80	80	40	80
Ec031SGH	80	80	80	80	40	40	5	10	40	80
Ec032SGH	80	ND	80	80	20	ND	5	10	ND	ND
MIC_{90}	80	>80	>80	>80	64	80	60	80	80	>80
Kp001SGH	80	80	ND	ND	5	10	2.5	5	40	ND
Kp002SGH	80	80	ND	ND	20	20	40	40	80	ND
Kp005SGH	80	80	ND	ND	ND	ND	5	10	80	ND
Kp006SGH	ND	ND	ND	ND	ND	ND	20	20	80	80
Kp007SGH	ND	ND	ND	ND	40	40	5	20	80	80
Kp008SGH	80	ND	80	80	40	80	5	10	80	ND
Kp009SGH	ND	ND	ND	ND	40	ND	5	10	80	ND
Kp010SGH	80	ND	80	80	80	80	80	ND	80	ND
Kp013SGH	80	80	80	ND	80	80	80	ND	80	ND
Kp016SGH	80	80	ND	ND	40	40	10	40	80	ND
MIC_{90}	80	80	NA	NA	80	80	26	40	80	NA

MIC:Minimum Inhibitory Concentration, MBC: Minimum Bactericidal Concentration, ND: Not Detected, X: missing extract, µg: microgram, µl: microliter; N/A: Not Applicable.

Results

Resistance Phenotypes of the tested strains

As shown in z1, the patterns of resistance of the tested strains could be devided into four categories:

- AmpC negative Quinolone resistant ESBL producers.
- AmpC negative Quinolone susceptible ESBL producers.
- AmpC positive Quinolone resistant ESBL producers.
- AmpC positive Quinolone susceptible ESBL producers.

Inhibitory and Bactericidal Activities of Eriobotrya japonica

Antimicrobial activity of leaves

As table 2 shows, both inhibitory and bactericidal effects of Eriobotrya japonica (leaves) on Escherichia coli and Klebsiella pneumoniae were mainly observed with the crude extract of the plant, the ethyl acetate, the Dichloromethane**,** and the aqueous fractions. The antibacterial effect of

the Petroleum ether fraction was limited. The lowest MIC_{90} was observed with ethyl acetate fraction at 26 µg/µl for Klebsiella pneumoniae.

The lowest MIC was recorded for the ethyl acetate fraction with Escherichia coli and Klebsiella pneumoniae at 2.5 µg/µl. In addition, the MIC and MBC effects were detected within 1 dilution. Eriobotrya japonica leaf crude extract, dichloromethane, ethyl acetate and aqueous fractions exhibited bactericidal effect against 63%, 53%, 100% and 40% of the strain respectively. The aqueous fraction showed comparable antibacterial activity with Escherichia coli and Klebsiella pneumoniae. Moreover, the concentration at which most of the bacterial suspensions were cleared were 80 µg/µl for the crude extract and the aqueous fraction, 40 µg/µl for the dichloromethane fraction and 10 µg/µl for the ethyl acetate fraction. Petroleum ether fraction exerted its bactericidal activity mostly at 80 µg/µl. The solvents' controls did not show any antibacterial activity. Positive controls showed Bacterial growth while no growth was observed for the negative controls.

Table 3: MICs and MBCs of the Different Fractions of Eriobotrya japonica branches on Escherichia coli and Klebsiella pneumonia.

Bacterial strain	Crude (µg/µl)		Petroleum ether (µg/µl)		Dichloromethane (µg/µl)		Ethyl acetate (µg/µl)		Aqueous (µg/µl)	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Ec001SGH	40	ND	40	80	X	X	10	10	80	80
Ec002SGH	40	40	10	10	X	X	5	10	80	80
Ec003SGH	40	40	ND	ND	X	X	10	10	80	80
Ec004SGH	40	80	ND	ND	X	X	2.5	2.5	40	80
Ec007SGH	20	20	ND	ND	X	X	40	40	80	ND
Ec010SGH	20	40	ND	ND	X	X	20	40	80	80
Ec011SGH	40	80	80	ND	X	X	2.5	5	80	80
Ec012SGH	20	80	80	80	X	X	5	5	80	80
Ec013SGH	20	80	80	ND	X	X	5	10	80	80
Ec016SGH	20	40	ND	ND	X	X	2.5	2.5	40	40
Ec017SGH	10	10	10	10	X	X	10	10	40	40
Ec018SGH	40	40	ND	ND	X	X	10	10	80	ND
Ec019SGH	20	20	ND	ND	X	X	5	5	80	80
Ec020SGH	80	80	ND	ND	X	X	10	10	80	80
Ec021SGH	80	80	40	80	X	X	2.5	2.5	20	20
Ec023SGH	80	80	40	80	X	X	2.5	2.5	40	80
Ec026SGH	20	40	10	ND	X	X	20	40	80	80
Ec030SGH	80	80	80	80	X	X	5	5	80	80
Ec031SGH	40	40	ND	ND	X	X	10	20	80	80
Ec032SGH	40	80	ND	ND	X	X	10	20	80	80
MIC_{90}	80	80	N/A	NA	X	X	20	40	80	80
Kp001SGH	40	80	80	ND	X	X	10	10	80	80
Kp002SGH	20	20	80	ND	X	X	10	10	80	80
Kp005SGH	80	ND	10	ND	X	X	10	20	80	ND
Kp006SGH	80	80	ND	ND	X	X	20	20	80	ND
Kp007SGH	40	40	ND	ND	X	X	10	20	80	80
Kp008SGH	40	40	40	80	X	X	5	10	80	80
Kp009SGH	80	80	80	ND	X	X	10	10	ND	ND
Kp010SGH	40	80	80	80	X	X	10	20	80	80
Kp013SGH	80	80	80	80	X	X	10	10	80	80
Kp016SGH	80	80	80	80	X	X	10	10	80	80
MIC_{90}	80	80	80	>80	X	X	11	20	80	80

MIC:Minimum Inhibitory Concentration, MBC: Minimum Bactericidal Concentration, ND: Not Detected, X: missing extract, µg: microgram, µl: microliter; N/A: Not Applicable

Antimicrobial Activity of branches

As table 3 shows, Antibacterial effects of Eriobotrya japonica branches on Escherichia coli and Klebsiella pneumoniae (Table 3) were observed with the crude extract of the plant, the ethyl acetate, the petroleum ether, and the aqueous fractions. The Dichloromethane fractionation did not reveal enough quantity to run all the experiments, it was therefore discarded. The best inhibitory activity represented by the lowest MIC_{90} was observed with ethyl acetate fraction at 20 µg/µl for Escherichia coli and at 11 µg/µl for Klebsiella pneumoniae. The concentrations at which most of strains were inhibited were 40 µg/µl for the crude extract, 10 µg/µl for the ethyl acetate fraction and 80 µg/µl for the aqueous fraction. The lowest MIC was recorded for the ethyl acetate fraction with Escherichia coli at 2.5 µg/µl. The MIC and MBC effects were detected within 1 dilution. The concentrations at which most of the strains were killed were 40 µg/µl for the crude extract, 10 µg/µl for the ethyl acetate fraction and 80 µg/µl for the aqueous and petroleum ether fractions. The solvents' controls did not exert any antibacterial activity. Bacterial growth was observed for the positive controls while no growth was observed for the negative controls.

Discussion

Due to the high level of resistance to third generation cephalosporins and monobactams, the choice of effective and safe antibiotic treatment is becoming limited. Alternative agents or extracts obtained from natural medicinal plants need to be introduced or combined with antibiotics for therapeutical use. In the present study, extracts from three different plants were tested for antimicrobial activity against ESBL producing Escherichia coli and Klebsiella pneumoniae.

Most studies [7, 15-18] found more antimicrobial activity in plant extracts tested against Gram-positive than Gram-negative bacteria. This was attributed to the fact that Gram-negative bacteria possess an outer membrane that acts as a barrier to many environmental substances [19].

The present study showed that different extracts/fractions exhibited antimicrobial activity against ESBL producing Escherichia coli and Klebsiella pneumoniae. The crude extracts, the ethyl acetate, the petroleum ether, the dichloromethane (where available) and the aqueous fractions of both leaf and branch extracts of the plant exhibited an inhibitory effect.

In most of the cases, inhibitory and bactericidal effects were detected by the same concentrations. The lowest MIC was recorded with ethyl acetate fraction of leaf extracts at 2.5 µg/µl.

In a previous study [7], we have shown that different extracts of Rosmarinus officinalis, Origanum majorana, and Trigonella foenum-graecum inhibited the growth of ESBL producing Escherichia coli and Klebsiella pneumoniae at different rates. In that study, the MICs ranged between 1.25 and 80 µg/µl. The majority of these microorganisms were inhibited by 80 and 40 µg/µl of the crude extracts and the petroleum ether fraction of Origanum majorana significantly inhibited 94% of the tested strains.

In the present study the individual MICs varied between 2.5 and 80 µg/µl. Some MICs of the same extracts varied against the different tested strains, although, some of the tested strains had the same antimicrobial susceptibility patterns. In their investigation, Ahmad and Aqil [21] postulated that the presence of different intrinsic levels of tolerance to antimicrobials in the tested microorganisms caused the variation of the MIC values among the isolates with relatively similar antimicrobial susceptibility patterns.

This study has shown that the highest antibacterial activity against ESBL producing Escherichia coli and Klebsiella pneumoniae was mainly manifested by ethyl acetate fractions of both leaf and branch extracts; the other fractions exhibited less antibacterial affect. However, the toxic effects of plant extracts were not explored or tested in this work. The selective toxicity of an antimicrobial agent on eukaryotic cells is crucial

and would impact on the usefulness of this extract as a medicinal compound. Antibacterial extracts that are toxic on human cells may be useful as non-medicinal antimicrobial agents, such as surface disinfectants. In addition, purification and identification of the bioactive components is needed to examine the mechanisms of action of these agents especially that these mechanisms probably differ from those of the commonly used antibiotics.

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