Journal of Clinical Chemistry and Laboratory Medicine

Sonbol et al., J Clin Chem Lab Med 2017, 1:1

Research Article Open Access

Antimicrobial Susceptibility of Some Natural Oils against *Acinetobacter* Species

Fatma Sonbol[1], **Tarek El- Banna**[2], **Ahmed Abd El-Aziz**[2] **and Nermin Gouda**[3*]

[1]*Professor & Chairman of Pharmaceutical Microbiology Department, Faculty of Pharmacy, Tanta University, Egypt*

[2]*Professor of Pharmaceutical Microbiology Department, Faculty of Pharmacy, Tanta University, Egypt*

[3]*Pharmacist at Aswan University Hospital, Egypt*

[*]**Corresponding author:** Nermin Gouda, Pharmacist at Aswan University Hospital, Egypt, E-mail**:** haleemsaad33@yahoo.com

Received date: November 02, 2017; **Accepted date:** November 14, 2017; **Published date:** November 22, 2017

Abstract

The *in vitro* activities of 18 natural oils and 14 antimicrobial agents against 72 *Acinetobacter* isolates isolated from 1000 patients in Aswan University hospital over 18 months obtained from urine cultures, burn swabs, sputum, wound swabs and endotracheal swabs were studied. MICs were determined by an Agar dilution method. The antimicrobial activity of plant oils has been recognized for many years. However, few investigations have compared large numbers of oils using methods that are directly comparable. In the present study, 18 plant oils were investigated for activity against *Acinetobacter* isolates, using an agar dilution method. Cinnamon, thyme, tea tree, rosemerry, peppermint, clove and lavender, inhibited all organisms at concentrations of ≤ 6 mg/ml. Four oils did not inhibit any organisms at the highest concentration, which was 6 mg/ml oil for tea, camphor, caraway and Nigella staive. Variable activity was recorded for the remaining oils. These results support the notion that plant essential oils and extracts may have a role as pharmaceuticals and preservatives.

Good activity against *Acinetobacter* isolates was demonstrated for imipenem, amikacin, and ciprofloxacin. Most of isolates were susceptible to imipenem, ciprofloxacin, expanded-spectrum cephalosporins, amoxicillin-clavulanate, and the aminoglycosides but were resistant to ampicillin and older cephalosporins.

Keywords: *Acinetobacter*; MIC; Antimicrobial activity; Natural plant oils

Introduction

Acinetobacter is a genus of Gram-negative bacteria belonging to the wider class of Gammaproteobacteria. *Acinetobacter* species are not motile and oxidase-negative, and occur in pairs under magnification. *Acinetobacter* species are a key source of infection in debilitated patients in the hospital, in particular the species *Acinetobacter baumannii*. Species of the genus *Acinetobacter* are strictly aerobic, nonfermentative, Gram-negative bacilli. They show preponderantly coccobacillary morphology on nonselective agar. Rods predominate in fluid media, especially during early growth .The morphology of *Acinetobacter* species can be quite variable in Gram-stained human clinical specimens, and cannot be used to differentiate *Acinetobacter* from other common causes of infection. They are oxidase-negative, nonmotile, and usually nitrate-negative [1]. *Acinetobacter* species are widely distributed in nature, and commonly occur in soil. They can survive on moist and dry surfaces, including in a hospital environment. Some strains have been isolated from foodstuffs. In drinking water, they have been shown to aggregate bacteria that otherwise do not form aggregates [2]. *Acinetobacter* is frequently isolated in nosocomial infections, and is especially prevalent in intensive care units, where both sporadic cases and epidemic and endemic occurrences are common. *A. baumannii* is a frequent cause of nosocomial pneumonia, especially of 'late-onset' ventilator-associated pneumonia. It can cause various other infections, including skin and wound infections, bacteremia, and meningitis, but *A. lwoffi* is mostly responsible for the latter. *A. baumannii* can survive on the human skin or dry surfaces for weeks. Epidemiologic evidence indicates *Acinetobacter* biofilms play a role in infectious diseases such as periodontitis, bloodstream infections, and urinary tract infections, because of the bacteria's ability to colonize indwelling medical devices (such as catheters). Antibiotic resistance markers are often plasmid-borne, and plasmids present in *Acinetobacter* strains can be transferred to other pathogenic bacteria by horizontal gene transfer. The ability of *Acinetobacter* species to adhere to surfaces, to form biofilms, and to display antibiotic resistance and gene transfer motivates research into the factors responsible for their spread [3].

The spread of drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases. Down the ages essential oils and other extracts of plants have interest as sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases [4]. World Health Organization (WHO) noted that majority of the world's population depends on traditional medicine for primary healthcare. Medicinal and aromatic plants which are widely used as medicine and constitute a major source of natural organic compounds. Essential oils have been shown to possess antibacterial, antifungal, antiviral insecticidal and antioxidant properties [5,6]. Some oils have been used in cancer treatment [7]. Some other oils have been used in food preservation [8], aromatherapy [9] and fragrance industries [10]. Essential oils are a rich source of biologically active compounds. There has been an increased interest in looking at antimicrobial properties of extracts from aromatic plants particularly essential oils [11]. Therefore, it is reasonable to expect a variety of plant compounds in these oils with specific as well as general antimicrobial activity and antibiotic

potential [12]. Essential oils (also called volatile oils) are aromatic oily liquids obtained from plant materials (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots). They can be obtained by expression, fermentation or extraction but the method of steam distillation is most commonly used for commercial production. An estimated 3000 essential oils are known, of which 300 are commercially important in fragrance market [8]. Essential oils are complex mixers comprising many single compounds. Each of these constituents contributes to the beneficial or adverse effects. Essential oils such as aniseed, calamus, camphor, cedar wood, cinnamon, citronella, clove, eucalyptus, geranium, lavender, lemon, lemongrass, lime, mint, nutmeg, orange, palmarosa, rosemary, basil, vetiver and wintergreen have been traditionally used by people for various purposes in different parts of the world. Cinnamon, clove and rosemary oils had shown antibacterial and antifungal activity [13]; cinnamon oil also possesses antidiabetic property [14]. Anti-inflammatory activity has been found in basil [15]. Lemon and rosemary oils possess antioxidant property [15,16]. Peppermint and orange oils have shown anticancer activity [17,18]. Citronella oil has shown inhibitory effect on biodegrading and storage-contaminating fungi [19]. Lime oil has shown immunomodulatory effect in humans [18]. Lavender oil has shown antibacterial and antifungal activity; it was also found to be effective to treat burns and insect bites [20].

Materials and Methods

Collection of isolates

Isolates were collected from different departments of Aswan University hospitals and these isolates collected from patients after history taking and complete clinical examination then sample collected from different types of samples according to methods explained in in references that follow the type like urine [21], burn swabs, wound swabs [21], sputum [22] and endotratracheal swabs [23] from 1000 patients over 18 months. 72 isolates were identified as *Acinetobacters* isolates by using VITEK 2.

Identification

Identification with the VITEK 2 system was performed with ID-GNB cards, according to the manufacturer's instructions. The 64-well plastic ID-GNB cards contain 41 tests, including 18 tests for sugar assimilation, 18 tests for sugar fermentation, 2 decarboxylase tests, and 3 miscellaneous tests (for urease, utilization of malonate, and tryptophane deaminase). With a vacuum device, the cards are inoculated with a 0.5 McFarland suspension of the organism prepared from an 18 to 20-h-old Columbia sheep blood agar plate (bioMérieux) and are then automatically sealed and manually inserted inside the VITEK 2 reader-inoculator module. Fluorescence is measured every 15 min, and the results of identification are determined after 3 h.

Natural oils

The study used 18 natural oil including cinnamon oil, lavander oil, peppermint oil, thyme oil, orange oil, lemon oil, garlic oil, ginger oil, jasmine oil, green tea oil, tea tree oil, parsely oil, carway oil, red rose oil, rosemerry oil, camphor oil, nigella sativa (black seed) all of these oil obtainted from Tanta University medical plants departments which was pursed these oils from sigma company except (tea tree oil, tea oil, garlic oil pursed from sigma company) and the oils diluted with DMSO to obtain serials of dilutions and DMSO was used as control for these oils.

Antimicrobial Susceptibility Testing

MIC agar dilution assay

MIC against all isolates was determined by the agar dilution method according to the procedure recommended by the National Committee for Clinical Laboratory Standards [24].

A. Preparation of essential oils containing media

Muller-Hinton agar (MHA) was used as a basal medium. Sterile Mueller-Hinton agar (MHA) was allowed to equilibrate to about 50°C in a water bath. A series of two fold dilution of each oil in DMSO ranging from 0.125 to 6 mg/ml were added to the molten agar prior to inoculation. A plate containing identical amount of the basal medium, but free from essential oil was used as control. Plates were dried at room temperature for 30 min prior to inoculation.

B. Preparation of the inoculum

Two or three discrete representative overnight colonies, of each tested isolate were inoculated into 2 ml sterile saline, homogenized by vortex mixer and diluted to obtain OD values of 0.05 at 600 nm. Aliquot of the prepared suspension of each isolate was transferred by automatic pipette to a certain well in the seed plate of a sterile multiinoculator according to a configuration of a record key. Each well in the seed plate was half filled to avoid the carry over problem.

C. Inoculation and incubation of the plates

The plates were inoculated as follows; the head with sterile inoculating rods was gently lowered into the well in the seed plate, gently raised and gently lowered onto the surface of the agar medium.

D. Recording the results

After 24 h of incubation at 37°C for bacteria, MIC was determined as the lowest concentration of essential oil inhibited visible growth of the microorganism compared with the growth in the control plate. The presence of one or two colonies was not taken into account for final assessment of MIC [25,26].

Susceptibility of Tested Isolates to Different Antimicrobial Agents

MICs of antimicrobial agents against all bacterial isolates were determined using the agar dilution method according to the procedure previously described but instead of essential oil, membrane filtered stock solution of each tested antimicrobial agent was prepared. Serial dilutions of the tested antimicrobial agent solutions ranged from 0.125 to 6 mg/ml were added to the molten agar prior to inoculation. There are 14 antibacterial agents tested in this study the break points for these antibiotics were used according to CLSI (2017) guidelines [27].

Results

Table 1 shows the incidence of *Acinetobacter* infection in ICU and burn unit. Table 2 and Figure 1 shows the incidence of *Acinetobacter* isolates in relation to other organisms in this study. Table 3 shows the

relation between frequency of *Acinetobacter* infection and underlying disease in ICU of the studied cases. Table 4 shows the invasive procedures in respiratory tract *Acinetobacter* infection as a risk factor. Table 5 shows the Invasive procedures in urinary tract *Acinetobacter* infections as a risk factor.

Sites	**Burn unit**		**ICU**		**Other unit**	
Acinetobacter isolates (n=63)	n	%	n	%	N	%
	38	52.8	25	34.7	9	12.5
It shows that *Acinetobacter* isolated from burn unit were (52.8%) and from ICU were (34.7%) among the nosocomial isolates.						

Table 1: Incidence of *Acinetobacter* infection in ICU and burn unit.

Total number of	*Acinetobacter*		**Other organisms**	
Samples (n=405)	N	%	n	%
	72	17.8%	333	82.2%
It shows that the incidence of *Acinetobacters* was 17.8% among the total isolates.				

Table 2: Incidence of *Acinetobacter* isolates in relation to other organisms in this study.

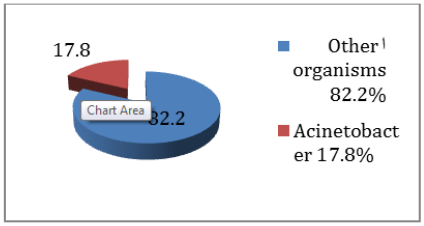

Figure 1: show that the incidence of *Acinetobacters* was 17.8% among the total isolates.

Diagnosis	*Acinetobacter* **infection**	
	N	**%**
Burn case	38	52.8
Stroke	8	11.1
Heart failure	6	8.3
Respiratory failure	4	5.6
Renal failure	2	2.8
Others	5	6.9

Table 3: Relation between frequency of *Acinetobacter* infection and underlying disease in ICU of the studied cases.

Discussion

This study were done to obtain detailed study about antimicrobial activity of 18 essential oil against *Acinetobacter* isolates that have considerable degree of resistance to a wide range of recommended antibiotics. All of the *Acinetobacter* included in this survey were isolated from clinical specimens and were considered to be significant by the referring laboratory.

Acinetobacter **Nis**	**Invasive procedure**			
Acinetobacter RTIs (n=52) isolates	Ventilated patients		Non Ventilated patients	
	N	%	N	%
	44	84.6	8	15.4
It shows that the rate of *Acinetobacter* respiratory tract infections was higher in ventilated patients.				

Table 4: Invasive procedures in respiratory tract *Acinetobacter* infection as a risk factor.

One of the most important nosocomial pathogen are *Acinetobacters* as they are often resistant to numerous antimicrobial agents and cause life-threating infections in patients with altered host-defense mechanisms. In addition, they have a tendency toward cross-transmission, especially in ICUs, where numerous outbreaks have occurred [28].

Acinetobacter **Nis**	**Invasive procedure**			
Acinetobacter RTIs (n=52) isolates	Catheterized Patients		Non Catheterized Patients	
	N	%	N	%
	11	73.3	4	26.7
It shows that *Acinetobacters* UTIs were higher in catheterized patents.				

Table 5: Invasive procedures in urinary tract *Acinetobacter* infections as a risk factor.

Antibiotic	**MIC (S-R)**	**Number of resistant isolates**	**Percent of resistance**
Ampicillin	8-256	55	76%
Piperacillin	2-128	43	59.7%
Augmentin	2-64	10	13.9%
Cefazolin	8-64	29	40.3%
Cefoxitin	8-16	33	45.8%
Cefotaxime	2-64	39	54.2%
Ceftazidime	4-64	30	41.7%
Imipenem	1-8	3	4%
Aztreonam	4-64	31	43%
Amikacin	16-128	22	30.5%

Gentamicin	16-64	39	54.7%
Tobramycin	2-16	22	30.5%
Ciprofloxacin	1-8	12	16.6%
Tetracycline	4-16	18	25%

Table 6: *In vitro* activities of 14 antimicrobial agents against 72 of *Acinetobacter* isolates.

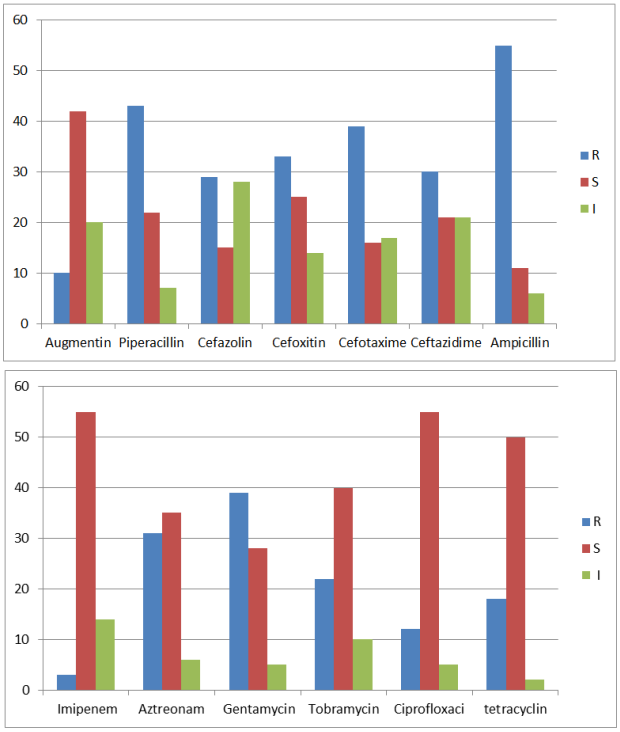

Figure 2: Demonstration of Good activity against *Acinetobacter* isolates.

As a little is known about *Acinetobacters* infection in our hospital, the current study aimed to identify the prevalence of *Acinetobacters*, in Aswan University Hospital as nosocomial pathogen. The present study also aimed to detect the resistance rate of *Acinetobacters* to different antimicrobial agents and different natural oils. For this purpose, 1000 samples were collected randomly from ICU of Anaesthesia Department and Internal Medicine Department as well as Burn Unit, Aswan University Hospital. Out of these 1000 samples 72 samples were detected as *Acinetobacters*. Several automated systems are available for the identification and susceptibility testing of the clinically most important bacteria [29]. The VITEK system (bioMérieux-Vitek, Hazelwood, Mo.) was originally designed as an onboard system for the detection and identification of *Acinetobacter* pathogens. It was first introduced in clinical laboratories in 1979 and has since been evaluated extensively [30]. More recently, the new VITEK 2 system (bioMérieux-Vitek) was introduced. The VITEK 2 system detects metabolic changes by fluorescence-based methods which facilitate the identification of gram-negative bacteria within 3 h. This system monitors the kinetics of bacterial growth and calculates MICs using a unique algorithm. In addition, the VITEK 2 system incorporates several technical improvements which automate many procedures that were performed manually with the previous VITEK system (Gayral et al., Clin. Microbiol. Infect. abstr. P254) and for this Evilo J Perea use VITEK 2 to identify *Acinetobacters* with high correct limits [31].

In this work the rate of NIs was 40.5% this result was smaller than that of Ozer [32] and his colleagues study who aimed to determine the types and risk factors for NIs in the ICU of Turkey University Hospital. They found that the rate of NIs was 68% which higher than that found in current study.

Natural oil	**MIC (mg/ml)**									
	0.125	0.25	0.5	1	2	3	4	5	6	7
Cinnamon	13	43	12	4	-	-	-	-	-	-
Clove	9	39	22	2	-	-	-	-	-	-
Thyme	-	19	35	18	-	-	-	-	-	-
Tea tree	-	3	-	39	30	-	-	-	-	-
Rose red	-	-	-	14	36	22	-	-	-	-
Peppermint	-	-	23	37	10	2	-	-	-	-
Lavander	-	2	12	35	19	4	-	-	-	-
Orange	-	-	-	-	3	12	35	20	2	-
Lemon	-	-	-	-	2	34	23	13	-	-
Rosemerry	-	-	7	10	33	14	8	-	-	-
Ginger	-	-	-	10	24	35	3	-	-	-
Garlic	-	-	-	-	-	23	41	8	-	-
Green tee	-	-	-	-	-	-	-	-	4	5
Parsely	-	-	-	-	-	4	-	-	7	14
Caraway	-	-	-	-	-	-	-	-	-	-
Nigella staive	-	-	-	-	-	-	-	-	-	-
Camphor	-	-	-	-	-	-	-	-	-	-
DMSO (solvent)	-	-	-	-	-	-	-	-	-	-

Table 7: MIC distribution of tested essential oils against clinical isolates of *Acinetobacters* (n=72).

While [31] who stated that the rate of NIs in their study was 20% and [33] detected lower incidence of NIs 12.2%, these results were lower than the current one. NIs varies between different studies in different countries according to the establishment of preventive measures and developmental status and between the hospitals

according to the spectrum of their patients and between the wards of the hospitals according to treatment and intervention [33].

In the present study, the incidence of *Acinetobacter* among the nosocomial isolates was 17.8% these results correlate with Radwa Essa result who reported that *Acinetobacters* account for 13.9% of NIs and [34] who reported that *Acinetobacters* 11% of NIs which was explained by previous exposure of the patients to various risk factors as ventilation, urinary catheterization and surgical operations. Also the results were similar to those of [35], who found that *Acinetobacters* represented 9% of bacteriologically positive samples collected from teaching hospital in India. This can be explained by previous report made by [36] which had shown that there is a relatively high frequency of *Acinetobacter* infection in hot countries as hot climate and humidity favors *Acinetobacter* infection. However, these finding were higher than those of [37] who stated that *Acinetobacters* represent 1.43% of all nosocomial isolates over a six year period. This may be attributed to the antimicrobial therapy received by his patients before *Acinetobacters* isolation. On the other hand, [38] found that *Acinetobacter* isolates among nosocomial infections represent 32.8% which was higher than the current study. This was attributed to exposure of all his patients to several risk factors especially prolonged stay in ICU for more than 15 days before *Acinetobacters* isolation. In this work *Acinetobacters* were isolated from urine, sputum, tracheal aspirates, surgical wounds and burn samples. *Acinetobacters* were most commonly isolated from urine samples 29.6% followed by respiratory tract samples including sputum and tracheal aspirates 26.9% and surgical wound samples 23.5% and lastly from the burn samples in which *Acinetobacter* isolates represent 17.8% these results are in line with that of [39], who isolated *Acinetobacters* most commonly from urine samples 31% followed by respiratory tract and wound samples which represent 26.7% and 17.8% respectively. On contrary to our results [40] stated that *Acinetobacters* is most commonly isolated from respiratory tract samples 38.5%. Also [41] estimated that *Acinetobacter* respiratory tract represented by 54% followed by wound infection 22%. This variation is explained by Bergogne-Berezin [42] who stated that the predominant sites of *Acinetobacter* nosocomial infection have varied with time. In early observations, UTIs predominated in ICUs. Recently the incidence of UTIs has decreased, possibly in relation to better care of urinary catheters, whereas the incidence of nosocomial pneumonia has increased significantly as reported in several recent surveys.

The incidence of *Acinetobacter* infection is high in ICU patients who always exposed to various risk factors as prolonged hospital stay, medical devices especially central venous catheters, heavy exposure to broad-spectrum antimicrobial drugs especially cephalosporins, surgical operations and severe illness. So in the current study, ICU admission may be considered as a risk factor for *Acinetobacters* acquisition. This runs in parallel with both [43] who reported that *Acinetobacters* are common pathogen particularly in ICUs where risk factors of colonization and infection with *Acinetobacters* are present and [44] who stated that ICU admission was associated with MDR *A. baumannii* acquisition while [45] who found that *Acinetobacters* are an important cause of clinical infection especially in patients hospitalized in ICUs. Also Blot and his colleagues observed that patients with *A. baumannii* had longer ICU stay [46]. This was also accepted by [47] who reported that outbreaks of HAIs caused by *Acinetobacters* are recognized particularly in ICUs. Medical device insertion play an important role in *Acinetobacter* infection, in the present work the incidence of *Acinetobacter* infection in ventilated patients is higher than in nonventilated patients, also in case of urinary catheterization the incidence of *Acinetobacter* infection in catheterized patients is higher than in non-catheterized patients. This is coordinated with the finding of [48] who stated that mechanical ventilation and urinary catheterization are risk factors for *Acinetobacter* NI .

The essential oils tested in the present study were found to inhibit *Acinetobacter* isolates. The MIC value does not depend on the level of bacterial resistance. Parkasm et al. [49] report that clove, peppermint possess antibacterial activity against *Acinetobacter* isolates obtained from variable clinical specimens as endotracheal aspirates, urine, burn swabs and sputum [50]. This study has demonstrated that seven natural oil inhibit *Acinetobacter* isolates at high concentrations which is cinnamon, thyme, rosemerry, clove, tea tree, peppermint and lavender, inhibited all organisms at high concentrations of ≤ 1024 µg/ml. Four oils did not inhibit any organisms at the highest concentration, which was 1024 µg/ml oil for tea, camphor, parsely and Nigella staive. However, the present finding suggest that while oils should be used in diluted form, especially when directly applied to skin, their antimicrobial properties are effective as those of chemical antibacterial agents. In addition, it is important that the microorganisms do not acquire resistance to the natural oils or to their components [51]. The lowest concentration of oils that inhibited the growth of *Acinetobacter* isolates were considered as MIC. The essential oils tested in the present study were found to inhibit *Acinetobacter* isolates from patients. The MIC value does not depend on the level of bacterial resistance [52] proved high antibacterial activity of essential oil from Cinnamon oil against gram negative bacteria in this study MIC of cinnamon oil at concentration ranging from 0.25 to 4 mg/ml and MIC of lavender at concentration ranging from 0.5 to 4 mg/ml and MIC of thyme ranging from 0.5 to 2 mg/ml which is similar to result proved by [53] and MIC of tea tree oil at concentration 0.5 to 1 mg/ml which is proved in paper by name Tea tree oil–natures miracle in fighting infections and other problems. MIC of rosemerry at concentrations 3 to 5 mg/ml. MIC of peppermint at concentration 0.5 mg/ml and MIC of clove at concentration 0.5 to 3 mg/ml, and all of these oils inhibit all isolates at high concentration. MIC of lemon oil at concentration 2 to 5 mg/ml which is similar to result proved by Edeltrudes de Oliveira Lima and orange oil have similar results. MIC of Red rose oil at concentration 1 to 4 mg/ml which similar to result in paper by name The Antimicrobial effect of Aqueous extract of garlic against resistant *Enterococci* and MIC of ginger is like garlic oil and this indicate that they are weak against *Acinetobacter* isolates which and this result similar to result e in paper by name antimicrobial active herbal compound against *A. baunmanni* and other pathogen. other natural oil not have any activity against *Acinetobacter* like nigella sative (black seed oil), caraway, camphor, parsely and tea oil and that result of nigella sative oil obtained from seed and leaves have no activity confirmed in Antibiotic Resistance: Mechanisms and New Antimicrobial Approaches edited by Kateryna Kon, Mahendra Ral in chapter 12. Hammer et al. determined the antimicrobial activity of 53 essential oils, including oil of thyme, against various bacterial species with the use of serial dilution technique, both agar and broth. The minimum inhibitory concentrations with thyme oil to the reference strain NTCT 7844 of *A. baumannii* was 0.12 µL/mL and *P. aeruginosa* NTCT 10662-over 2 µL/mL). In this study the most powerful oils were cinnamon, thyme, lavender, clove and tee tree oils with MIC range from 0.125 to 1 mg/ml and this very useful in the treatment of *Acinetobacter* nosocomial infection which is a big problem this result is similar to results [52] and oils with moderate effect like lemon and orange, which their MIC >2 mg/ml which also similar to results given

by [52]. Also peppermint oil also has powerful antimicrobial activity with MIC range 0.5 to 3 mg/ml and this results similar to that given by [52] with MIC range 0.5 to 1 mg/ml and red rose oil have moderate effect with MIC 1 to 4 mg/ml which similar to results of [54] whose results was MIC from 2 to 4 mg/ml. and ginger give moderate antimicrobial effect with MIC from 1 to 4 mg/ml which is similar to results given by [55]. And this author has similar result in the MIC of tee tree oil, MIC from 1 to 2 mg/ml which similar to the results in our study. And support our results also which is similar to MIC for clove and cinnamon oils also and Nigla sativa oil seeds (black seed oil) has no antimicrobial activity and this is similar to results in a book by name Antibiotic resistance.

And rosemerry oil have also moderate antimicrobial effect with MIC range from 0.5 to 4 mg/ml which close to results given by Widad Jumaa that made investigation in Iraq 2015 [56] that gave MIC range from 0.312 to 5 mg/ml. and green tee oil has very weak effect that kill only 4 isolates at 6 mg/ml and this results supported by results that gave MIC is 15.6 mg/ml that given by [57] and this study was made at Isfahan University.

This study presents 14 antimicrobial agents against 72 *Acinetobacter* clinical isolates. The activity of different agents against *Acinetobacter* isolates are shown in the (table 6) shows the percentage of resistant isolates at CISI breakpoints [58]. In terms of MICs for 90% of isolates, the most active agent against *Acinetobacter* isolates was imipenem. Few resistant strains for imipenem [3] isolates 4% of the total isolates was found which was very small according to the total number 72, others have reported moderate activity like Amoxicillin-clavulanate, Whereas ampicillin, broad-spectrum penicillins, cephalosporins, aminoglycosides, and ciprofloxacin were less active. The trend towards resistance to expanded-spectrum cephalosporins was demonstrated by and seemed to be related to the presence of cephalosporinases. Recently, the presence of an extended broad-spectrum beta-lactamase was reported (Others have also found increasing resistance of *Acinetobacter* isolates to modern quinolones as well as to amikacin and tobramycin. Resistance to amikacin was shown to be due to the presence of aminoglycoside phosphotransferase. Excellent activity against *Acinetobacter* isolates was shown for imipenem, amikacin, ciprofloxacin, Amoxicillin clavulanate. Of the other beta-lactamase tested, only Ceftazidine showed moderate *in vitro* activity, some isolates show greater susceptibility to ciprofloxacin and aminoglycosides [59].

This study presents 14 antimicrobial agents against 72 *Acinetobacter* clinical isolates. The activity of different agents against *Acinetobacter* isolates shows the percentage of resistant isolates at ClSI breakpoints (2017). In terms of MICs for 90% of isolates, the most active agent against *Acientobacter* isolates was imipenem. Few resistant strains for imipenem [3] isolates of the total isolates was found which was very small according to the total number 72 and this results are in consistence with [60] who stated that most of his *Acinetobacter* isolates were susceptible to imipenem (Table 7). Also [61] mentioned the same result. In line with these results [44], who reported that 93% of his *Acinetobacter* isolates were susceptible to imipenem, also [62] reported that most of his *Acinetobacter* nosocomial isolates sensitive to imipenem. Coming to aminoglycoside, [41] stated that *Acinetobacter* resistant to gentamycin was 70%. Also Spence and his colleagues reported that *Acinetobacter* were found resistant to 4 or more aminoglycoside. On the other hand, [63] reported an outbreak of infections due to *Acinetobacter* resistant to carbapenems that occurred in a New York hospital after increased use of imipenem. The prolonged use of carbapenems for the treatment of nosocomial infections can favor the development of resistance to these antimicrobial agents. The spread of these strains within the hospital environment is a serious problem that could contribute to poor patient outcome [64].

Acinetobacter is resistant to most β-lactam antibiotics, particularly penicillins and cephalosporins, especially in ICU patients [65-68]. Ceftazidine, Piperacillin and carbapenems are among the β-lactam antibiotics most active against *A. baumannii*. The main mechanism of resistance to β-lactam antibiotics in *Acinetobacter spp*. is the production of β-lactamases encoded either by the chromosome or by plasmids [69]. In a study from Germany in the early 1990s, imipenem was found to be the most active agent against *A. baumannii*. All 180 *Acinetobacter spp*. isolates tested were fully susceptible to imipenem. Amoxycillin-clavulanate showed moderate activity, whereas ampicillin, broad-spectrum penicillins and cephalosporins were less active. Similarly, in another report dating from 1991, 23 *Acinetobacter spp*. isolates were obtained from ICU patients in ten German hospitals. Ceftazidine and imipenem were the most active β-lactam antibiotics, with 96% of the isolates remaining susceptible. Susceptibilities to Piperacillin and Cefotaxime were 65% and 61%, respectively [70]. All 11 *Acinetobacter spp*. strains isolated in 1990 from patients in eight Dutch hospitals were susceptible to imipenem, and ten (91%) of the strains were susceptible to ceftazidine, ceftriaxone and amoxycillin-clavulanate [71]. In a study from Germany in the early 1990s, imipenem was found to be the most active agent against *A. baumannii*. All 180 *Acinetobacter spp*. isolates tested were fully susceptible to imipenem. Amoxycillin-clavulanate showed moderate activity, whereas ampicillin, broad-spectrum penicillins and cephalosporins were less active. Similarly, in another report dating from 1991, 23 *Acinetobacter spp*. isolates were obtained from ICU patients in ten German hospitals. Ceftazidime and imipenem were the most active β-lactam antibiotics, with 96% of the isolates remaining susceptible. Susceptibilities to Piperacillin and Cefotaxime were 65% and 61%, respectively [70]. All 11 *Acinetobacter spp*. strains isolated in 1990 from patients in eight Dutch hospitals were susceptible to imipenem, and ten (91%) of the strains were susceptible to ceftazidime, ceftriaxone and amoxycillin-clavulanate [72]. Resistance of *A. baumannii* to the fluoroquinolones has been attributed to changes in the structure of DNA gyrase or topoisomerase IV as mutations. In Germany, 96% of *Acinetobacter spp*. isolates from ICU patients were susceptible to ciprofloxacin [70], and all 11 *Acinetobacter spp*. isolated in 1990 from patients of eight Dutch hospitals were susceptible to ciprofloxacin [73]. In 1994-1995, susceptibilities to ciprofloxacin in isolates from ICU patients were 82% in Belgium, 22% in France, 25% in Portugal, 19% in Spain and 81% in Sweden [74,75]. In Belgium, 51% of the 70 *Acinetobacter spp*. isolates from ICU patients in 1990 were susceptible to ciprofloxacin [75], while 76% of the 41 *Acinetobacter spp*. isolated in 1997 from Belgian ICUs were susceptible to ciprofloxacin, compared with 56% of the 11 isolates in 1999 [76]. Of the 268 *A. baumannii* isolated from the ICUs of 39 French teaching hospitals in 1991, were 18% were susceptible to ciprofloxacin [77]. In Spain, [67] found ciprofloxacin (70%) and ofloxacin (72%) to be more active against clinical isolates of *A. baumannii* than norfloxacin (18%), but in a separate study, ciprofloxacin resistance in clinical isolates of *Acinetobacter* increased in Spain from 54.4% in 1991 to 90.4% in 1996 [78]. Of the 279 clinical *Acinetobacter spp*. isolates from 20 European university hospitals participating in the 1997-1998 SENTRY study, 45.2%, 46.6% and 47.3% were susceptible to ciprofloxacin, ofloxacin and levofloxacin, respectively. Gatifloxacin and trovafloxacin showed the best *in-vitro* activities against *Acinetobacter spp*. [79]. Quinolones

showed poor activity against *Acinetobacter spp*. from blood cultures. Only 50.6%, 52.6% and 54.7% of the 247 isolates showed *in-vitro* susceptibility to ciprofloxacin, ofloxacin and levofloxacin, respectively. Similar resistance rates were seen throughout the different European centers [80]. Of the 41 *Acinetobacter spp*. isolates associated with skin and soft tissue infections, 41.5%, 46.3% and 48.8% were susceptible to ciprofloxacin, ofloxacin and levofloxacin, respectively [81]. Different publications have reported excellent activity of doxycycline or minocycline, but not tetracycline, against *Acinetobacter spp*. Shown in Figure 2 [67,82]. This may result from the fact that Tetra, the major tetracycline resistance determinant, confers resistance to tetracycline, but not to minocycline. In a Spanish study of the early 1990s, 98% of 54 A. baumannii isolates tested were susceptible to doxycycline [67]. Of 109 *A. baumannii* isolates tested in Spain between 1997 and 1999, 85% were resistant to tetracycline [83].

Conclusion

Cinnamon oil, thyme, clove, tee tree oils have very powerful antimicrobial activity against *Acinetobacter* isolates so it can be used as alteranative to antibiotics which is very useful in the treatment of *Acinetobacter* nosocomial infections which was a very big problem in the recent years.

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