Pathologie Biologie 64 (2016) 29–34

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com

Original article

# Antibacterial and antibiofilm activities of *Laurus nobilis* L. essential oil against *Staphylococcus aureus* strains associated with oral infections

## *Les activités antibactériennes et antibiofilm de* Laurus nobilis *L. huile essentielle contre* Staphylococcus aureus *tensions associées à infections orales*

A. Merghni [a,*], H. Marzouki [a], H. Hentati [b], M. Aouni [a], M. Mastouri [a,c]

[a] *Laboratory of infectious diseases and biological agents (LR99ES27), faculty of pharmacy, Monastir university, avenue Avicenne, 5000 Monastir, Tunisia*
[b] *Buccodental surgery department of Monastir dental clinic, laboratory of oral health and orofacial rehabilitation (LR12ES11), Monastir, Tunisia*
[c] *Laboratory of microbiology, university hospital of Fattouma Bourguiba, Monastir, Tunisia*

ARTICLE INFO

*Article history:*
Received 20 June 2015
Accepted 30 October 2015
Available online 4 December 2015

*Keywords:*
*Laurus nobilis* L.
Essential oil
GC–MS analysis
Anti-staphylococcal activity
Antibiofilm activity

ABSTRACT

*Background.* – *Laurus nobilis* L. is an aromatic herb with relevant medicinal properties due to its important chemical composition and its potential therapeutic effects. In this study, we investigate the chemical composition, the antibacterial and the antibiofilms activities of Tunisian *L. nobilis* L. essential oils against clinical *Staphylococcus aureus* strains.
*Methods.* – The chemical composition of *L. nobilis* L. essential oils was analysed by Gas Chromatography-Mass Spectrometry (GC–MS). The antibacterial activity of *L. nobilis* L. essential oils was evaluated in vitro against oral *S. aureus* ($n = 21$) strains using broth microdilution method. The antibiofilm activity was assessed via Crystal Violet staining and MTT assays.
*Results.* – Our results revealed that GC–MS assay exhibited 1.8-Cineole, methyl eugenol and α-terpinyl acetate as the major compounds in the essential oils. Moreover, the essential oil from Sousse exhibited the best bactericidal activity (MICs values ranged from 3.91 to 15.62 mg $m^{-1}$). Furthermore, this oil showed a strong biofilm inhibition effect above 70%, from a low sub-inhibitory concentration (1/16 × MIC). MTT assay revealed that both essential oils displayed an excellent antibiofilm activity with eradication percentages ranging from $79.6 \pm 2.27$ to $95.2 \pm 0.56$.
*Conclusion.* – Our finding demonstrated that *L. nobilis* L. essential oils are able to inhibit oral *S. aureus* strains with important antibiofilm efficacy. It could have a promising role in the prevention of oral diseases.



*Mots clés :*
*Laurus nobilis* L.
Huile essentielle
CG–SM
Activité anti-staphylococcique
Activité antibiofilm

RÉSUMÉ

*Laurus nobilis* L. est une plante aromatique avec des propriétés médicinales pertinentes en raison de sa richesse en composantes chimiques importantes et ses effets thérapeutiques potentiels. Dans cette étude, nous avons évalué la composition chimique, l'effet antibactérien et antibiofilm d'huiles essentielles tunisien *L. nobilis* L. contre des souches cliniques de *Staphylococcus aureus* isolées à partir de la cavité buccale.
*Méthodes.* – La composition chimique des huiles essentielles de *L. nobilis* L. a été analysée par chromatographie en phase gazeuse couplée au spectrométrie de masse (CG–SM). L'activité anti-bactérienne des huiles essentielles a été évaluée in vitro contre les souches de *S. aureus* ($n = 21$) en utilisant la méthode de microdilution de bouillon (MH). L'activité antibiofilm a été évaluée par coloration au cristal violet et par réduction de MTT.
*Résultats.* – Les résultats d'analyse par CG–SM ont montré que les principaux composés des huiles essentielles de *L. nobilis* L. sont représentés par : 1,8-cinéole, l'eugénol de méthyle et l'acétate α-terpinyle.

* Corresponding author.
*E-mail address:* abderrahmen_merghni@yahoo.fr (A. Merghni).

http://dx.doi.org/10.1016/j.patbio.2015.10.003
0369-8114/

D'autre part, l'huile essentielle de Sousse présentait la meilleure activité bactéricide (CMI variant de 3,91 à 15,62 mg $m^{-1}$). En outre, cette huile a montré une forte activité inhibitrice du biofilm (> 70 %), à partir d'une faible concentration (1/16 × CMI). Le test MTT a révélé que les deux huiles essentielles affichaient une excellente activité antibiofilm avec des pourcentages d'éradication allant de 79,6 ± 2,27 % à 95,2 ± 0,56 %.

*Conclusion.* – Notre étude a démontré que les huiles essentielles *L. nobilis* L. sont capables d'inhiber les souches de *S. aureus* avec une importante activité antibiofilm. Cette huile pourrait avoir un rôle prometteur dans la prévention des infections buccodentaires.



## 1. Introduction

The oral cavity shelters one of the most complex bacterial ecosystems of the human body. This cavity is inhabited by more than 700 species of Gram-positive and Gram-negative commensal bacteria, of which many have been of keen interest due to their pathogenicity in oral diseases [1]. In recent years, the attention has been focused on the mouth as a reservoir of opportunistic pathogens [2], such as *Staphylococcus aureus* which causes a variety of self-limiting to life-threatening diseases [3]. To prevent oral diseases, natural products have been recently investigated as promising agents against oral infections, and especially dental caries [4]. For instance, surfactants and essential oils have been successfully formulated into toothpastes and mouthrinses to control plaque biofilms and subsequently to prevent dental caries and periodontal diseases [5].

The interest in medicinal plants has burgeoned due to their bioactive compounds and their antimicrobial and antibiofilm properties [6]. Essential oils are volatile fractions obtained from medicinal and aromatic plants and have been used widely as natural food preservatives [7]. Furthermore, essential oils are more potent than antimicrobial agents, due to their rich and diverse composition, as well as their low toxicity levels [8]. Thereby, there has been an increased interest in the study of the compositions and biochemical properties of the essential oils of several aromatic and medicinal plant species for their potential exploitations [9].

*Laurus nobilis* L. (Lauraceae) is a native plant from the Southern Mediterranean region, found in warm climate regions with high rainfall [10]. The leaves of *L. nobilis* L. are traditionally used to treat the symptoms of gastrointestinal problems, and more recently as a fragrance component in the cosmetics and food industry [11]. The phytochemical studies conducted in all parts of *L. nobilis* L. characterize the presence of monoterpene 1,8-cineole as a commonly predominant essential oil compound [12]. This chemical specie is used as a food flavouring agent, and also frequently in the pharmaceutical industry for drug formulations [13]. Moreover, the biological activities of *L. nobilis* L. essential oils are well recognized namely their antibacterial [14], antifungal [15] and antiviral activity [16].

The present work aims to assess the chemical composition, antibacterial and antibiofilm effects of Tunisian *L. nobilis* L. essential oils against oral *S. aureus.*

## 2. Materials and methods

### 2.1. Plant material and essential oil extraction

Leaves of *L. nobilis* L. trees were sampled from tow Tunisian localities. Laurel was collected from Gafsa (34°28′N, 8°43′E) in the southwest of Tunisia, and Sousse (35°49′N, 10°37′E) in the central-east of the country.

*L. nobilis* L. leaves were collected and air-dried in the shade for several days. 100 g of dried leaves boorishly crushed and mixed with 600 mL distilled water were subjected to hydrodistillation (HD) for 4 h using a modified Clevenger-type apparatus [17].

### 2.2. GC–MS analysis

An Agilent Technologies Inc. gas chromatograph (Santa Clara, CA, USA) model 6890 N was employed for analysis of the essential oils. It was equipped with a split-splitless injector, an autosampler Agilent model 7683 and an Agilent HP5 fused silica column; 5% phenyl-methylpolysiloxane, 30 m × 0.25 mm i.d., film thickness 0.25 μm. GC conditions used were: programmed heating from 60 to 280 °C at 3 °C/min, followed by 30 min under isothermal conditions. The injector was maintained at 250 °C. Helium was the carrier gas at 1.0 mL/min; the sample (1 μL) was injected in the split mode (1:20). The GC was fitted with a quadrupole mass spectrometer (MS, Agilent model 5973 detector). MS conditions were as follows: ionization energy 70 eV, electronic impact ion source temperature 200 °C, quadrupole temperature 100 °C, scan rate 1.6 scan/sec, mass range 50–500 u. The software adopted to handle MS and chromatograms was a ChemStation NIST02 and LIBR (TP) [18,19] Mass Spectra Libraries were used as references. Samples were run in chloroform with a dilution ratio of 1:100.

### 2.3. Identification of compounds

Compounds were identified by matching their MS and retention index with those reported in the literature [18,19]. Moreover, identification of important constituents has been confirmed by injection of authentic samples (Table 1). A quantitative analysis of each oil component (%) was carried out by peak area normalization measurement. The response factors were estimates using standard compounds having the same molecular weight as the compound families that constitute the essential oil (hydrocarbon and oxygenated monoterpenes, hydrocarbon and oxygenated sesquiterpenes).

### 2.4. Microorganisms and antimicrobial assay of Laurus nobilis L. essential oils

Bacteria used in this study for antibacterial activity of *L. nobilis* essential oil were: a reference strain *S. aureus* ATCC 6538 (a known biofilm former) and twenty one *S. aureus* strains isolated from the oral cavity of Tunisian patients. Bacterial strains were phenotypically characterized for their ability to form a biofilm using the Congo red agar (CRA) method [20]. Among the 21 *S. aureus* isolates, 19 strains were slime producers and two other strains showed a biofilm negative phenotype [20]. Determination of antibiofilms activity of *L. nobilis* L. essential oils was carried out against: *S. aureus* ATCC 6538, biofilm positive strain (L37) and biofilm negative strain (L36) as previously published [20].

The antibacterial activity of *L. nobilis* L. essential oils was evaluated by the agar disk diffusion assay [21] with a bacterial inoculum of 0.5 Mcfarland. Absorbent discs (Whatman disc No. 3, 6 mm diameter) were impregnated with 10 μL of the essential oils, and then placed on the surface of inoculated plates. Positive control discs of standard antibiotic Oxacillin (5 μg/mL) were tested. After 24 h of incubation at 37 °C, the inhibition zones were measured and expressed in mm. All experiments were performed in triplicate.

### 2.5. Minimum inhibitory and minimum bactericidal concentrations (MIC and MBC)

The minimum inhibitory concentration (MIC) values for each essential oil against oral *S. aureus* isolates were determined according to the standard protocols [22]. An overnight culture (37 °C) of the tested strains was prepared by adjusting the turbidity of each bacterial culture to reach an optical density of 0.5 McFarland standards. The broth dilution method was carried out in 96-well microtitre plates using microbial reference strain and field isolates. The essential oils were prepared aseptically and transferred to sterile 96-well microtitre plates (190 μL per well) by two-fold serial dilutions using dimethylsulfoxide (DMSO) and then diluted in Muller Hinton (MH) broth. The resultant doses of the tested essential oils ranged between two and 250 mg/mL and the inocula (10 μL) of each strain were added to