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Efficacy of the Clove Oil, Cinnamon Oil, Thyme Oil and Origanum Oil against Multidrug Resistant *Pseudomonas aeruginosa* and *Burkholderia cepacia* Complex

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A B S T R A C T

The increased frequency in clinically observed cases of antibiotic resistance has been attributed to many factors such as the misuse and overuse of antibiotics since in some countries, antibiotics are sold over the counter without a prescription, the large quantities of antibiotic waste produced from livestock rearing, overconfidence in human control over infectious diseases and the continued decline in the number of newly approved antibiotics. Few studies have focused on the investigation of antimicrobial activities of medicinal plants against clinically isolated antibiotic resistant pathogens. Hence the aim of this work is to investigate the antimicrobial effect of clove, cinnamon, thyme and origanum on clinically isolated multidrug resistant strains of *Pseudomonas aeruginosa* and *Burkholderia cepacia complex*.

Introduction

Antibiotics have always been considered one of the wonder discoveries of the 20th century. However, the resistance of various microbial species to different antimicrobial drugs has emerged as a cause of public health threat all over the world at a terrifying rate, due to the pacing advent of new resistance mechanisms and decrease in efficiency of treating common infectious diseases, it results in failure of microbial response to standard treatment, leading to prolonged illness, higher expenditures for health care, and an immense risk of death (Tanwar, 2012).

Pseudomonas aeruginosa and *Burkholderia cepacia* complex are considered the most

resistant Gram-negative bacteria. Infections in the intensive care unit and burn unit represent a great challenge, especially those caused by Gram-negative organisms (Sader, 2014). The most serious complication of infection with *Pseudomonas aeruginosa* and *Burkholderia cepacia complex* is the delay of healing and resistances to available of antibiotics among these infections are burn and infection in intensive care units (Patil, 2015; Afify, 2012; Azzopardi *et al*., 2014; Sader, 2014).

Nature is a generous source of a number of compounds with potential application for the treatment of several diseases including the infectious diseases. The presently investigated natural products derived from local botanical are promising candidates that could be used against multidrug resistant (MDR) pathogens. Nevertheless, there is still a vast flora that once systemically explored could provide additional antimicrobial leads and drugs (Palombo, 2011). The increasing resistance of microorganisms to conventional chemicals and drugs has prompted scientists to search for novel sources of biocides with broad-spectrum activities. Vegetable, fruits and spices with high level of essential oils are excellent sources of natural elements with activity against microorganisms of agricultural and health interest (Palaniappan, 2010).

It worth to mention that this is the first report for natural products illustrated significant activities against *Burkholderia cepacia complex*, although many studies were carried out before to evaluate essential oils activities against several microorganisms (Lang, 2012).

Materials and Methods

The fine powder of clove, cinnamon, thyme and origanum (1500 g, each) were subjected to hydro distillation to prepare their essential oils using Clevenger-type apparatus.

Forty essential oil specimens, 10 per each plant is required for *Pseudomonas aeruginosa* and *Burkholderia cepacia complex* to estimate an average minimal difference =11.5 mm with SD = 2.8 mm and 5 mm, respectively, α = 0.05 will provide a power of 80%.

Determination of MIC

Preparation of 10 fold dilution of each essential oil

We used two sets of 10 ml glass tubes with cover (one set for each organism), each set consisted of 24 test tubes arranged in 4 rows (labeling each row with one of the tested herbal essential oil). In each of these 48 tubes we put 1 ml of cooked meat media using graduated glass pipette with broth to be sterilized in autoclave after sterilization. From the first tube of each row 100 µl of cooked meat broth was removed and discarded using a sterile automatic pipette and sterile tips. Then we put 100 µl of each essential oil in the first tube of each row using a sterile automatic pipette and sterile tips and mixed the content of the tubes of first row well by using vortex then from the first tube of each row we took 100 µl of its mixture (cooked meat broth + essential oil) put it in the second tube of each row and mixing them well by using vortex then 100 µl of the mixture to third tube of each row was removed and so on till the sixth tube of each row, from the tube number six in each row 100 µl of the mixture was removed and discarded to make all volume constant in the six tubes, so we prepare 10 fold dilution of the essential oils starting with 10^{-1} and ending with 10^{-6} µl/ml. Then the tubes were incubated at 37°C for 24 hours, after the incubation the suspension of each tube was inspected by naked eye for turbidity (growth) or clearance (no growth) to determine the MIC (the least concentration or the highest dilution that give the clearance suspension).

Cultivation of the two type of the bacteria

We used *Pseudomonas aeruginosa* and *Burkholderia cepacia complex* by bringing the stocks of glycerol broth we kept at -80 ^{0}C to the room temperature, and then in the surface of nutrient agar plate we took a loopful from each glycerol broth and streaked it in the surface of nutrient agar plate and put in the incubator at 37°C for 24 hours then transferred sum of it using a sterile loop, put it in tube contain sterile distilled water to obtain a suspension and standardize to McFarland standard 0.5 then added 10 µl of each bacterial suspension measured by McFarland standard 0.5 by calibrated loops in all six tubes (one type of bacteria in first rack tubes and another tube in another rack tubes).

Determination of MBC

After inspection of turbidity in MIC method, we took one loopful of suspension showing apparently clearance (the test showed the last clearance was recorded) by calibrated loops 10 µl from each clear tube and streaked the surface in nutrient agar then incubated at 37°C for 24 hours, after the incubation, the plate examined for any colonial growth to determine the MBC (plate showing complete no growth).

Results and Discussion

Pseudomonas aeruginosa and *Burkholderia cepacia complex* are MDR organisms as illustrated in their antibiograms (Table 1).

Table.1 Antibiogram of multidrug resistant *Pseudomonas aeruginosa* and *Burkholderia cepacia* complex

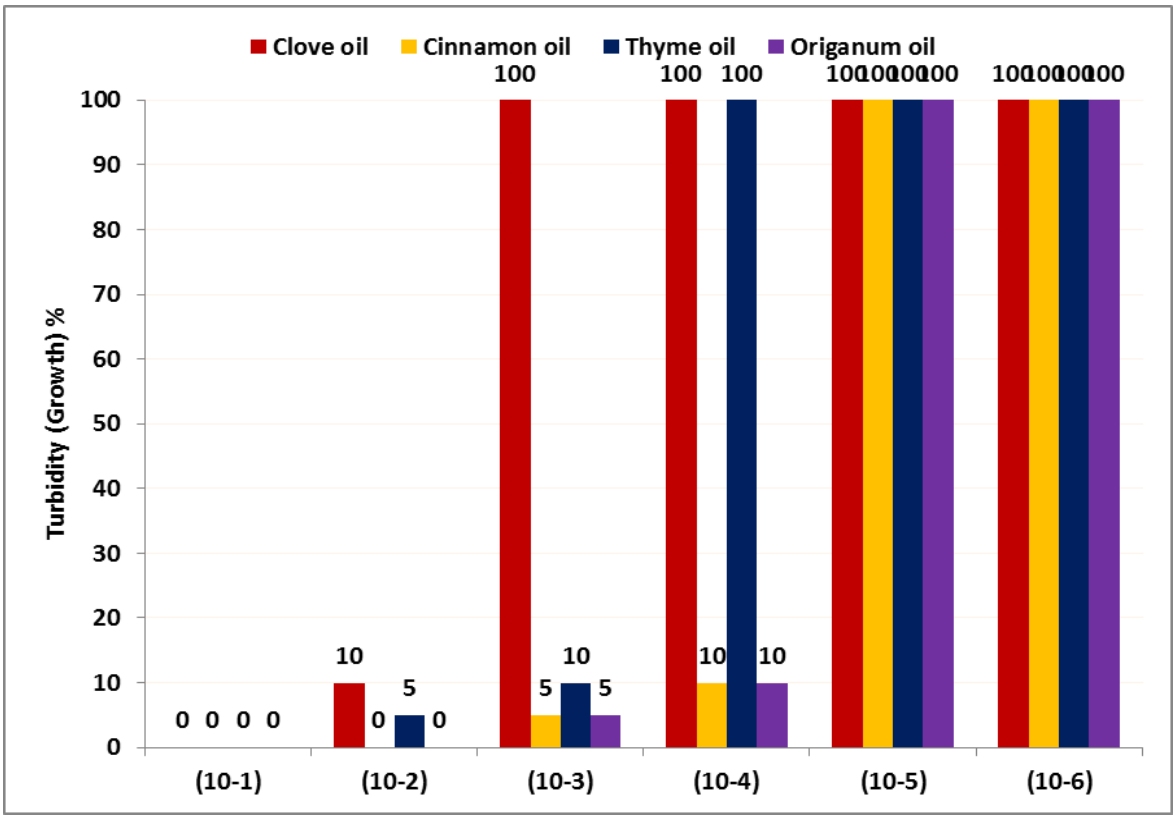
Organisms	No. of isolates	Gentamycin (GN)%	Amikacin (AK)%	Ampicillin/Sulbactam (SAM)%	Amoxacillin/Clavulanate(AMC)%	Cefipime(FEP)%	Cefoperazone(CFP)%	Cefotaxime(CTX)%	Ceftriaxone(CRO)%	Piperacillin(PIP)%	Imipinem(IPM)%	Meropenem(MEM)%	Ciprofloxacin(CIP)%	Levofloxacin(LEV)%	Trimethoprim/Sulfamethoxazole(SXT)%	Aztreonam(ATM)%	Ceftazidime(CAZ)%	Chloramphenicol(C)%	Colistine(c)%	Tetracyclin(TE)%
P.aeruginosa	40	55.8	55.8	74.4	74.4	55.8	55.8	100	-	74.4	55.8	55.8	55.8	55.8	-	55.8	74.4	55.8	55.8	-
BCC	40	100	87.5	100	100	100	100	100	100	100	62.5	62.5	100	100	100	100	100	100	50	61.25

Table.2 Distribution of MIC and MBC of the four essential oils through six different concentrations on *Pseudomonas aeruginosa* and *Burkholderia cepacia* complex isolates

Concentration µl/ml	Method	Clove oil		Cinnamon oil		Thyme oil		Origanum oil		*MCP*
		No	%	No	%	No	%	No	%	
(10^{-1})	**MIC**									
	▪ Clear	40	100	40	100	40	100	40	100	-
	▪ Turbid	0	0	0	0	0	0	0	0	
	MBC									
	▪ Growth	0	0	0	0	0	0	0	0	-
	▪ No growth	40	100	40	100	40	100	40	100	
(10^{-2})	**MIC**									
	▪ Clear	36	90	40	100	38	95	40	100	0.253
	▪ Turbid	4	10	0	0	2	5	0	0	
	MBC									
	▪ Growth	4	10	0	0	2	5	0	0	0.253
	▪ No growth	36	90	40	100	38	95	40	100	
(10^{-3})	**MIC**									
	▪ Clear	0	0	38	95	36	90	38	95	0.001*
	▪ Turbid	40	100	2	5	4	10	2	5	
	MBC									
	▪ Growth	40	100	2	5	4	10	2	5	0.001*
	▪ No growth	0	0	38	95	36	90	38	95	
(10^{-4})	**MIC**									
	▪ Clear	0	0	36	90	0	0	36	90	0.001*
	▪ Turbid	40	100	4	10	40	100	4	10	
	MBC									
	▪ Growth	40	100	4	10	40	100	4	10	0.001*
	▪ No growth	0	0	36	90	0	0	36	90	
(10^{-5})	**MIC**									
	▪ Clear	0	0	0	0	0	0	0	0	-
	▪ Turbid	40	100	40	100	40	100	40	100	
	MBC									
	▪ Growth	40	100	40	100	40	100	40	100	-
	▪ No growth	0	0	0	0	0	0	0	0	
(10^{-6})	**MIC**									
	▪ Clear	0	0	0	0	0	0	0	0	-
	▪ Turbid	40	100	40	100	40	100	40	100	
	MBC									
	▪ Growth	40	100	40	100	40	100	40	100	-
	▪ No growth	0	0	0	0	0	0	0	0	
P		0.001*		0.029*		0.005*		0.029*		
Agreement rate between MBC & MIC		100%								

MCP: Mont Carlo exact probability * P < 0.05 (significant)

Fig.1 Comparison between the four essential oils according to turbidity and growth on *Pseudomonas aeruginosa* and *Burkholderia cepacia* complex isolates, Alexandria, 2016



Antimicrobial activity results (Table 2) revealed that the cinnamon essential oil and origanum essential oil gave the best results as it showed MIC and MBC against *Pseudomonas aeruginosa* and *Burkholderia cepacia complex* at concentration 10^{-4} µl/ml. Table (2) shows that MIC and MBC of clove oil is at (10^{-2} µl/ml), the MIC and MBC of thyme oil is at (10^{-3} µl/ml), while MIC and MBC for both cinnamon oil and origanum oil are at (10^{-4} µl/ml). All the concentrations were statically none significant except the concentration (10^{-3} µl/ml) and (10^{-4} µl/ml).

Figure (1) prove that that the essential oil of cinnamon oil and origanum oil that gave clearance and no growth till concentrations (10^{-4} µl/ml), then thyme oil that gave clearance and no growth till concentrations (10^{-3} µl/ml), and the least one is clove oil that gave clearance and no growth till concentrations (10^{-2} µl/ml).

In this context a study of antimicrobial activities of clove, cinnamon, thyme and origanum against *Pseudomonas aeruginosa and Burkholderia cepacia complex* demonstrated that, the four studied essential oils displayed activities towards all the tested clinical isolates.

From the results of this study the cinnamon essential oil and origanum essential oil gave the best results as they showed MIC and MBC against *Pseudomonas aeruginosa and Burkholderia cepacia complex* at concentration 10^{-4} µl/ml.

In case of comparing the antimicrobial activity of each pair of the studied essential

oils: Thyme essential oil was more active than clove essential oil at conc. 10^{-3} µl/ml. Regarding cinnamon essential oil, it was more active than both clove and thyme essential oils at conc. 10^{-4} µl/ml, while cinnamon and origanum essential oils both illustrated the same activity at conc. 10^{-4} µl/ml. Concerning origanum essential oil it was more active than both clove and thyme essential oils at conc. 10^{-4} µl/ml.

Cinnamon essential oil is enriched in phenyl propanoids as cinnamaldehyde, cinnamic acid, cinnamyl acetate and eugenol, in addition to the monoterpene hydrocarbons; terpinolene and α-thujene and the 3^{ry} alcohol linalool. On the other hand, origanum essential oil contains many phenyl propanoid compounds as eugenol, carvacol, p-cymene, estragole and thymol. Moreover, it contains monoterpene hydrocarbons; α-pinene, myrcene, β-ocimene and α-terpinene. Consequently the higher activities of both cinnamon and origanum essential oils are attributed to the synergism of all their volatile components.

Volatile oil components are reported to exhibit antimicrobial activity via various potential pathways, they induce structural changes in cytoplasmic membrane, affecting its permeability, resulting in release of ATP and interacts with both membrane and intracellular proteins damaging a variety of cellular functions (Xu, 2008).

From the results of this study, the following could be recommended: Routine screening for *P.aeruginosa* and BCC from burn and intensive care units to prevent spread among susceptible individuals and to allow for appropriate management. Further in vitro and in vivo studies on cinnamon oil, origanum oil, thyme oil and clove oil to determine the bioavailability of active compounds, dose and toxicity before using them as therapeutic agents for *P.aeruginosa* and BCC infection.

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