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Assessment of the antibiofilm and antiquorum sensing activities of *Eucalyptus globulus* essential oil and its main component 1,8-cineole against methicillin-resistant *Staphylococcus aureus* strains

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ABSTRACT

Antibacterial resistance is an increasingly serious threat to global public health. The search for new anti-infection agents from natural resources, with different mode of actions and competitive effects became a necessity. In this study, twenty height methicillin-resistant *Staphylococcus aureus* (MRSA) strains were investigated for their biofilm formation ability. Subsequently, the antibiofilm effects of *Eucalyptus globulus* essential oil and its main component 1,8-cineole, against MRSA, as well as their antiquorum sensing potential, were evaluated. Our results displayed the potent efficacy of both *E. globulus* essential oil and 1,8-cineole against the development of biofilms formed by the methicillin-resistant strains. Additionally, *E. globulus* essential oil showed more potent of *anti*-QS activity, even at a low concentration, when compared to 1,8-cineole. All these property of tested agents may pave the way to prevent the development of biofilm formation by MRSA and subsequently the spreading of nosocomial infection.

1. Introduction

Staphylococcus aureus is accredited as an opportunistic pathogen, responsible for nosocomial infections and, high mortality and morbidity rates [17]. Several inherent capabilities *viz*. secretion of multiple toxins and exoenzymes [40], adhesion and biofilm formation on biomaterials and medical devices [24], along with multidrug resistant phenotype [25] attributes to high pathogenicity of this micro-organism. Posing resistance towards multiple antibiotics has been identified as a global threat. Biofilm formation adds to the resistance phenotype of the microbial pathogens towards host defences and conventional drug therapy, often resulting in serious and persistent infections [9]. Multidrug resistance by *S. aureus* represents a rapidly growing public health concern, particularly pressing in developing world. Appearance and spread of multidrug resistance results in increased morbidity, mortality, and whooping cost of health care. Hence, a multifaceted approach is needed to combat this problem, warranting the discovery of novel antimicrobial agents and/or new methodological concepts [4].

Several pathogenic bacterial strains use signal molecules, like N-acyl homoserine lactones (AHLs) to monitor their own population density [44]. At a threshold population density, AHLs interact with the cellular receptors and trigger the expression of a set of target genes, including virulence, antibiotic production, biofilm formation, bioluminescence, mobility and swarming, in a process called quorum sensing (QS) [44]. Quorum sensing has been identified as one of the alternate approach for combating multidrug-resistant pathogens [26].

The interest in medicinal and aromatic plants and their components, chiefly bioactive compounds has increased remarkably over the last decade [16]. Essential oils (EOs) from medicinal and aromatic plants have been reported to possess potent antimicrobial activities [3]. Such recent reports have attracted an increased interest in the compositions and biochemical properties of the plant EOs for their potential exploitations [32]. Essential oil from leaves of *Eucalyptus globulus* is among the various types of oils targeted for their biological activities

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Table 1
Exopolysaccharide production (CRA), glass adhesion (Safranin 1%) and biofilm formation on polystyrene by clinical *S. aureus* strains.

Strains	Origin	Slime phenotype (CRA)	Safranin assay (Glass)	Biofilm formation on polystyrene	
				OD 570 ± SD	Capability
ATCC 6538	–	S+	+++	2.90 ± 0,055	H.B. Producer
Sa1	Blood culture	S-	++	0.19 ± 0.01	W.B. Producer
Sa3	Blood culture	S+	++	0.13 ± 0.02	W.B. Producer
Sa4	Blood culture	S+	++	0.59 ± 0.05	W.B. Producer
Sa16	Blood culture	S+	+++	0.22 ± 0.03	W.B. Producer
Sa17	Blood culture	S+	+++	0.17 ± 0.03	W.B. Producer
Sa18	Blood culture	S+	++	2.62 ± 0.39	H.B. Producer
Sa21	Blood culture	S+	+++	0.24 ± 0.06	W.B. Producer
Sa26	Blood culture	S+	++	0.21 ± 0.01	W.B. Producer
Sa2	Divers	S+	++	0.12 ± 0.02	W·B. Producer
Sa8	Divers	S+	++	0.42 ± 0.13	W.B. Producer
Sa10	Divers	S+	+++	0.80 ± 0.30	W.B. Producer
Sa12	Divers	S+	+++	2.96 ± 0.11	H.B. Producer
Sa15	Divers	S-	+++	1.77 ± 0.60	H.B. Producer
Sa19	Divers	S+	+	0.15 ± 0.01	W.B. Producer
Sa23	Divers	S+	+++	0.13 ± 0.01	W.B. Producer
Sa5	Pus	S+	+++	0.46 ± 0.05	W.B. Producer
Sa6	Pus	S-	+++	0.26 ± 0.03	W.B. Producer
Sa7	Pus	S-	+++	0.22 ± 0.02	W.B. Producer
Sa13	Pus	S+	++	0.15 ± 0.02	W.B. Producer
Sa24	Pus	S+	++	0.62 ± 0.08	W.B. Producer
Sa25	Pus	S+	+++	0.12 ± 0.01	W.B. Producer
Sa28	Pus	S+	++	0.14 ± 0.02	W.B. Producer
Sa29	Pus	S+	++	0.14 ± 0.03	W.B. Producer
Sa30	Pus	S+	+++	1.00 ± 0.08	H.B. Producer
Sa31	Pus	S-	+++	0.18 ± 0.04	W.B. Producer
Sa32	Pus	S-	+	0.16 ± 0.07	W.B. Producer
Sa9	Pus	S+	+++	0.15 ± 0.01	W.B. Producer
Sa27	Pus	S+	++	0.14 ± 0.03	W.B. Producer

SD: standard deviation.
H.B.: Highly biofilm; W.B.: Weakly biofilm.
S+: Slime producer; S-: Non slime producer.
+: Weak adherent; ++: Moderately adherent; +++: Strongly adherent.

[33], namely antimicrobial [2], antioxidant [6] and anti-inflammatory [34] potential. The phytochemical studies conducted in different parts of *E. globulus* reveals the presence of terpene 1,8-cineole (eucalyptol) as the major component, making upto 84% of the total chemical components of the essential oil [19,22].

The present study assessed the antibacterial and antibiofilm effects of *E. globulus* EO and its main component 1,8-cineole, against methicillin resistant *S. aureus* strains and also evaluated their antiquorum sensing potential.

2. Materials and methods

2.1. Tested agents and bacterial strains

The essential oil of *Eucalyptus globulus* and its main component 1,8 cineole were procured from Huiles & Sens (France). Reference strain of *Staphylococcus aureus* (ATCC 6538) was procured from American Type Culture Collection (ATCC) USA. Methicillin-resistant *S. aureus* (MRSA) strains (n = 28) were obtained from the laboratory of microbiology at the University Hospital of Monastir, Tunisia. A total of 13, out of 28 clinical isolates were from pus samples, 8 from blood culture, and remaining 7 strains from diverse cultures. Molecular identification and confirmation was carried out using species specific PCR primers (Sa442-1 and Sa442-2), as previously described by Ref. [47]. The strains were routinely sub-cultured and maintained on Luria-Bertani (LB, Liofilchem, Milan, Italy) broth, and a set preserved as glycerol stocks at −80 °C. Prior to each assay, the strains were sub-cultured thrice and incubated at 37 °C for 24 h to ensure optimal growth.

2.2. Biofilm formation ability of tested isolates

2.2.1. Phenotypic characterization of bacteria-producing slime

Detection of slime producing strains was carried out by culturing the isolates on Congo Red Agar (CRA) plates prepared by mixing 36 g of saccharose (Sigma Chemical Company, St. Louis, MO) with 0.8 g of Congo red in 1 L of Brain Heart Infusion (BHI) agar (Biorad, USA), as previously described [46]. The strains were incubated at 37 °C for 24 h under aerobic conditions. Black colonies with rough surface were identified as slime-producers and those with red and smooth surface as non-slime producers. Colonies with variable phenotype viz. black center and red outline, or red center and black outline were considered as positive slime producers [46].

2.2.2. Test tube method

Slime production over glass surfaces was determined using the Safranin method as described earlier for coagulase negative staphylococci with minor modifications [14]. In brief, a loopful of bacterial culture was inoculated into a glace test tube containing 10 ml of LB broth supplemented with glucose (final concentration, 8%). The tubes were incubated at 37 °C for 24 h and examined for the presence of a viscid slime layer. Slime production by each isolate was scored as negative, weak (1+), moderate (2+), or strong (3+). Each isolate was tested at least three times and read independently by two different observers.

2.2.3. Biofilm formation in 96-well polystyrene plates

Biofilm production by *S. aureus* strains grown in BHI (Bio-Rad, France) was assessed using crystal violet staining assay on 96-well tissue culture plates (Nunc, Roskilde, Denmark), as described previously [27]. Adherent bacteria were fixed with 95% ethanol and