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Original article

Investigation of the antimicrobial effect of some natural products on Egyptian *Acinetobacter baumannii* isolates

Kholoud Baraka[1*] , Rania R Abozahra[1], Alaa Youssef [2], Sarah M Abdelhamid [1]

1- Microbiology and Immunology Department, Faculty of Pharmacy, Damanhour University, El Behira, Egypt.

2- Damanhour Regional Laboratory, Ministry of Health, El Behira, Egypt.

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ABSTRACT

Background: Extremely high mortality rates were associated with *Acinetobacter baumannii* infections because of their resistance to first-and second-line drugs. This study aimed to investigate the antimicrobial effect of some natural products against *A. baumannii*. **Methods:** Fifty *A. baumannii* isolates were collected from patients at Damanhour Medical National Institute. Antibiotic resistance of isolates was determined by VITEK 2 system. Biofilm formation was detected using microtiter plate method. MIC and anti-biofilm effect of oils, liquorice and ferric chloride were determined by the broth microdilution method. RND and QS genes were detected by conventional PCR. The effect of liquorice and ferric chloride on the expression of *adeB* and *abaI* was detected using real-time PCR. **Results:** 72% of isolates were MDR and 86% of isolates were biofilm forming. The 4 reagents had anti-bacterial and anti-biofilm effects. 86% and 84% of isolates were positive for *abaI* and *adeB*, respectively. Liquorice and ferric chloride caused down regulation of *adeB* and *abaI* genes. **Conclusion:** The 4 reagents had antibacterial and anti-biofilm effects against *A. baumannii* isolates. Liquorice extract and ferric chloride caused down regulation of QS and efflux pump genes, and liquorice extract had a stronger effect. Therefore, it's recommended to further investigate their antibacterial effect to use them as single natural antibiotics or in combination with traditional antibiotics.

Introduction

Acinetobacter baumannii (*A. baumannii*) is an aerobic Gram-negative, non-motile, coccobacillus. It causes several nosocomial multi-drug resistant (MDR) infections. Extremely high mortality rates were associated with *A. baumannii* infections because of their resistance to first-and second-line drugs [1, 2]. It has the ability to resist desiccation, disinfectants, and major antimicrobials. It is persistent in hospitals, especially intensive care units (ICUs). It causes a variety of infections such as bloodstream infections, meningitis, ventilator-associated pneumonia, wound infection, urinary tract infections, and endocarditis [3].

Bacterial resistance to antibiotics constitutes a serious public health problem. *Acinetobacter baumannii* can resist the effect of antibiotics through several mechanisms such as the transporting system (efflux pumps), biofilm formation, and quorum sensing (QS). There are five families of efflux-pump proteins that are associated with multidrug resistance. RND protein is comprised of a transporter efflux protein. It is located in the inner cytoplasmic membrane, the

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* *Corresponding author:* Kholoud Baraka

E-mail address: *loody_mri@yahoo.com*

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membrane fusion protein, and the outer membrane protein. RND coding genes help for the drug export across both inner and outer membranes being responsible for the intrinsic resistance of *A. baumannii* to many antibiotics such as aminoglycosides, tetracycline, erythromycin, chloramphenicol, trimethoprim, and fluoroquinolones [4].

Bacterial cells of *A. baumannii* were found to demonstrate strong biofilm formation in skin and soft-tissue infections, as well as in wounds and on occlusive dressings [5]. They can also form biofilm communities on most abiotic surfaces, including health-care-associated equipment, such as endotracheal tubes. Biofilms are supported by polymer matrices that are secreted by microorganisms themselves, facilitating the formation of bacterial communities. This viscous matrix can protect bacteria from harmful external factors, thereby increasing the resistance of the microbial community [6]. The autoinducer synthase *AbaI* is necessary for quorum sensing and biofilm formation and plays an important role in the late stages of biofilm maturation [7, 8].

Quorum sensing is a bacterial cell–cell communication process that involves the detection, and response to extracellular signaling molecules called auto-inducers. It mediates many physiological processes of bacteria such as swarming, twitching, antibiotics biosynthesis, biofilm formation, and conjugation [9]. *A. baumannii* exhibits N-acyl homoserine lactone (AHL)-mediated cell density dependent quorum sensing which highly contributes to its virulence [10, 11]. The AHL synthase enzyme is responsible for the synthesis of the signaling molecules, AHLs. In *A. baumannii*, *abaI* synthase enzyme produces AHLs which bind to the receptor molecules on cell surfaces and initiate the QS process [12]. Quorum quenching was found to attenuate the organism's virulence rather than killing it. Therefore, it has become the main strategy for new therapeutics against several bacterial pathogens.

It was reported that natural substances such as liquorice, star anise, coriander oil, eucalyptus glabra, lemon grass, and euginol acetate had an anti-virulence effect on *A. baumannii*. Upon exposure to ferric chloride, it was reported that bacterial genes involved in QS and biofilm formation showed significant differences. Moreover, AHL production and biofilm formation are regulated by iron concentration in *A. baumanii* strains. Iron limitation was reported to enhance the expression of QS genes. In the presence of low concentrations of iron-III, a higher amount of AHL was detected and more biofilms were formed [12].

Objectives of the study

- Investigate the antibacterial and anti-biofilm effect of coriander oil, star anise oil, liquorice extract and ferric chloride against clinical *A. baumanii* isolates.
- Molecular detection of RND and QS genes by conventional PCR.
- Molecular detection of the effect of liquorice extract and ferric chloride on the expression of *adeB* and *abaI* genes using real-time PCR.

Materials and Methods

Sample collection, isolation, and identification

One hundred different samples (sputum, blood, wound, and pleural) were collected from patients at Damanhour Medical National Institute, El-Behira, Egypt from June 2021 to April 2022. For the detection of *A. baumannii*, samples were cultured on MacConkey agar plates. Gram staining was performed on non-lactose-fermenting colonies. Several biochemical tests including oxidase, triple sugar iron agar, indole, methyl red, Voges Proskauer, citrate, and catalase were carried out [13]. *Acinetobacter baumannii* isolates were identified at the species level using the automated VITEK 2 system (Bio-Merieux, l'Etoile, France).

Antibiotic susceptibility testing

The antibiotic resistance of *A. baumanii* clinical isolates was determined automatically by using the VITEK 2 system against eleven antibiotics representing different antibiotic classes. These antibiotics were: meropenem, piperacillin/tazobactam, cefazolin, ceftriaxone, cefepime, ceftazidime, tobramycin, gentamycin, trimethoprim / sulfamethoxazole, ciprofloxacin, and levofloxacin.

Biofilm assay

The biofilm-forming ability of *A. baumanii* clinical isolates was tested using the polystyrene microtiter plate method described by **O'Toole et al.** [14]. Overnight broth cultures in nutrient broth were diluted 1:100 into fresh nutrient broth, and then 200 µL of the freshly inoculated medium were dispensed into the wells of a 96-well microtiter plate. The plate was incubated at 37ºC

overnight without agitation. Biofilms were detected by staining the wells with 200 µL of crystal violet (0.1%[w/v] in H2O). The plate was incubated for 15 min. at room temperature and then it was washed thoroughly with distilled water to remove planktonic cells and the residual dye. Then, 200 µL of ethanol (95%) was used to elute crystal violet from biofilms. The absorbance of the solubilized dye was measured at 590 nm using a microtiter plate reader. Assays were performed in triplicate and the average was calculated [14].

Determination of minimum inhibitory concentration (MIC) of coriander oil, star anise oil, liquorice extract and ferric chloride

MICs of oils, liquorice extract and ferric chloride against the *A. baumanii* clinical isolates were determined by the broth microdilution method described by **Duarte et al.** using 96-well microtitre plates [15]. One hundred µL of luria broth (LB) were supplemented with DMSO to a final concentration of 2% (v/v) in order to increase oils' solubility. In 96-well microtitre plates, two-fold serial dilutions were prepared of coriander and star anise oils from 2048 µL to 128 µL and of liquorice extract from 120 µL to 7.5 µL. For ferric chloride, it was first supplemented with ethanol 96% to a final concentration of 10% (v/v), in order to increase ferric chloride solubility [16]. Then, two-fold serial dilutions of it were prepared from 100 µM to 12.5 µM in a 96-well plate (100 µL per well). Three bacterial wells without any added reagent and 3 wells without any bacteria were used as positive and negative controls, respectively. Microtiter plates were incubated for 16–20 h at 37°C and the bacterial growth was assessed visually. The assay was done three independent times. MIC was determined as the lowest reagent's concentration without visible growth [15].

Anti-biofilm effect of coriander oil, star anise oil, liquorice extract and ferric chloride

The effect of different concentrations of coriander oil, star anise oil, liquorice extract, and ferric chloride on biofilm formation of *A. baumanii* clinical isolates was detected using polystyrene flat-bottomed microtiter plates. Bacterial cultures were incubated overnight in LB broth at 37°C and were diluted 1:100 into fresh LB. Then, 100 µL of diluted bacterial cultures were pipetted into each well, followed by the addition of 100 µL of different concentrations of each reagent to yield a final cell concentration of 1×10^7 CFU/mL. Each concentration was pipetted in 3 replicates. Plates were incubated at 37°C for 24 h [17]. Microtiter plates were washed twice with 150 µL of NaCl (0.90%), stained with 100 µL of 0.1% crystal violet, and left at room temperature for 30 min. Then, the stain was discarded, and wells were washed 3 times to remove excess stain. Wells were eluted with 200 µL of 95% ethanol, and the absorbance was measured at 570 nm. [18].

Genomic DNA extraction of bacterial isolates:

Genomic DNA extraction was performed using the boiling method [19]. Briefly, 4_5 pure bacterial colonies of *A. baumannii* isolates cultured on MacConkey's agar plates were suspended in 500 µL of sterile water in a sterile Eppendorf tube, and heated at 100 °C for 10 min. Bacterial suspensions were cooled on ice for 5 min, then centrifuged at 14,000 rpm for 5 min. Supernatants were transferred to sterile Eppendorf tubes and stored at_20 °C till the PCR assay.

Molecular detection of RND and QS genes by conventional PCR technique

DNA extracts of all *A. baumannii* isolates were tested for the presence of structural and regulatory RND (*adeB*) and QS (*abaI*) genes by conventional PCR technique using a thermal cycler (BOECO- BOE8085240, hamburg, Germany) and specific primers **(Table 1)**. Cycling conditions were initial denaturation at 95 °C for 2 min; followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 52 °C for 1 min, and extension at 72 °C for 30 s, and a final extension at 72 °C for 5 min [18]. PCR amplicons were then resolved on 1.5 % agarose gel stained with ethidium bromide and visualized via ultraviolet illumination.

Molecular detection of the effect of liquorice extract and ferric chloride on the expression of *adeB* and *abaI* genes using real-time PCR

RNA extraction

Initially, RNA extraction of all *A. baumannii* isolates was performed by the RNAeasy kit (Qiagen , USA). First, a loopful of well-isolated colony was inoculated into sterile LB broth supplemented with 50 µM of $FeCl_3$. For liqourice extract, the LB broth was supplemented with it to a final concentration equals 25 µL/mL. They were then incubated at 37 °C overnight. One mL of each sample was centrifuged at 4 °C for 10 min at 3000 g and cell pellets were used for RNA extraction according to the manufacturer's instructions.

Quantitative real time PCR assay

RT-PCR was performed by using the TOPreal™ One-Step SYBR Green RT-qPCR Kit, a Real-Time PCR thermal cycler (Applied Biosystems; StepOne™ Real-Time PCR, Foster City, CA, USA), and specific primers **(Table 1)**. A typical RT-PCR sample (25 µL) contained 4.5 µL of a serial dilution of RNA template, 2.5 µL of nuclease-free water, 10 µL of RT-PCR reaction mixture, 1 µL of TOPreal™ one-step RT-qPCR enzyme mix, and 1 µL of forward and reverse gene-specific primers. Relative quantification of genes' expression was performed by iCycler iQ5™ software using a normalized expression analysis method. The housekeeping *cpn60* and *gltA* genes served as endogenous controls. ATCC19606 served as a reference strain. Relative expressions were calculated using the $2^{-\triangle\triangle Ct}$ method.

Results

Isolation, identification and antimicrobial resistance of clinical isolates

Fifty (50%) of the 100 collected clinical isolates were initially identified as *Acinetobacter* species by their growth appearance on MacConkey agar plates and their morphological and biochemical characteristics. Non-lactose fermenting colonies appeared as Gram-negative coccobacilli upon Gram staining. Biochemically, all isolates were oxidase negative, catalase positive, indole negative, methyl red negative, Voges Proskauer negative, and citrate positive. On triple sugar iron agar slants, all isolates produced alkaline butt and slant without gas or H_2S production. Identification of *A. baumannii* isolates was confirmed to the species level using the automated vitek 2 system. Twenty-six (52%) of the fifty *A. baumannii* isolates were obtained from blood, 20 (40%) from sputum, 3 (6%) from wound swabs, and 1 (2%) from pleural samples.

Thirty-six (72%) of the 50 *A. baumannii* isolates were MDR (resistant to more than 3 different antibiotic classes). High resistance against beta-lactam antibiotics was observed by our isolates; 92% of isolates were resistant to cefazolin, 72% to each of piperacillin/tazobactam, ceftriaxone, cefepime, and ceftazidime, and 68% to meropenem. Moreover, moderate to high resistance was observed against quinolones, macrolides, and sulfa drugs: ciprofloxacin (72%), levofloxacin (68%), tobramycin (40%), gentamycin (60%), and trimethoprim/sulfamethoxazole (41%).

Biofilm assay

Upon detecting biofilm formation of our 50 *A. baumannii* isolates using the microtiter plate method, 7 (14%) were non-biofilm forming, 14 (28%) were strong-biofilm forming, 20 (40 %) were moderate-biofilm forming, and 9 (18 %) were weak-biofilm forming.

Determination of MICs of coriander oil, star anise oil, liquorice extract and ferric chloride against the 50 *A. baumannii* isolates

The four reagents had an antibacterial effect against all *A. baumannii* isolates with varying intensities **(Table 2)**. MICs of coriander oil ranged from 1 to 16 µL/mL of star anise oil from 8 to 32 µL/mL of liquorice extract from 1.5 to 6 µL/mL, and of ferric chloride was 100 µM.

Anti-biofilm effect of 1/2 MIC of coriander oil, star anise oil, liquorice extract and ferric chloride on the 50 *A. baumannii* isolates

The four reagents had an obvious inhibitory effect on biofilm formation. Coriander oil showed the strongest anti-biofilm effect followed by liquorice extract, star anise oil, and then ferric chloride **(Table 3)**.

Molecular detection of RND and QS genes by conventional PCR technique

Molecular detection of RND and QS genes using conventional PCR technique showed that 43 (86%) isolates were positive for the *abaI* gene, 42 (84%) were positive for *adeB* gene, and 36 (72%) were positive for both genes. By agarose gel electrophoresis, *abaI* and *adeB* genes were detected at 121 bp and 168 bp, respectively **(Figure 1)**.

Molecular detection of the effect of 1/2 MIC of liquorice extract and ferric chloride on the expression of *adeB* and *abaI* genes in selected 15 *A. baumannii* isolates using real-time PCR

Liquorice extract caused down regulation of *adeB* and *abaI* genes in 100% and 80% of isolates, respectively; however, ferric chloride caused down regulation of *adeB* and *abaI* genes in 73% and 60% of isolates, respectively. On the other hand, ferric chloride caused over expression of *adeB* and *abaI* genes in 27% and 40% of isolates, respectively **(Table 4)**.

Table 1. Primers used in conventional PCR and RT-PCR.

The gene	The primer's Sequence	The amplicon size (bp)
AdeB	***AdeB*-F:** CTTGCATTTACGTGTGGTGT ***AdeB*-R:** GCTTTTCTACTGCACCCAAA	168 bp
abaI	***AbaI*-F:** CCACACAACCCTATTTACTCGC ***AbaI*-R:** GGCGGTTTTGAAAAATCTACGG	121 bp
glt A	***gltA*-F:** AATTTACAGTGGCACATTAGGTCCC ***gltA*-R:** GCAGAGATACCAGCAGAGATACACG	722 bp

Table 2. MICs of coriander oil, star anise, liquorice extract, and ferric chloride against the 50 *A. baumannii* isolates results.

Reagent	Number of inhibited isolates (%)	MIC
coriander oil	**14 (28%)**	1 µL/mL
	7(14%)	4 µL/mL
	8 (16%)	8 µL/mL
	21 (42%)	16 µL/mL
Star anise oil	**21 (42%)**	8 µL/mL
	15 (30%)	16 µL/ mL
	14 (28%)	32 µL/mL
Liquorice extract	**6 (12%)**	1.5 µg/mL
	16 (32%)	3 µg/mL
	28 (56%)	6 µg/mL
Ferric chloride	**50 (100%)**	100 µM

Table 3. Inhibitory effect of half MIC of coriander oil, star anise oil, liquorice extract and ferric chloride on biofilm formation of the 50 *A. baumannii* isolates.

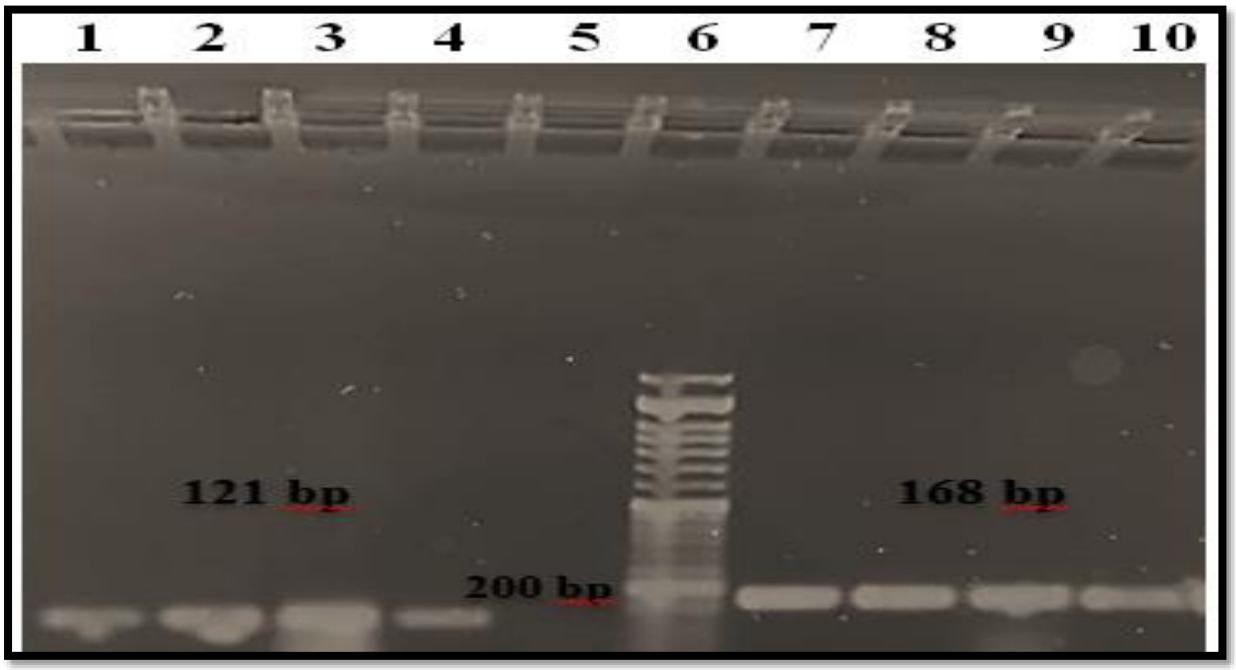
	Biofilm formation before treatment	Biofilm formation after treatment with			
		Coriander oil	Star anise oil	Ferric chloride	Liquorice extract
	No (%)	No (%)	No (%)	No (%)	No (%)
No biofilm formation	7 (14%)	24 (48%)	21 (42%)	20 (40%)	23 (46%)
Weak biofilm formation	9 (18%)	26 (52%)	28 (56%)	30 (60%)	27 (54%)
Moderate biofilm formation	20 (40%)	0	0	0	0
Strong biofilm formation	14 (28%)	0	0	0	0

Table 4. Molecular detection of the effect of 1/2 MIC of liquorice extract and ferric chloride on the expression of *adeB* and *abaI* in selected 15 *A. baumannii* isolates using real-time PCR.

Reagent	Gene	No. of isolates	Level of expression
Liquorice extract	*abaI*	12	0.5 ≤
		0	2 ≤
		3	0.5 ≤ 2
	adeB	15	0.5 ≤
		0	2 ≤
		0	0.5 ≤ 2
Ferric chloride	*abaI*	9	0.5 ≤
		0	2 ≤
		6	0.5 ≤ 2
	adeB	11	0.5 ≤
		0	2 ≤
		4	0.5 ≤ 2

- $p \leq .5$: down regulation, p .5 ≤ 2: normal regulation, p 2 ≤: over expression.
- Fold change (fc) was calculated using following formula; 2_ddctd = ct (target gene) _ ct (reference gene)

Figure 1. Detection of *abaI* and *adeB* genes on 1.5% agarose gel following amplification with conventional PCR.



Lanes 1 - 4: four isolates positive for the *abaI* gene detected at 121 bp, lane 5: one isolate negative for the 2 genes, lane 6: DNA ladder, lanes 7 - 10: four isolates positive for the *adeB* gene detected at 168 bp.

Discussion

Over the last years, *A. baumannii* has become a highly problematic nosocomial pathogen on a global scale. Being one of the most successful MDR organisms endangering modern antibiotic therapy, its amazing ability to acquire or up-regulate multiple resistance determinants has played a major role in its clinical importance [20]. The increased antimicrobial resistance of *A. baumannii* and the occurrence of strains resistant to virtually all available drugs are quite alarming [21]. Besides being intrinsically resistant to a number of commonly used antibiotics, *A. baumannii* has a quite notable ability to acquire resistance to numerous other agents and thus swiftly respond to changes in environmental pressure [22].

In this study, 26 (52%) of our *A. baumannii* isolates were collected from blood, 20 (40%) from sputum, 3 (6%) from wounds, and 1 (2%) from plural specimens. Similar to our results, **Nojookambari et al.** reported that the highest percentage of their isolates were collected from blood (30%); however, in contrast to our results, the lowest percentage were collected from sputum (1%) [23]. In contrast to our results, **Boulesnam et al.** reported that only 2 isolates were collected from blood, and no isolates were collected from sputum [24].

In this study, our *A. baumannii* isolates showed high antimicrobial resistance against most antibiotics. The most resistant antibiotic was cefazolin (92%) and the most sensitive antibiotic was tobramycin (40%); however, resistance to other antibiotics was: piperacillin/tazobactam (72%), meropenem (68%), ceftriaxone (72%), cefepime (72%), ceftazidime (72%), gentamycin (60%), trimethoprim/sulfamethoxazole (41%), ciprofloxacin (72%), and levofloxacin (68%). Higher resistance percentages were reported by **Boulesnam et al.** as 100% of their isolates were resistant for gentamycin, piperacillin and tobramycin, 96.7% for ceftazidime, 86.7% for levofloxacin, 93.3% for trimethoprim/sulfamethoxazole, and 93.3% for meropenem [24]. On the other hand, **Khoshnood et al.** reported that meropenem was the most resistant antibiotic (92%); however, ampicilin/sulbactam was the most sensitive one (42%) [25]. In addition, **Mahmoudi et al.** reported that 90% of their *A. baumannii* isolates were resistant to ciprofloxacin and imipenem [26] and Ardebili *et al.* reported that 100% of the their isolates were resistant to ciprofloxacin [27]. Higher resistance percentages were also reported by **Nogbou et al.** against all antibiotics [28]. In this study, 36 (72%) of our isolates were MDR. In contrast, **Shivaprasad et al.** reported that all of their isolates were MDR [29].

In this study, antibiotic resistance was higher among biofilm producing isolates than in non-biofilm producers. On the contrary, Rodriguez *et al*. reported that higher biofilm production was detected in antibiotic sensitive isolates than in resistant isolates [30]. Prevention and treatment of *A. baumannii* infections were linked to bacterial biofilms [31]. In this study, it was found that 7 (14%) of our isolates were non-biofilm forming, 14 (28%) were strong-biofilm forming, 20 (40 %) were moderate-biofilm forming, and 9 (18 %) were weak-biofilm forming. Similarly, **Boulesnam et al.** reported that 2 (6.6%) of their 30 isolates were non-biofilm producers, 15 (50%) were moderate producers, and 13 (43.4%) were strong biofilm producers [24]. On the other hand, Khoshnood *et al*. reported that 52% of their isolates were strong biofilm formers, 36% were moderate biofilm formers, and 12% were weak biofilm formers [25]. In addition, **Al-Shamiri et al.** reported that only 2.9% of their isolates were non-biofilm formers, 51.4% were strong biofilm formers, 41.4% were moderate biofilm formers, and 4.3% were weak biofilm formers [32]. **Yang et al.** also reported that only 6.4% of their isolates were not biofilm producers, 15.6% were weak biofilm formers, 32.4% were moderate biofilm formers, and 45.4% were strong biofilm formers [33].

Medicines developed from plants have had a significant positive impact on human health and wellbeing. It has long been understood that plant essential oils contain antimicrobial characteristics and are effective against infectious diseases. It was reported that plant-based antimicrobial agents had higher minimum inhibitory concentrations against bacteria and fungi than traditional antibiotics [34].

The antibacterial activity of star anise extracts has been assessed previously for *A. baumannii* [35]. In this study, star anise demonstrated an antibacterial activity against our *A. baumannii* isolates with MIC values of 8, 16, and 32 μL mL^{-1}**.** Our MIC values were much higher than those reported by **Cos et al.** [36]. Star anise EO can be used as an alternative medicine and as a disinfectant. It was reported that the mechanism of action of essential oils was to damage bacterial cell membranes, resulting in cell lysis and consequent cell death [37]. Hence one of the most important virulence factors of *A. baumannii* is biofilm formation which is controlled by quorum sensing [38], the anti-biofilm properties of star anise EO was evaluated in this study. It was found that star anise EO was able to inhibit biofilm formation and reduce its strength among our isolates and these results had been also described for the extracts of this plant by **Rahman et al.** [39].

In this study, coriander oil EO also exhibited antibacterial activity against most of our *A. baumannii* isolates with MIC values of 1, 4, 8, and 16 μL /mL. Similarly, MIC values of 1 and 4 μL/mL were reported by Durate *et al.* [15]. The antibacterial activity of coriander EO against *A. baumannii* isolates was also described by **Hammer**

et al. [40]. In addition, **Silva et al.** [41] also investigated the antibacterial activity of coriander EO against gram-negative bacteria and suggested that its mechanism of action was membrane damage. In this study, coriander EO was found to have anti-biofilm effect more than star anise EO.

In this study, liquorice extract also exhibited an antibacterial activity against most of our *A. baumannii* isolates with MIC values of 1.5, 3, and 6 µg /mL. Similarly, the same MIC values were reported by **Eissa et al.** [42]. In contrast, **Aziz et al.** [43] reported that MIC of liqourice extract was 15 mg/mL. In this study liqourice extract was found to have anti-biofilm effect more than star anise EO and less than coriander EO.

Regarding ferric chloride, it also exhibited an antibacterial activity against all *A. baumannii* isolates with a MIC value of 100 µM in this study. It was reported that biofilm formation was under the regulation of iron concentration. In this study, ferric chloride was able to inhibit biofilm formation and reduce its strength among our *A. baumannii* isolates.

The efflux of antibiotics to the external medium by membrane efflux is one of the most often recognized mechanisms of antibiotic resistance. Efflux pumps are the most extensively researched mechanisms of resistance in *A. baumannii* by which bacterial cells expel antibiotics out [44]. Therefore, the presence of the RND gene (*adeB*) and the QS gene (*abaI*) was investigated among our isolates by conventional PCR technique. In this study, 86% of our isolates were positive for the *abaI* gene, 84% were positive for *adeB* gene, and 72% were positive for both genes. Similarly, **Tang et al.** in China reported that 84% of their isolates were positive for *abaI* gene [45]. Higher percentage was reported by **Rafiei et al.** in Iran who reported that 100% of their *A. baumannii* isolates were positive for *adeB* gene [46].

In this study, the inhibitory effect of liqourice extract and ferric chloride on the expression of *abaI* and *adeB* genes was investigated by real time PCR. Liquorice extract caused down regulation of *adeB* and *abaI* genes in 100% and 80% of isolates, respectively; however, ferric chloride caused down regulation of *adeB* and *abaI* genes in 73% and 60% of isolates, respectively. On the other hand, ferric chloride caused over expression of *adeB* and *abaI* genes in 27% and 40% of isolates, respectively. Indeed, we didn't find any comparative studies published by other authors.

In conclusion, coriander oil, star anise, liquorice extract and ferric chloride were found to have an antibacterial effect against our MDR *A. baumannii* isolates. In addition, the four reagents had also an anti-biofilm effect. Moreover, liquorice extract and ferric chloride were found to cause down regulation of QS and efflux pump genes, and liquorice extract had a stronger effect. Therefore, it's recommended to further investigate their antibacterial effect to use them as natural antibiotics either as single agents or in combination with traditional antibiotics. They could potentiate the antibacterial effect of traditional antibiotics. Due to their natural origin and reported low to no toxicity, EOs are typically regarded as safe antimicrobials. This explains the basis of the investigation of the effects of plants as a potential therapy.

Declarations

Ethical approval and Consent to participate

This study adhered to the accepted principles of ethical conduct according to the approval reference number (421PM20) given by the Research Ethics Committee of the Faculty of Pharmacy, Damanhour University. Before testing and molecular analysis of their materials, all the available samples and patient data were gathered with informed ethical consent.

Consent for publication

Not applicable

Availability of data and materials

All data supporting the findings of this study are available within the paper and its supplementary information.

Competing interests

All authors declare that they don't have any competing interests to declare.

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Authors' contributions

Sarah Abdelhamid and Rania Abozahra conceived and designed research. Kholoud Baraka and Alaa Youssef conducted experiments. Sarah Abdelhamid and Rania Abozahra analyzed data. Kholoud Baraka and Alaa Youssef wrote the manuscript. All authors read and approved the manuscript.

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