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Research Article

In vitro Antibacterial Effects of *Salvia sclarea*, *Eucalyptus Globulus* and *Eugenia Caryophyllata* Essential oils Against Multidrug Resistant *Corynebacterium spp* Clinical Isolates

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Abstract

Objectives: Multidrug resistant *Corynebacterium* species are increasingly reported as the ethiological agent of various clinical infections. Thus, the purpose of this research was to evaluate the in vitro antimicrobial activity of three essential oils *Salvia sclarea*, *Eucalyptus globulus* and *Eugenia caryophyllata* against Corynebacterium species.

Methods: Twenty-four multidrug resistant strains including *C. striatum*, *C. amycolatum*, *C. urealyticum*, *C. aurimucosum*, *C. imitans*, and *C. jeikeium* were used in the study. Inhibition diameter zone, minimum inhibitory concentration and minimum bactericide concentration of these oils were determined using agar disc diffusion method and microdilution method. Tigecycline was used as positive control.

Results: Our study showed that *Eugenia caryophyllata* had the best activity. *Eucalyptus globulus extract exhibited a moderate activity and Salvia sclarea was inactive against all the species tested.* We found that *C. amycolatum* was more resistant to the essential oils than other species. On the other hand, tigecycline was effective on the majority of the strains (37.5%), but his action was lower than *Eugenia caryophyllata* oil.

Conclusion: These results support the use of clove oil as a natural alternative to treat infections caused by multidrug resistant corynebacteria.

Key Words: Essential oil; *Corynebacterium*; antibacterial effect; tigecycline

1. Introduction

Essential oils from aromatic and medicinal plants have been known since antiquity to possess biological activity, notably antibacterial properties [1,2]. Nowadays, the use natural, effective and nontoxic antimicrobial agents have been developed interestingly in particular with the emergence of many drug resistances among a wild range of bacteria. In fact, the prolonged exposure to antibiotics in clinic significantly increases the manifestation of opportunistic pathogens notably *Corynebacterium spp*. The spread of antimicrobial resistance *Corynebacterium* species is a known problem worldwide [3-5]. Thus, there is growing interest in using natural antimicrobial compounds to treat patients suffering from infections due to multidrug resistance *Corynebacterium* species. *Previous studies reported the antimicrobial activity of essential oils from clove* (*Eugenia caryophyllata*), *eucalyptus (Eucalyptus globulus*), *and sage (Salvia sclarea*) *against bacteria [6-9]. Thereby, in this study we evaluated the* antimicrobial potency of Eos extracted from these plants against MDR *Corynebacterium* strains responsible for clinical infections and we compared their activity to tigecycline.

2. Materials and Methods

2.1. Essential oils obtention

Three medicinal plants selected for this study were *Salvia sclarea*, *Eucalyptus globulus* and *Eugenia caryophyllata*. Brut EOs were purchased from Gandiva Cosmética Vegetal (Spain) and stored at room temperature until tested.

2.3. Bacterial strains

A total of 24 MDR *Corynebacterium* spp strains including *C. striatum* (n=15), *C. amycolatum* (n=5), *C. urealyticum* (n=2), *C. aurimucosum* (n=1), *C. imitans* (n=1), and *C. jeikeium* (n=1) were collected from patients hospitalized at the University Hospital of F. Hached, Sousse Tunisia. Clinical strains were recovered from different specimens (Table I) as follows: surgical wound exudates (n=10), vaginal swabs (n=1), ear effusions (n=3), urine (n=3), sputum (n=2), tracheal aspirates (n=2), eye effusions (n=2), central venous catheter tips (n=2).
Isolated bacterial strains were firstly identified by conventional biochemical methods and Api Coryne V.2 (BioMerieux®, Marcy-l'Etoile, France). Then, MALDI-TOF-MS (Vitek MS, BioMérieux®) was used for final identification.
Susceptibility to tigecycline (15µg) used as control was determined using disc diffusion method on Muller-Hinton blood agar (biorad, France)

Strains number	Species	Specimens
1	*C. striatum*	Sputum
2	*C. striatum*	Surgical wound exudates
3	*C. striatum*	urine
4	*C. striatum*	Tracheal aspirates
5	*C. striatum*	Ocular wound exudates
6	*C. striatum*	Surgical wound exudates
7	*C. striatum*	Surgical wound exudates
8	*C. striatum*	Surgical wound exudates
9	*C. striatum*	Tracheal aspirates
10	*C. striatum*	Tracheal aspirates
11	*C. striatum*	Central venous catheter tips
12	*C. striatum*	Surgical wound exudates
13	*C. striatum*	Ear wound exudates
14	*C. striatum*	Ear wound exudates
15	*C. striatum*	Ear wound exudates
16	*C. amycolatum*	Surgical wound exudates
17	*C. amycolatum*	Surgical wound exudates
18	*C. amycolatum*	Vaginal swab
19	*C. amycolatum*	Central venous catheter tips
20	*C. urealyticum*	Urine
21	*C. urealyticum*	Urine
22	*C. aurimucosum*	Surgical wound exudates
23	*C. imitans*	Surgical wound exudates
24	*C. jeikeium*	Surgical wound exudates

according to Clinical and Laboratory Standards Institute recommendations [10]. The minimum inhibitory concentrations (MICs) for 12 drugs including: Kanamycin, erythromycin, clindamycin, doxycycline, ciprofloxacin, moxifloxacin, penicillin, cefotaxime, rifampicin, vancomycin, linezolid and daptomycin were determined by using micro-dilution in cation adjusted Muller-Hinton broth in accordance with guidelines of the CLSI [10]. All the strains tested were resistant to kanamycin, erythromycin, clindamycin, ciprofloxacin, moxifloxacin, cefotaxime, penicillin, and rifampicin.

2.4. Essential oils antibacterial activity

2.4.1. Determination of diameter of inhibition zone

The susceptibility of MDR pathogens to the 3 essential oils was tested by disc diffusion method Muller Hinton Agar. Sterile blank discs of 6 mm diameter (Biomérieux, Marcy-l'Etoile, France) loaded with 20µl of each oil were placed on solidified medium, previously inoculated on the surface agar with 200 μL of 10^6cfu/mL suspension. The plates were incubated at 37 ºC for 24 hours. Diameter of zone of inhibition of growth was measured.

2.4.2. Determination of the MIC and MBC

The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) for The essential oils, were determined using broth dilution method. Serial dilutions (10 dilutions) of essential oils (50µl/ml) were prepared with BHI broth medium. One colony of each bacterial strain was sampled with a loop, then inoculated in 25 mL BHI broth and incubated for 18–24 h at 37°C. The suspension was diluted to obtain 10^6 cfu/mL and 50µl of the solution were placed in each well. Final solutions were incubated at 37°C overnight. The MIC was considered as the lowest concentration that prevented the visible growth. The MBC was determined by subculturing 100 μL from each test well ≥ MICs onto plate count agar plates. MBC was defined as the lowest concentration resulting in a negative subculture or giving presence of only one colony after incubation. Bacteriostatic activity has been defined as a ratio of MBC to MIC >4 [11].

3. Results and Discussion

The antimicrobial activities of *S. sclarea*, *E. globulus* and *E. caryophyllata* against 24 MDR clinically relevant *Corynebacterium spp* strains was evaluated.

Bacterial susceptibility to the essential oils, as determined by the agar diffusion method, showed that the selected essential oils have variable antibacterial activity.

E. caryophylatta EO showed remarkable activity against the all the tested strains (Table I). Our study was in concordance with other findings reporting the potent antibacterial effect of this EO [12]. It was active against food-borne bacteria [13,14] and *H. pylori* [15,16].

This activity is due to the eugenol, the major compound of *E. caryophylatta* EO [17] .

Our results revealed that *E. globulus* EO had a moderate activity against the strains tested. The inhibition diameters zones were lower than those obtained with the clove oil, ranging from 7 mm to 20 mm (Table II).

	Inhibition zone diameter (mm)			
Strains	*Eugenia caryophyllata*	*Eucalyptus globulus*	*Salvia sclarea*	Tigecyclin
1	30	18	15	20
2	25	10	9	20
3	25	16	10	20
4	23	20	10	20
5	25	12	10	30
6	20	15	15	22
7	22	15	10	22
8	25	18	10	24
9	20	15	8	22
10	20	7	9	20
11	20	18	10	24
12	18	13	10	18
13	22	8	8	20
14	22	10	10	20
15	20	13	8	18
16	8	8	8	24
17	28	17	15	26

18	28	18	15	30
19	22	10	8	26
20	24	7	10	28
21	20	15	10	24
22	22	10	10	20
23	10	12	10	18
24	10	13	10	20

Table II. Inhibition diameter zone (mm) of different plant essential oil on Corynebacterium spp strains

For the majority of the strains (52%), the diameters were comprised between 10 and 18 mm. The lowest values were exhibited by *C. amycolatum* strains. The MICs values ranges proportionally to the inhibition diameter. They ranged from 0.05µl to 50µl. MBCs results showed either bacteriostatic or bactericide effect of this EO (Table III).

Oils	*Eugenia caryophyllata*		*Eucalyptus globulus*		*Salvia sclarea*	
Strains	MIC (µl/ml)	MBC (µl/ml)	MIC (µl/ml)	MBC (µl/ml)	MIC (µl/ml)	MBC (µl/ml)
1	0.05	1.6	0.2	6.25	6.25	NA
2	0.4	12.5	25	25	50	NA
3	0.4	12.5	12.5	50	50	NA
4	<0.05	1.6	0.05	12.5	50	NA
5	<0.05	1.6	25	50	25	NA
6	<0.05	1.6	0.2	50	6.25	NA
7	<0.05	3.125	25	50	50	NA
8	<0.05	3.125	3.125	25	25	NA
9	<0.05	1.6	25	50	50	NA
10	0.2	12.5	25	25	6.25	NA
11	0.2	12.5	0.8	25	50	NA
12	0.2	12.5	25	50	50	NA
13	<0.05	1.6	0.2	50	50	NA
14	<0.05	1.6	50	50	6.25	NA
15	<0.05	1.6	6.25	50	50	NA
16	0.125	3.125	50	50	50	NA
17	<0.05	1.6	50	50	50	NA
18	<0.05	1.6	50	50	50	NA
19	0.125	6.25	50	50	50	NA
20	<0.05	3.125	50	50	50	NA
21	0.4	12.5	50	50	50	NA
22	<0.05	1.6	50	50	25	NA
23	<0.05	1.6	25	50	50	NA
24	<0.05	1.6	25	50	50	NA

Table III: MIC and MBC obtained for the 3 essential oils tested against orynebacterium spp strains.

Similarly, eucalyptus oil was used in various studies where its activity was found either moderate [8,18,19] or low [20]. Singh et al [8] found that Eucalyptus oil inhibited growth in *S. indicum* and *E. coli* but the area of inhibition zone was much less in comparison to clove oil, while *Staphylococcus* and *Bacillus* strains were found completely insensitive to eucalyptus oil. However, other studies reported that the extract of *E. globulus* potently inhibited the growth of *E. coli*, *S. aureus* [21] and fish pathogenic bacteria [22].

The antibacterial activity of *Eucalyptus* extracts has been due to his principal constituents oxygenated monoterpenes and terpinen-4-ol and other components such as α-pinene, β-pinene, α-phellandrene, 1,8-cineole, limonene, terpine-4-ol, aromadendrene, epiglobulol, piperitone and globulol [23].

It seemed that the presence of 1,8-cineole and α-phellandrene coupled with low antioxidant activity and high cytotoxic effect makes eucalyptus oil less effective against the control of bacterial growth. Thus, eucalyptus oil is not much suited for medicinal purposes, but can be used as repellant or anti-feedant in insecticidal formulation [8].

In this study, sage oil has shown the lowest effect against our strains. All the isolates tested exhibited diameter ≤15mm. Only undiluted oil was active against the strains. The MICs value obtained were high for almost all the strains. Sepahvand et al [24] has demonstrated that *S. sclarea* essential oil did have different anti-bacterial effects among the microorganisms tested. In accordance to our study, it has a low effect on *P. aeruginosa*. Unlikely, Cui et al [9] proved that this oil was highly lethal to *E. coli*, *S. aureus*, *B. pumilus*, *B. subtilis*, *K. pneumoniae*, *S. typhimurium* and *P. aeruginosa*. *S. sclarea* oil was an effective bacterial inhibitor and bactericide with a broad antibacterial spectrum. It damaged the cell membrane and changed its permeability, leading to the release of some cytoplasm such as macromolecular substances, ATP and DNA. The antimicrobial action of *S. sclarea* essential oil is not only attributable to a unique pathway, but also involves a series of events both on the cell surface and within the cytoplasm. Two diterpenoids, salvipisone and aethiopinone contained in *Salvia sclarea* are known for their high antibacterial activity [24].

In our study, we noted that *C. amycolatum* was the most insensitive specie among the strains tested. This may be explained by the particular structure of his cell wall which lacked mycolic acid but contains a cation-selective cell wall channel that may be responsible for the limited permeability of the cell wall to EO and notably to different antibiotics. The structure of the cellular walls justifies also the differences observed between Gram-positive and Gram-negative bacteria [9].

We observed that the susceptibility an EO varied among different species included in the study and among strains belonging to the same species. These discrepancies may be explained by the same mechanisms of antibiotic resistance including adaptation of a strain to ecological environment, selection pression and cross breeding [25].

Among the 24 strains tested, 9 (37.5%) strains showed resistance to tigecycline. Salas et al. [26] and Fernandez-Roblas et al. [27] reported good activity of this antibiotic against coryneform bacteria. Tigecyclin

was more potent than *E. globulus and S. sclarea.* However, the diameters of inhibition zone of *E. caryophyllata* were higher than the tigecycline for the majority of the strains. This fact proved the potential activity of clove oil against *Corynebacterium* spp.

Conclusion

In this study, results obtained showed that clove EO had the highest activity against MDR *Corynebacterium* species. His activity was slightly higher than tigecycline. However, the sage oil was inactive on all the species tested. *E. caryophyllata* EO seems to be an important alternative treatment in nosocomial *Corynebacterium* infections ranging from cutaneous infections to urinary tract infections.

Conflict of interest: None.

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