The Antibacterial Activity of Clove Essential Oil Against Propionibacterium acnes and Its Mechanism of Action

Propionibacterium acnes is a gram-positive anaerobic microorganism recognized as the major skin bacterium causing acne.1 Clove essential oil is the extract of Syzygium aromaticum (L) Merr et Perry. Its antimicrobial property has been reported,2 but to our knowledge its antibacterial activity toward P acnes and the mechanism of action have not been reported.

Methods. Agar diffusion and broth microdilution methods were used to evaluate the activity of clove essential oil toward P acnes, and the mechanisms of action were investigated by flow cytometry (FCM), atomic force microscopy (AFM), and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Results. Clove essential oil exerted significant antibacterial activity toward P acnes (Table). The mean (SD) bacteriostatic diameter was 24.0 (0.5) mm. Both the minimum inhibitory concentration and minimum bactericidal concentration were 0.31 mg/mL.

Propidium iodide staining and FCM showed a dose-dependent induction of cell death by essential oil due to cell wall and membrane damage (Figure 1). The relative amount of dead cells among untreated cells was 7.85%, whereas the proportion increased to 71.67% after treatment with 0.62-mg/mL essential oil. This result indicates that the mode of action of clove essential oil toward P acnes is associated with a loss of membrane integrity.

By AFM, a large concentration-dependent variation in the morphologic characteristics of bacteria was observed after treatment with clove essential oil (Figure 2). At low concentrations, the essential oil first attached to the surface of P acnes, and the surface became rougher. At higher concentrations, ruptured cell walls and membranes resulted, causing severe cell damage. Further-

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Clove Essential Oil</th>
<th>Erythromycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibition diameter, mean (SD), mm</td>
<td>24.0 (0.5)</td>
<td>21.7 (0.7)</td>
</tr>
<tr>
<td>Minimum inhibitory concentration</td>
<td>0.31 mg/mL</td>
<td>3.13 µg/mL</td>
</tr>
<tr>
<td>Minimum bactericidal concentration</td>
<td>0.31 mg/mL</td>
<td>3.13 µg/mL</td>
</tr>
</tbody>
</table>

*Results are expressed as the mean (SD) of 3 measurements.

Figure 1. Fluorescence cell number–per-channel (A1–D1) and light scatter–intensity (A2–D2) cytometric histograms of control Propionibacterium acnes (A) and P acnes grown in the presence of different concentrations of clove essential oil and stained with propidium iodide. B–D, Treatment with 0.04-mg/mL, 0.16-mg/mL, and 0.62-mg/mL, respectively, clove essential oil. FL3 indicates fluorescence level; FSC, forward light scatter; and Q, quadrant.
more, the cytoplasmic volume decreased, ultimately leading to bacterial death. By SDS-PAGE, we found that 2 specific proteins at 18 kDa and 33 kDa disappeared after treatment with 10- or 20-mg/mL essential oil for 1 hour. At concentrations below 10 mg/mL, no changes in protein expression were observed (Figure 3). After treatment for 4 hours, a global decrease in protein expression was observed.

Comment. Clove essential oil exhibited significant activity against \textit{P. acnes}; the minimum inhibitory concentration indicates a high lipophilicity and promises potential in vivo activity. The bacteriostatic mechanism involves damage to the cell walls and membranes of bacteria. At longer incubation times, cytoplasmic proteins may diffuse from the cytoplasm. Alternatively, essential oil might inhibit protein synthesis. Under short-term incubation, the disappearance of 18- and 33-kDa proteins might point to a specific mechanism, ultimately leading to bacterial death.

The role of \textit{P. acnes} in acne development seems to be related to the activity of several enzymes, including lipase and protease. \textit{Propionibacterium acnes} lipase (GehA, 33 kDa) and a heat shock protein (PPA737, 18 kDa) have been recognized as the virulence factors involved in the pathogenesis of acne. Therefore, it was supposed that the downregulated 33-kDa and 18-kDa proteins in the present analysis might be GehA and PPA737. The findings would provide a scientific basis for the application of clove essential oil as a therapeutic agent for acne, and in vivo studies should be conducted to investigate further.

**YuJie Fu, PhD**
**LiYan Chen, PhD**
**YuanGang Zu, PhD**
**ZhiGuo Liu, PhD**
**Xia Liu, MS**
**Ying Liu, PhD**
**LiPing Yao, PhD**
**Thomas Efferth, PhD**
Correspondence: Dr Zu, Key Laboratory of Forest Plant Ecology, Ministry of Education, Northeast Forestry University, Harbin 150040, People’s Republic of China (yujie_fu2002@yahoo.com).

Author Contributions: Study concept and design: Fu, Chen, Zu, and Efferth. Acquisition of data: Chen and Z. Liu. Analysis and interpretation of data: X. Liu, Y. Liu, and Yao. Drafting of the manuscript: Fu, Chen, Z. Liu, X. Liu, Y. Liu, and Efferth. Critical revision of the manuscript for important intellectual content: Zu, Yao, and Efferth. Statistical analysis: Fu and Chen. Obtained funding: Fu and Zu. Administrative, technical, and material support: Fu. Study supervision: Fu, Chen, Zu, Z. Liu, X. Liu, Y. Liu, Yao, and Efferth.

Financial Disclosure: None reported.

Funding/Support: This study was funded by grants 30770231 from the National Natural Science Foundation of China, Beijing, China; JC200704 from the Heilongjiang Province Science Foundation for Excellent Youths, Harbin, China; 108049 from the Key Project of Chinese Ministry of Education, Beijing; 2006BAD18B0401 from the National Key Technology Research and Development Program, Beijing; 2006-4-75 from the Innovative Program for Importation of International Advanced Agricultural Science and Technology, National Forestry Bureau, Beijing; and 2006RFXS001 from the Research Foundation for Science and Technology Innovation Talents of Harbin.

Role of the Sponsors: The sponsors had no role in the design and conduct of the study; in the collection, analysis, and interpretation of data; or in the preparation, review, or approval of the manuscript.


Long-term Maintenance of a Successful Occupational Sun Safety Intervention

Skin cancer prevention specialists should attempt to reduce UV radiation (UVR) exposure among outdoor workers, as pointed out in a recent review.1 To address this need, Project SUNWISE,2,3 a randomized controlled sun safety intervention trial with Southern Californian US Postal Service letter carriers, was conducted from 2001 through 2004.

Methods. The intervention consisted of providing free sunscreen, free wide-brim hats, a series of 6 brief on-site educational sessions, and sun safety prompts. The primary outcome measures were validated questionnaire items that asked participants how often they had used specific sun protection strategies over the past 5 weekdays while delivering mail.2,4 The questionnaires were administered at baseline and 3, 12, 24, and 36 months after baseline. The 5 response options ranged from “never” to “always”; we considered “always” as “consistent use” in analyses.

Results. At the 2-year follow-up evaluation, participants at the intervention postal stations had significantly higher rates of consistent sunscreen and wide-brim hat use than those at control stations.2 Details about the study procedures and sample characteristics have been published previously.1 Immediately following the 2-year evaluation, control station participants received the free items and 3 of the educational sessions (ie, introduction and protection strategies, sun safety for eyes, and review and encouragement to maintain sun safety practices). At the intervention stations, we continued to provide free sunscreen during that year. Herein, we describe the behavioral outcomes at the 3-year follow-up evaluation.

Evaluation cohort retention rates (of those completing questionnaires) from the 2- to 3-year follow-up periods were 93.2% (927 of 994) for the intervention group and 94.4% (1130 of 1196) for the control group. The trends over 3 years in 2 key outcomes—consistent use of sunscreen and wide-brim hats—were analyzed using generalized linear mixed models treating 3 months, 1 year, 2 years, and 3 years as a set of repeated measures on each postal worker. In addition, we adjusted for postal workers clustered within post offices within a multilevel model. The intraclass correlation coefficients for sunscreen and hats were 0.015 and 0.067, respectively. All analyses were adjusted for the baseline level of the outcome variable.

For each analysis, we tested (1) the time × condition interaction to determine if the intervention effect remained constant over time and (2) the condition main effect. Consistent sunscreen use rates for intervention participants at the 2- and 3-year follow-up periods were 39.2% and 38.3%, respectively, and for control participants, the rates were 26.3% and 34.3%, respectively. Wide-brim hat use rates for intervention participants during these periods were 40.0% and 43.8%, respectively, and for control participants, the rates were 22.3% and 33.0%, respectively. Results of the analyses for each of these outcomes showed significant time × condition interaction effects (P < .001 for all), in-

| Table. Results of Generalized, Linear, Mixed-Model Analyses During 3-Year Follow-upa |
|-----------------------------------------------|----------------|----------------|
| Follow-up Period | Sunscreen | Wide-Brim Hat |
| 3 mo             | 2.69 (2.13-3.39) | 3.13 (2.43-4.03) |
| 1 y              | 2.06 (1.642.58) | 2.40 (1.87-3.09) |
| 2 y              | 1.96 (1.54-2.48) | 2.64 (2.03-3.43) |
| 3 y              | 1.68 (1.35-2.13) | 1.44 (1.12-1.85) |

a All data are reported as odds ratios (95% confidence intervals) for the intervention group always using the listed sun protection vs the control group; all models were adjusted for baseline level of the outcome variable and postal station clustering.

©2009 American Medical Association. All rights reserved.