HPLC-PDA-ESI-MS/MS Profiling and Anti-Biofilm Potential of *Eucalyptus sideroxylon* Flowers

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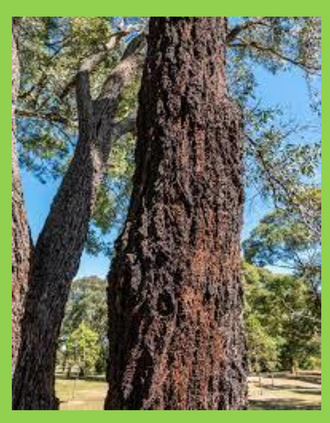
Introduction

Several pathogenic organisms form a polymeric matrix called biofilm. It increases the resistance of its aggregated cells to different groups of antibiotics and the host's immune system. The regulation of biofilm formation is considered one of the effective solutions against the worldwide emerging problem of increased bacterial resistance. Various studies have explored different plant extracts secondary metabolites to prevent the biofilm formation [Shehabeldine, *et. al.* 2020**].**

Eucalyptus is a diverse genus of Myrtaceae flowering trees. Eucalyptus species are reported to be used traditionally in wound healing and in the treatment of infections. However, there is a lack of enough scientific data to support the mechanism by which *Eucalyptus* species can be used as antimicrobial agents.

***E. Sideroxylon* is known as iron wood and has been previously tested for its effective use as alternative medicine in treating various bacterial, viral and fungal infections [**Okba, et. al. 2017**]. In the current work, the anti-biofilm potential of *E. sideroxylon* flowers against two strong biofilm forming bacteria (*Pseudomonas aeruginosa* and *Staphylococcus aureus*) in addition to the pathogenic yeast *Candida albicans* was investigated. A complete map of the *E. sideroxylon* flower's secondary metabolites was also studied using LC-MS/MS.**



Material & Methods

Plant Material and Extraction: ***Eucalyptus sideroxylon*** **Cunn. ex Woolls flowers were collected in May 2018, from El-Kobba Palace, Cairo, Egypt. The air-dried flowers were extracted by maceration in methanol and evaporated under reduced pressure till dryness, yielding *E. sideroxylon* flowers extract (ESFE). Strains: potent biofilm-producing *Staphylococcus aureus* (ACL51) clinical strain was used. The susceptibility profile of this strain was investigated using the VITEK®2 automated system (BioMerieux, Marcy-l'Étoile, France) and denoted as the methicillin-resistant *S. aureus* MRSA strain. In addition, a coded methicillin sensitive *S. aureus* MSSA (ATCC 29213) strain as well as *Bacillus subtilis* (ATCC 6051), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 35218) and *Candida albicans* (ATCC 90028) strains were used in this study. Time–Kill Curves: were used to monitor bacterial growth and death over a gradient of ESFE concentrations versus time. Biofilm Inhibition Assay: The MTP method was used. For the quantitative determination of biofilm formation, 30% acetic acid was added to the wells and the colour absorbance was measured at O.D 540 nm by an automated microplate reader. HPLC-PDA-ESI-MS/MS: The ESFE was analysed utilizing a ThermoFinnigan LCQ-Duo ion trap mass spectrometer (ThermoElectron Corporation, Waltham, MA, USA) coupled with an ESI source. A ThermoFinnigan HPLC system using a Discovery HS F5 column was used. Water and acetonitrile (ACN) (0.1% formic acid each) were used as a mobile phase. Xcalibur software was used to control the system. Statistical Analyses: Student's t-test was used. Differences were considered significant if the *p* values were <0.05.**

Results

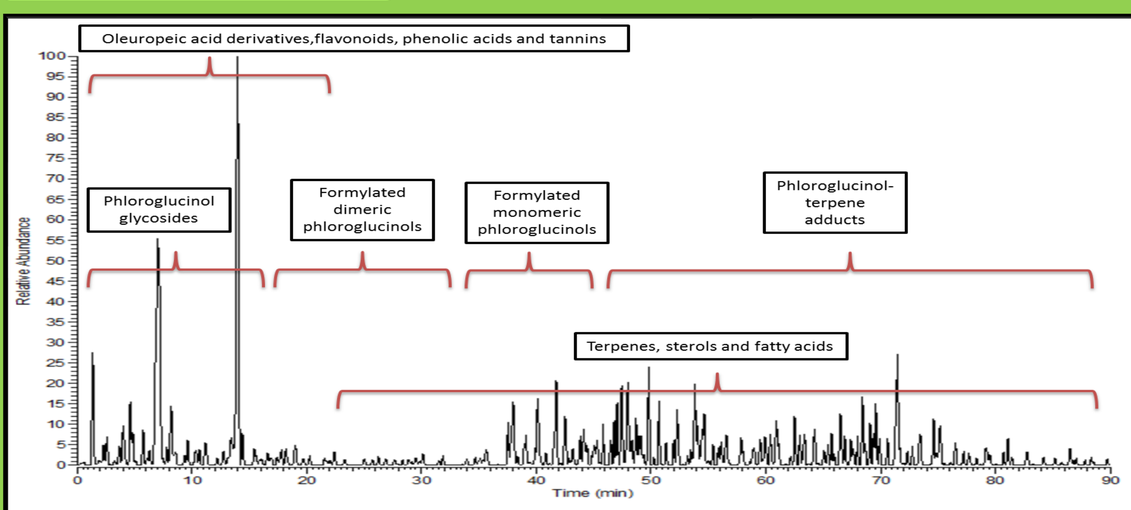


Figure (1) LC-MS/MS profile of *E. sideroxylon* flower

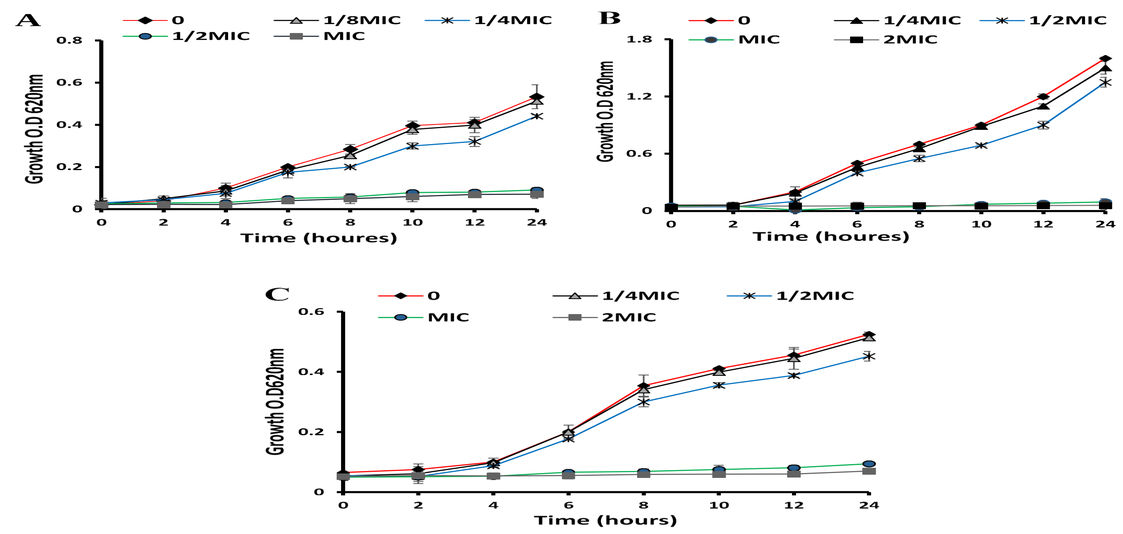


Figure (2) Time killing curve illustrates the effect of different concentrations of *E. sideroxylon* flower extract ESFE on the growth of (A) MRSA (B) *P. aeruginosa* (C) C. *albicans*.

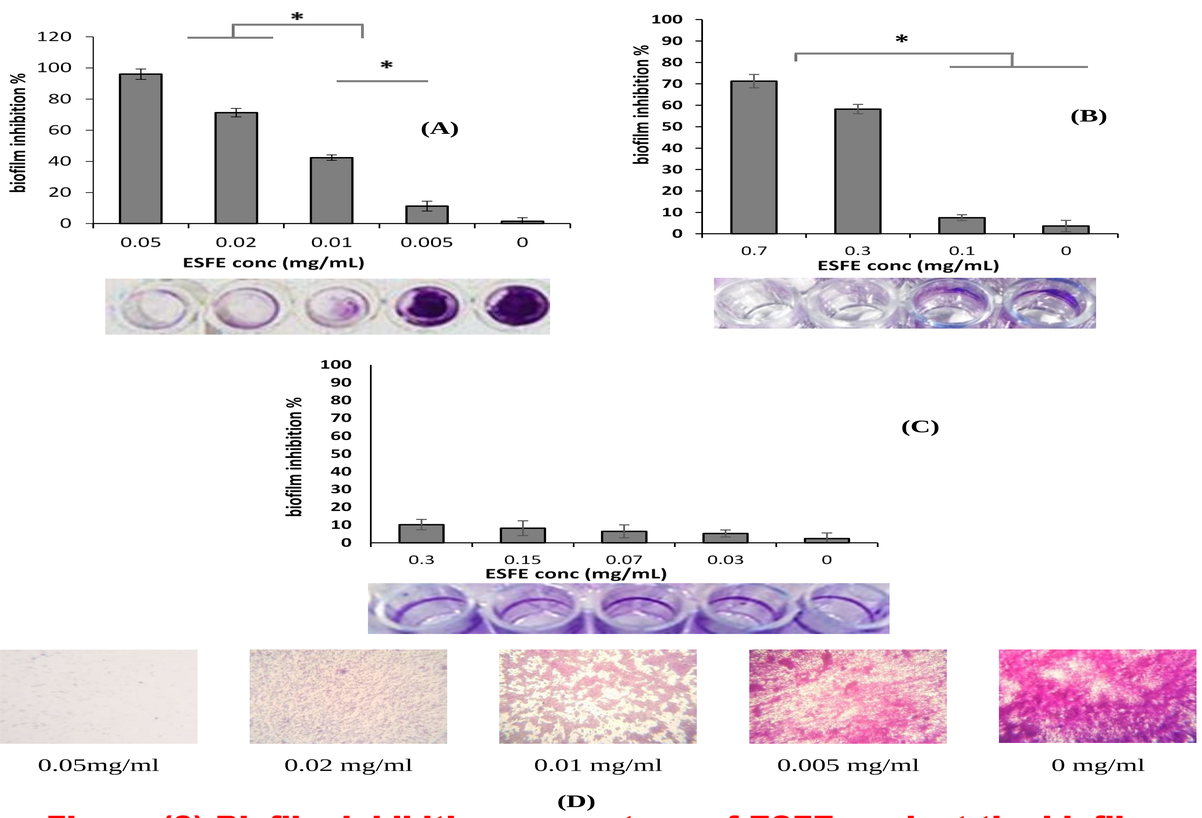


Figure (3) Biofilm inhibition percentage of ESFE against the biofilm formation of (A) MRSA (B) *C. albicans* (C) *P. aeruginosa* (D) Microscopic images (X 150) illustrate the effect of different sub MIC of ESFE on the biofilm formation of the highly producing biofilm MRSA

Conclusion

ESFE demonstrated promising MRSA biofilm inhibition potential up to 95.9% and eradicated *C. albicans* biofilm formation up to 71.2%. Apart from the essential oil, this is the first detailed chemical profile of *E. sideroxylon* flowers. LC-MS analysis allowed the identification of 83 secondary metabolites: 21 phloroglucinol, 18 terpenes, 16 flavonoids, 7 oleuropeic acid derivatives, 7 ellagic acid derivatives, 6 gallic acid derivatives, 3 phenolic acids, 3 fatty acids and 2 miscellaneous.

***E. sideroxylon* is a rich source of effective constituents that promote its valorization as a promising candidate in the management of multidrug-resistant bacterial infections.**

Acknowledgment

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