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Antibacterial Effect of Eucalyptus Essential Oil

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Abstract

Background: To determine minimum inhibitory concentration (MIC) of the Eucalyptus essential oil (EEO) and different antibiotics on *Staphylococcus aureus* ATCC 29213 and *Pseudomonas aeruginosa* ATCC 27853, and on twenty bacterial isolates from wound swabs (10 *S. aureus* and 10 *P. aeruginosa*). In addition, to evaluate the antibacterial effect of combinations of EEO with selected antibiotics. **Methods:** Skin infection swabs were cultured; all bacterial isolates were identified according to conventional methods. Ten-gram positive isolates (*S. aureus*), and ten gram negative isolates (*P. aeruginosa*) were used to determine MIC of some antibiotics and EEO by broth microdilution methods**.** Checkerboard method was used to calculate fractional inhibitory concentration indexes. **Findings:** EEO exhibited a synergistic activity against *S. aureus* ATCC 29213 but only gave additive effect against *P. aeruginosa* ATCC 27853. Outcome of oil/vancomycin combination found to be synergistic in all tested clinical *S. aureus* isolates from infected wound swabs. While 80% of clinical *P. aeruginosa* isolates showed additive outcome of EEO/ceftazidime combination, and only 20% of them gave indifference outcome. **Application:** Dermatological applications of EEOs have been growing with great popularity worldwide. It can be used as ointments to treat various dermatological conditions such as abscesses, athlete's foot, dermatitis, bacterial infections, blisters, boils, burns, cuts, and wounds

Keywords: Bacterial Drug Resistance; Essential Oils; Infected Wounds; Natural Compounds, Synergism.

1. Introduction

New antimicrobial compounds are needed to fight through the battle between humans and disease-causing pathogens, especially with the appearance of multidrug resistance [1]. Nature is a precious reservoir of natural antibacterial compounds extracted from marine animals, microorganisms, and plants [2].

Essential oils (EOs) are an odorous and volatile compound produced from only 10% of the plant kingdom [3]. The antibacterial activity of EOs depends on their chemical

composition [4]. Different antibacterial mechanisms such as disruption of the cell wall, and penetration of cell membrane had been proposed [5]. Difference in bacterial cell structure caused gram-negative bacteria to be more resistant to EOs than gram-positive bacteria. A new concept to face bacterial resistance is to combine conventional antimicrobial agents and EOs to reduce the minimum effective dose of antibiotics and thus minimize their adverse effects [6]. Oil of Eucalyptus plant (EEO) is one of the most promising essential oils to treat wound infections [7].

This research aims to discover the antibacterial synergystic effect of EEO in Egypt, as medicinal plants differ in their effect according to ecophysiological properties of plants grown in different geographical areas [8].

2. Material and Methods

2.1. Study Area

This study was carried during two-month period from beginning of September to end of October 2019. Infected wound swabs were obtained from different private hospitals in Alexandria.

2.2. Laboratory Investigation

2.2.1. Volatile Oil Preparation

Commercial EEO from Imtenan health shop (Imtenan) was dissolved to a final concentration of 0.001% Tween 80 to enhance oil solubility and diffusion [9].

2.2.2. Isolation and Identification of the Clinical Isolates

Skin infection swabs were inoculated on blood and MacConkey agarplates and incubated aerobically at 37 °C for 24 h. All bacterial isolates will be identified according to conventional methods [10].

2.2.3. Determination of Minimal Inhibitory Concentration of the Eucalyptus Essential Oil and Some Antibiotics on Isolated Bacteria

The minimal inhibitory concentrations (MICs) were determined by broth microdilution methods. MIC was determined as the lowest concentration without bacterial growth [11].

2.2.4. Determination of Effect of Combination of Eucalyptus essential oil with Some Antibiotics

Checkerboard method was used to calculate Fractional inhibitory concentration indexes (FICIs): FICI = FIC A (MIC of substance an in combination/MIC of substance an alone) + FIC B (MIC of substance B in combination/MIC of substance B alone). It was considered synergistic when the FICI value is ≤0.5, additive when it was 0.5 to ≤1, indifferent when it was 1–4.0, and antagonistic when it was >4 [12].

2.2.5. Statistical Analysis

Data were tabulated analyzed using statistical software SPSS version 24 (IBM Corp., Chicago, IL). Descriptive statistics of demographic variables were calculated including frequencies, percentages.

3. Results

The FIC and FICI values of the EEO and some antibiotics against *Staphylococcus aureus* ATCC 29213 and *Pseudomonas aeruginosa* ATCC 27853 were determined with the broth microdilution method (Table 1).

The oil exhibited a synergistic activity against *S. aureus* but only gave additive effect against *P. aeruginosa.* Determination of FIC of some antibiotics against *S. aureus* and *P. aeruginosa* isolates from infected wound swabs (Table 2). The MIC values of the EEO and vancomycin against ten *S. aureus* isolates from infected wound swabs were determined with broth microdilution method (Table 3). Outcome of oil/vancomycin combination

TABLE 1. Determination of FICI of EEO and some antibiotics on S. aureus ATCC 29213 and P. aeruginosa ATCC 27853

Antimicrobial substances	***S. aureus* ATCC 29213**		***P. aeruginosa* ATCC 27853**		
	FIC	**FICI**		**FIC**	**FICI**
EEO	0.11	0.26	EEO	0.25	0.75
Vancomycin	0.25		Ceftazidime	0.50	
EEO	0.25	0.37	EEO	0.20	1
Ampicillin	0.12		Ciprofloxacin	0.80	
EEO	0.18	0.21	EEO	0.25	0.96
Ceftriaxone	0.03		Gentamicin	0.71	

TABLE 2. Determination of FIC of some antibiotics against S. aureus and P. aeruginosa isolates from infected wound swabs

Strains	***S. aureus* isolates**			***P. aeruginosa* isolates**		
	Vancomycin	**Ampicillin**	**Ceftriaxone**	**Ceftazidime**	**Ciprofloxacin**	**Gentamicin**
No 1	0.125	0.13	0.17	0.002	0.80	0.75
No 2	0.0625	0.18	0.31	1	0.75	0.90
No 3	0.125	0.25	0.20	0.062	0.50	1.13
No 4	0.0625	0.12	0.29	0.002	0.80	1
No 5	0.125	0.75	0.18	0.002	0.50	0.63
No 6	0.125	0.13	0.28	0.002	0.71	0.38
No 7	0.0625	0.19	0.18	0.002	0.83	0.63
No 8	0.125	0.20	0.13	0.002	0.96	0.50
No 9	0.0625	0.20	0.26	0.002	0.80	0.38
No 10	0.125	0.16	0.12	0.002	0.50	0.38

TABLE 3. Antibacterial effect of EEO and vancomycin combination against S. aureus isolates from infected wound swabs

Strains	Agents	MIC		FIC		Outcome
S. aureus		**Alone**	**Combination**	**FIC**	**FICI**	
No 1	EEO	0.05	0.00625	0.125	0.25	Synergistic
	Vancomycin	0.002	0.00025	0.125		
No 2	EEO	0.05	0.01250	0.25	0.31	Synergistic
	Vancomycin	0.002	0.000125	0.0625		
No 3	EEO	0.04	0.00624	0.156	0.28	Synergistic
	Vancomycin	0.002	0.00025	0.125		
No 4	EEO	0.05	0.01250	0.25	0.31	Synergistic
	Vancomycin	0.002	0.000125	0.0625		
No 5	EEO	0.05	0.00625	0.125	0.25	Synergistic
	Vancomycin	0.002	0.00025	0.125		
No 6	EEO	0.05	0.00625	0.125	0.25	Synergistic
	Vancomycin	0.002	0.00025	0.125		
No 7	EEO	0.03	0.01250	0.41	0.47	Synergistic
	Vancomycin	0.002	0.000125	0.0625		
No 8	EEO	0.04	0.00624	0.156	0.28	Synergistic
	Vancomycin	0.002	0.00025	0.125		
No 9	EEO	0.03	0.01250	0.41	0.47	Synergistic
	Vancomycin	0.002	0.000125	0.0625		
No 10	EEO	0.05	0.00625	0.125	0.25	Synergistic
	Vancomycin	0.002	0.00025	0.125		

found to be synergistic in all tested clinical *S. aureus* isolates. The MIC values of the EEO and ceftazidime against ten *P. aeruginosa* isolates from infected wound swabs were determined with broth microdilution method (Table 4). 80% of *P. aeruginosa* showed additive outcome of oil/ceftazidime combination, and only 20% of them gave indifference outcome.

TABLE 4. Antibacterial effect of EEO and ceftazidime combination against P. aeruginosa isolates from infected wound swabs

Strains	Agents	MIC		FIC		Outcome
P. aeruginosa		**Alone**	**Combination**	**FIC**	**FICI**	
No 1	EEO	0.05	0.03	0.6	0.60	Additive
	Ceftazidime	0.125	0.00025	0.002		
No 2	EEO	0.05	0.00625	0.125	1.12	Indifference
	Ceftazidime	0.0125	0.0125	1		
No 3	EEO	0.05	0.05	1	1.06	Indifference
	Ceftazidime	0.002	0.000125	0.062		
No 4	EEO	0.05	0.04	0.8	0.80	Additive
	Ceftazidime	0.125	0.00025	0.002		
No 5	EEO	0.08	0.05	0.62	0.622	Additive
	Ceftazidime	0.125	0.00025	0.002		
No 6	EEO	0.05	0.03	0.6	0.602	Additive
	Ceftazidime	0.125	0.00025	0.002		

No 7	EEO	0.05	0.04	0.8	0.80	Additive
	Ceftazidime	0.125	0.00025	0.002		
No 8	EEO	0.08	0.05	0.62	0.622	Additive
	Ceftazidime	0.125	0.00025	0.002		
No 9	EEO	0.07	0.04	0.57	0.57	Additive
	Ceftazidime	0.125	0.00025	0.002		
No 10	EEO	0.05	0.04	0.8	0.80	Additive
	Ceftazidime	0.125	0.00025	0.002		

4. Discussion

Thick lipopolysaccharide layers of gram-negative bacteria serve as a barrier to entry of several antimicrobial especially those with lipophilic characteristics. In the present study, EEO exhibited a synergistic activity against *S. aureus* ATCC 29213, and all tested clinically isolated *S. aureus* indicating that the oil has a different mode of action to penicillin. EEO had a synergistic antibacterial activity against gram positive bacteria (*S. aureus*), while against gram negative bacteria (*P.aeruginosa*) it was found to be additive or indifference. This agree with the results reported earlier [11], that an inhibitory activity of essential oil was found against all gram-positive bacteria and yeasts but no activity against gram-negative bacteria. Different investigations had discussed the efficacy of essential oils against gram positive and negative bacteria, and showed that gram positive bacteria more susceptible to oils [13–14]. However, in [15], reported different results where EO in combination with antimicrobial drugs considerably reduced the effective doses of the drugs used with *E. coli* isolates despite relatively high MIC values of this EO.

In our study, missing data like the minimum bactericidal concentration were not estimated; more numbers of isolates should be tested to assess the efficacy of EEO and different antibiotics.

5. Conclusion

EEO had synergistic antibacterial activity against gram positive bacteria, while against gram negative bacteria it was found to be additive.

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