

## Supporting Information

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# Chemical Composition, and Evaluation of Antibacterial, Antibiofilm and Synergistic Effects with Conventional Antibiotics of Essential Oil from *Mallotus repandus*

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### **S1:** Isolation and identification of the essential oil

The plant material was subjected to hydrodistillation (600 g) by using a Clevenger-type apparatus for 5 h. The obtained essential oil, after drying over anhydrous sodium sulfate, was stored in the dark at 4 °C until further analysis. The yield was calculated in % (w/w) of dried plant material. The essential oil is pale yellow with agree able balsamic odor, optical rotation -5.53.

Detailed chemical compositions of the essential oil were obtained by GC/FID and GC/MS using an Agilent 6890 GC was equipped with HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm film thickness). Capillary column details and temperature conditions for the analysis were as previously described [1]. Composition relative percentage was calculated based on GC peak areas. Components were identified based on retention indices (relative to C<sub>7</sub>-C<sub>30</sub> *n*-alkanes, under the same experimental conditions) by matching their mass spectra with Wiley and NIST 14 library data, and comparisons of their Kovats retention indices with reference libraries (Adams, 2017 and Andriamaharavo, 2014) [2, 3].

### **S2:** Antibacterial activity test

The *in vitro* antibacterial activity test was done according to the protocol described by CLSI (Clinical and Laboratory Standards Institute) [1]. The microbial strains applied in this investigation were *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), *Paenibacillus larvae* (ATCC 9545), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853).

### S3: Evaluation of Synergistic Effects

The synergistic activity was determined by checkerboard assay in 96-well microtiter plates [4]. The interaction results was performed by the fractional inhibitory concentration index (FICI), defined as the sum of the MIC of the combined substances divided by the MIC of the isolated substances and categorized as: Synergism ( $FICI \leq 0.5$ ), additive ( $0.5 < FICI \leq 1$ ), indifferent ( $1 < FICI < 2$ ), or antagonism ( $FICI \geq 2$ ) [5].

### S4: Inhibition of Biofilm Formation

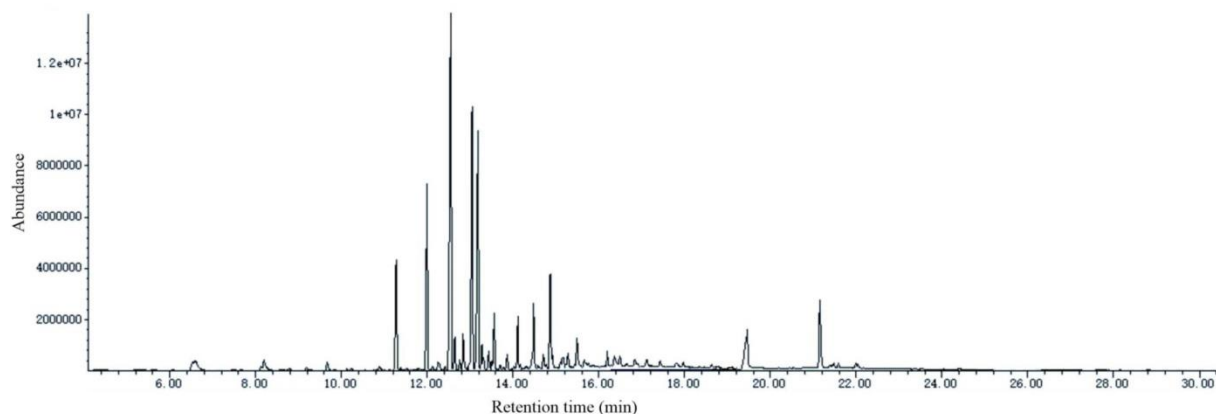
Biofilm formation of *Staphylococcus aureus* (ATCC 6538) was evaluated in the presence of the essential oil. Quantification of biofilm production was determined using crystal violet staining (CV) based on previously reported methodology [6].

### S5: Statistical Analysis

Data were statistically analyzed using IBM SPSS software (version 21.0) (IBM Corp., Armonk, NY, USA). The statistical significance of differences between controls and experimental groups was evaluated using Student's t-test.  $P < 0.05$  was considered statistically significant.

### S6: References

- [1] X. D. Su, Yang Gao, Y. X. Xiang, P. X. Lai and X. Xing (2019). Chemical composition and biological activities of the essential oil from *Aristolochia fordiana* Hemsl, *Rec. Nat. Prod.* **13**, 346-354.
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- [5] EUCAST. (2000). European Committee for Antimicrobial Susceptibility Testing (EUCAST) of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID): terminology relating to methods for the determination of susceptibility of bacteria to antimicrobial agents, *Clin. Microbiol. Infect.* **6**, 503-538.
- [6] M. Artini, A. Patsilnakos, R. Papa, M. Božović, M. Sabatino, S. Garzoli and L. Selan (2018). Antimicrobial and antibiofilm activity and machine learning classification analysis of essential oils from different mediterranean plants against *Pseudomonas aeruginosa*, *Molecules* **23**, 482.



**Figure S1:** The GC/MS spectrum of essential oil