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Cinnamon oil: A possible alternative for contact lens disinfection

Rasha H. Bassyouni[a,*], Zeinat Kamel[b], Maha Mohssen Abdelfattah[c], Eman Mostafa[b]

[a] Medical Microbiology and Immunology Department, Faculty of Medicine, Fayoum University, Egypt
[b] Botany Department, Faculty of Science, Cairo University, Egypt
[c] Microbiology Department, Research Institute of Ophthalmology, Cairo, Egypt

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ABSTRACT

Objective: To investigate the antibacterial activity of cinnamon oil alone and in combination with a multipurpose contact lens disinfectant solution (MPS) as well as tobramycin against multi drμg resistant conjunctival bacteria both in planktonic and sessile forms.
Methods: Minimum inhibitory concentrations (MIC) of tobramycin and cinnamon oil against 19 bacterial strains were investigated against planktonic and sessile cells by micro-dilution methods. Synergistic effects were determined by well diffusion and micro-dilution tissue culture plate methods for planktonic and sessile cells respectively. Time kill assay was performed to study the bactericidal effect of cinnamon oil in concentrations ranging from 5% to 0.312% combined with an MPS with respect to time.
Results: MICs of cinnamon oil against planktonic bacteria ranged from 0.04% to 1.25% versus 0.156% to 5% for sessile cells. Combination of cinnamon oil with tobramycin had a synergistic effect against most tested organisms. The MIC values of cinnamon oil in combination with tobramycin was significantly lower than cinnamon oil alone against biofilm production ($P = 0.004$). Time kill assay revealed that combination of cinnamon oil and disinfectant successfully eradicated the tested microorganisms at all tested concentrations within 2 h contact time except for 0.312% concentration (3 h) versus 24 h for MPS alone.
Conclusion: Cinnamon oil has a promising antimicrobial effect. It could be a probable candidate for contact lens disinfection.

1. Introduction

The use of contact lens is implicated in ulcerative keratitis occurrence worldwide, being a vehicle for delivery and transmission of microorganisms to the eye [1]. Bacterial keratitis accounts for approximately 90% of cases with *Staphylococcus* spp. and *Pseudomonas* spp. being the most common implicated pathogens. Untreated or severe bacterial keratitis may lead to perforation and endophthalmitis [2].

Ulcerative keratitis generally occurs when the normal natural resistance to infection has been interrupted either from trauma or the use of contact lenses. Many factors are implicated in keratitis associated with the use of contact lenses; bacterial adherence to the lenses themselves, the formation of a biofilm on lens or storage case, the resistance of microorganisms to disinfectant used, stagnation of tear film behind contact lenses, and reduced resistance of the cornea to infection [2,3].

Bacteria in biofilms show increased tolerance to antibiotics and disinfectants with concentrations ranging from 10 to 1000 times more than that needed to inactivate equivalent planktonic bacteria, due to poor antimicrobial penetration, induction of phenotypic variability, nutrient limitation, and adaptive stress responses [4–6]. The incidence of contact lens keratitis can be reduced by maintaining high standards of lens and lens case hygiene and following the recommended guidelines [6,7]. Contact lenses usually need to be stored overnight in a disinfectant solution to remove colonizing microbes from the lens surface resulting in a non-contaminated lens to be worn on the following day [8]. There are two groups of disinfecting and cleaning solutions for soft contact lens available for use; the first group is multipurpose solutions (MPS) which contain biguanides or polyquaternium-1, this group disrupts the microbial membranes leading to microbial death. The second group involves use of hydrogen peroxide (H_2O_2) which is a strong oxidizing agent. It acts on lipids, proteins and DNA leading to cell death. To obtain effective lens disinfecting solutions, they may also contain variable types of surfactants. The

* Corresponding author. Fax: +20 84 636583.
E-mail addresses: rhb00@fayoum.edu.eg, rashabassyoni@yahoo.com (R.H. Bassyouni).

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two-step H_2O_2 solutions are very efficient disinfectants, but they do require the addition of a neutralizing agent after a recommended disinfection time (4–6 h) [9].

The goal of treatment of contact lens related keratitis is the complete eradication of the pathogens. The gold standard of treatment in this case is the use of cefazolin 5% with tobramycin 1.3% or the use of second generation fluoroquinolones as a mono therapy [2].

Plant essential oils have long been used in preservation of food, alternative medicine and pharmaceutical therapies [5], many of these oils have a variable degree of antimicrobial activity against a wide range of bacteria [10]. Cinnamon oil is an essential oil commonly used in the food industry. It was reported that the acute toxicity resulted from cinnamon in animals is very low (Lethal dose 50 (LD50) = 1300 mg/kg rat) [11] althoμgh effects on the eye are not known. Previous studies have demonstrated the antibacterial properties of cinnamon oil, especially against biofilm production by *Streptococcus mutans*,*Lactobacillus plantarum* and *Staphylococcus epidermidis* [12]. Our previous work which screened the antimicrobial activity of 16 plant essential oils against conjunctival bacteria isolated from patients submitted to cataract surgery indicated the promising antibacterial properties of cinnamon oil [10].

The current research is focused on the investigation of the effectiveness of the antimicrobial properties of cinnamon oil alone and in combination with one commercially available MPS as well as tobramycin, against various multi-drμg resistant strains of conjunctival bacteria both in planktonic and sessile forms.

2. Material and methods

2.1. Bacterial isolates

Thirty six multi-drμg resistant bacterial strains (MDR) previously isolated from the conjunctiva of patients submitted to cataract surgery were used in this study [10]; 7*Staphylococcus aureus*, 17 coagulase-negative Staphylococci (CoNS), one *Enterococcus* spp., 2 *Streptococcus pneumoniae*, 3 *Moraxella* spp., 2 *Pseudomonas* spp., 2 *Klebsiellae pneumoniae,* one *Acinetobacter baumannii* and one *Escherichia coli.*

2.2. Screening of biofilm production

All isolates were tested for their ability to form biofilm by modified tissue culture plate method as described previously [13]. The negative control wells were filled with broth only. The strains were classified into the following categories: non biofilm producer ($OD \leq ODc$), weak biofilm producer ($ODc < OD \leq 2ODc$), moderate biofilm producer ($2\ ODc < OD \leq 4ODc$), strong biofilm producer ($4\ ODc < OD$), where ODc is the mean optical density of the negative control and OD is the mean optical density of the isolate.

2.3. Determination of minimum inhibitory concentration (MIC) of tobramycin

The MICs of tobramycin against 19 bacterial strains with different biofilm forming abilities were determined by the microdilution method for both planktonic and sessile cells in concentrations ranging from 512 μg/ml to 0.25 μg/ml. The MICs for planktonic cells were investigated according to the Clinical and Laboratory Standards Institute [14], while that of sessile cells were investigated by the method described by Nuryastuti et al. [12].

2.4. Determination of the antimicrobial effectiveness of the cinnamon oil and tobramycin combination

2.4.1. For planktonic cells

Susceptibility of 19 pathogenic strains to tobramycin in combination with cinnamon oil was determined using well diffusion method [15]. The bacterial strains (10^8CFU) were cultured on Mueller Hinton Agar (Oxoid Ltd., Basingstoke, UK) by spread plate technique. Wells of 8 mm diameter were made and filled as follows: 50 μl of tobramycin (MIC of tested strain) +50 μl sterile Mueller Hinton broth in the first well, four wells filled with 50 μl cinnamon oil in concentrations ranging from 5% to 0.625% (v/v) (two fold dilutions) +50 μl of tobramycin (MIC of tested strain). Another well filled with 100 μl sterile Mueller Hinton broth which served as negative control. The plates were allowed to stand for 1 h at room temperature for diffusion of the essential oil and antibiotic into agar then incubated at 37 °C for 24 h. The diameters of the zones of inhibition were measured and synergistic effect was considered when combinations exhibited enlargement of inhibition zone size by 5 mm versus tobramycin alone [16].

2.4.2. For sessile cells

The ability of cinnamon oil to inhibit biofilm formation in association with tobramycin in MIC concentration was assessed by tissue culture microtiter plate method described by Nuryastuti et al. [12]. Cinnamon oil was tested in concentrations ranging from 5% to 0.0097% (2 fold dilutions).

2.5. Time kill assay

A time-kill assay was performed as described by Zu et al. [17] in order to study the bactericidal effect of cinnamon oil in concentrations ranging from 5% to 0.312% (2 fold dilution) combined with one of the commercially available MPS with respect to time (ReNu MultiPlus Solution, Bauch & Lomb, Rochester, USA) (Formula: Hydroxyalkylphosphonate, Poloxamine, Polyaminopropyl Biguanide (0.0001%), Boric Acid, Disodium Edetate, Sodium Borate, and Sodium Chloride). Counts of viable cells were carried out at different intervals (0, 2, 4, 6, 24 h) after incubation for 24 h at 37 °C. kill curves were plotted with time against the logarithm of the viable colony counts (CFU/ml). A bactericidal effect is considered when there is a $3\ \log_{10}$ decrease in the CFU/ml or a 99.9% kill over a specified time [18].

2.6. Chemical analysis of cinnamon oil

Cinnamon oil was analyzed using gas chromatography mass spectrometry (GC/MS) (Shimadzu capillary GC-quadrupole MS system QP 5000) as described previously [19].

2.7. Statistical analysis

Statistical analysis were done using computer programs SPSS (Statistical Package for the Social Science) version 16 for Microsoft Windows. Data were statistically described in terms of frequencies and percentages. *P* values less than 0.05 were considered statistically significant.

3. Results

3.1. Biofilm formation

Thirty four out of 36 of tested bacteria had the ability to form biofilm (94.4%). Thirty isolates (83.3%) were strong biofilm producers, 4 isolates (11.1%) were moderate biofilm producers and 2 isolates (5.5%) were non biofilm producers (Table 1).