Amish Burn Ointment and Burdock Leaf Dressings: Assessments of Antimicrobial and Cytotoxic Activities

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Amish burn wound ointment (ABO) contains honey, lanolin, oils, glycerin, bees wax, and other natural additives. Although there are many anecdotal reports that this ointment covered with a burdock leaf (BL) dressing promotes burn wound healing, little scientific testing of this treatment has occurred. The goal of this study was to evaluate in vitro some of the components of this treatment modality for antimicrobial and cytotoxic activities. The ABO was tested for sterility using standard microbiological techniques. Because of the semisolid, lipid-based nature of the salve, the at-use product could not be tested in bioassays. Samples of BL and the dry ingredients (DI) used in the ointment were provided by the Amish vendor. Aqueous extracts of the DI and of the BL were prepared and freeze dried. The freeze-dried extracts were reconstituted, filtered, and tested separately on keratinocyte and fibroblast cell cultures for cytotoxicity (growth inhibition assay) and against a panel of susceptible and resistant microbes for antimicrobial activity (Nathan's agar-well diffusion assay) in a series of concentrations (% wt/vol). Neither DI nor BL extracts demonstrated antimicrobial activity against any of organisms tested. The DI extract inhibited growth of both keratinocytes and fibroblasts at the 0.1% concentration. The 0.1 and 0.03% concentrations of the BL extract were cytotoxic to both keratinocytes and fibroblasts. Although tests for microbial growth from the at-use preparation of the ABO were negative, extracts of the DI and BL did not demonstrate any antimicrobial activity. Additionally, both extracts inhibited the growth of skin cells in vitro at higher concentrations. These results suggest caution in the use of ABO and BL dressings if there is more than a minimal risk of complications from the burn injury. (J Burn Care Res 2014;35:e217–e223)

Interest in complementary and alternative medicine (CAM) is increasing as the general public pursues more holistic care. Complementary therapies are used in conjunction with conventional medicine whereas alternative therapies are not in conformity with standards of the medical community and generally not available in the hospitals of North America.[1] Patients want to be able to discuss CAM therapeutic options with their physicians to obtain guidance[2]; however, a lack of knowledge and scientific evidence to support nonconventional therapies prevent the medical community from accepting CAM. The British and American Medical Associations have called upon health care providers to learn about CAM.[3] The National Center for CAM was mandated by Congress in 1991 as the Office of Alternative Medicine at the National Institutes of Health to support research on alternative medical practices.[4] Many CAM therapies are culture-based, and the World Health Organization estimates that 80% of the world's population use herbal medicines.[4]

Amish healers perpetuate traditional herbal practices within their communities across North America. Their culture is centered on the family, home, and church in a segregated farming lifestyle, intentionally separated from the rest of society. Medical conditions are often treated with home remedies and natural/alternative therapies consistent with their plain

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lifestyle and their spiritual beliefs. They maintain that natural cures are best and sincerely believe they work.

One treatment unique to the Amish culture is the Amish burn wound ointment (ABO) originally devised by a plain Amish farmer who sought the help of God to heal his young son's scald injuries. What began more than 40 years ago has become a practice that is very much alive today. Most Amish insist on using their "comfortable dressing … provided by nature"[5] for a variety of wounds including significant, life-threatening burn injuries.[6]

ABO is an all-natural, inexpensive treatment retailing at $1.46 to $3.25 per ounce.[7] The formulation of the ABO ingredients was designed to melt at body temperature for rapid absorption into the wound. New applications are repeated every 12 hours. The previous application is removed by a light pressure with clean gauze to gently lift off any residue without scrubbing. The scalded burdock leaves (BL) provide a conforming dressing that does not stick to the wound and serves as a moisture barrier.[8–10]

The ABO contains more than a dozen naturally occurring medicinal ingredients blended into a salve. These include honey, lanolin, olive oil, wheat germ oil, marshmallow root, aloe vera gel, wormwood, comfrey root, white oak bark, lobelia inflata, glycerin, beeswax, and myrrh.[11] These ingredients were confirmed by the Amish manufacturer,[12] who also confirmed that 9 lbs of dry ingredients (DI) are used per 113 lb batch of final product (~8.0% by weight). An actual percentage of each of the DI has not been made available to the authors.

Claims of medicinal properties of individual ingredients are accompanied with this disclaimer on the product label as well as Internet sources: "These statements have not been evaluated by the Food and Drug Administration. The product is not intended to diagnose, treat, cure, or prevent any disease." Though there are many anecdotal reports that the ointment covered with a boiled BL dressing promotes burn wound healing, there has been no published report of scientific testing of this treatment.

Recently, the Amish have enlisted the cooperation of doctors who are willing to allow Amish burns teams to use the ABO treatment under medical supervision at certain hospitals in Michigan, Ohio, Pennsylvania, and Kentucky.[7–9] This cooperative treatment strategy provides precise (professional) assessment of burn depth and extent, as well as medical monitoring for dehydration, infection, and any other complications of burn injuries. It may also result in the collection of accurate data that could be published in peer-reviewed journals to document the effect of the Amish burn treatment in human subjects.

The purpose of this study was to evaluate the antimicrobial activities and cytotoxicity of some of the components of this treatment modality in vitro to determine its potential for effectiveness as a burn wound dressing.

METHODS

Samples of raw ingredients used to make the ointment (honey and DI) as well as the BL used as a dressing material were provided by the Amish vendor.[7] The ointment consisted of a nonaqueous vehicle combined with herbal medicines from dried plant material (DI). BL consisted of whole leaves harvested and dried from the previous year (2010). None of the materials were considered sterile at the start of the experiments. Because the cytotoxicity assays were performed in aqueous media, the ointment component of the ABO was not evaluated. Similarly, because of the semisolid, lipid-based nature of the salve, it was not possible to test the at-use product in other standard antimicrobial tests.

Preparation of Plant Extracts

The Engineered Skin Laboratory at the Shriners Hospital for Children—Cincinnati prepared 5% aqueous extracts of the DI and of the BL that were lyophilized and stored at –20°C for subsequent cytotoxicity and antimicrobial testing. Five grams of the DI or BL were weighed, shredded, and transferred to separate beakers containing 100 ml sterile, pyrogen-free water heated to 180°F (82.2°C). DI or BL were allowed to extract for 4 minutes, and then were allowed to cool. Next, the extracts were centrifuged at 350*g*, sterile filtered, transferred to glass Petri dishes, frozen on the refrigerated shelves of a manifold-type lyophilizer, and freeze dried. Dry extracts of DI or BL were reconstituted in Hepes-buffered saline, at a concentration of 0.1 g/10 ml (1% wt/vol) and sterile filtered. The reconstituted extracts were diluted into culture media for human epidermal keratinocytes (hK), or for human dermal fibroblasts (hF) at final wt/vol concentrations of 0.1, 0.03, 0.01, 0.003, and 0.001% (growth inhibition assay).[13,14] The same extract was used in assays for both antimicrobial activity and cytotoxicity.

Microbiology Tests

Sterility. The at-use ABO was cultured using standard microbiological techniques for both bacterial (aerobic and anaerobic) and fungal sterility tests. Tests were performed by inoculating the ointment directly onto BBL TSA II agar (Baltimore Biological

Laboratory tryptic soy agar; Becton, Dickinson & Company, Sparks, MD) with 5% sheep's blood, thioglycollate medium, and BBL inhibitory mold agar. The three media were incubated under the following conditions: TSA II agar with 5% sheep's blood (aerobic) at 35°C in 5.4% carbon dioxide for 5 days, thioglycollate medium (aerobic and anaerobic) at 35°C for 5 days, and inhibitory mold agar at 30°C for 14 days.

Antimicrobial Activity. The DI and BL extracts were tested for antimicrobial activity using the highest concentration of the extracts that were generated as stated above (0.1% wt/vol). The honey component of the ABO was also tested using the concentration received from the Amish supplier. Nathan's agar-well diffusion method[15] was used to determine the antimicrobial efficacy of the extracts and honey against the following panel of common burn wound organisms: *Candida albicans*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus*, and methicillin-sensitive *S. aureus*. A 5% mafenide solution was used as a positive control in the well assay. With regard to scoring in the agar-well assay, a measurable clear ring around the well indicated that the agent tested had antimicrobial activity against the microorganism on the test plate, and the diameter of the clear ring was recorded. If no area of clearing was visible, "no zone" was recorded, indicating that there was no sign of antimicrobial activity for that agent against the test organism in this assay. Occasionally, no clear zones were present, but there was an area of decreased microbial growth around the well, which was also recorded as no zone.

Cytotoxicity Assays. Subconfluent cultures of hK or hF were harvested and inoculated into 6-well plates (3 wells/condition) at 2e3 cells/cm^2 and allowed to grow for 2 days. On culture day 2, the media were changed and the DI or BL extracts were added at the final concentrations specified above. On incubation day 5, the cultures were photographed, and fluorescence was measured using the Alamar Blue assay, which correlates directly with cell numbers.[16] Fluorescence values (590 nm) are expressed as "cell growth" in Figures 1–4.

RESULTS

Microbiology Tests

Sterility. Because of concern about microbial contamination in the at-use ABO, the ointment was tested for both aerobic and anaerobic bacteria and fungi. No organisms grew in any of the tests, indicating an essentially sterile product.

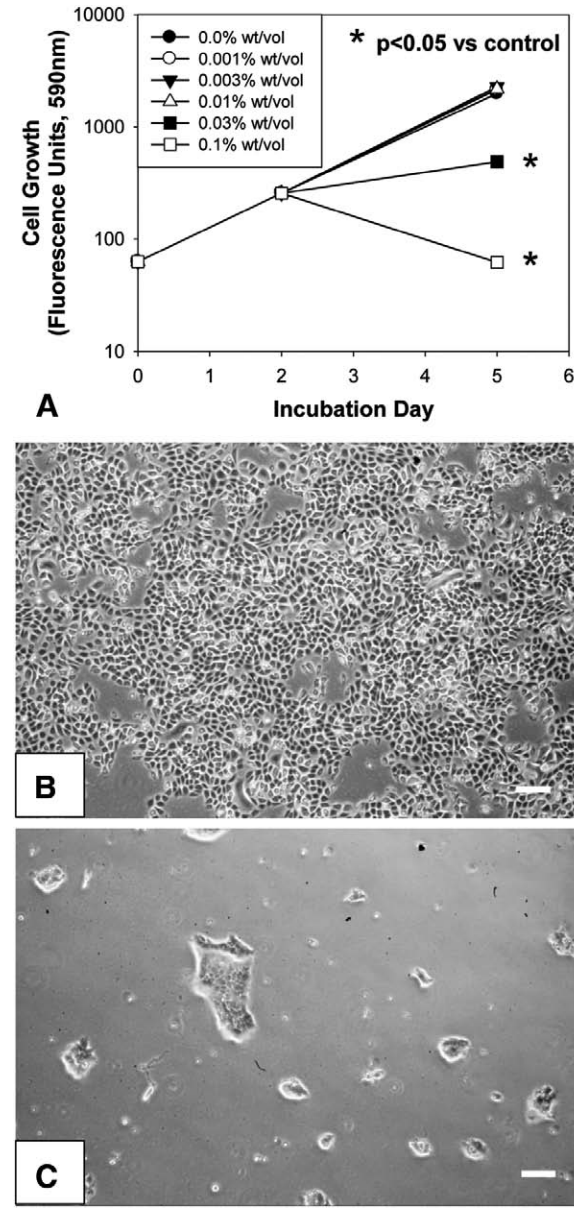

Figure 1. Cytotoxicity testing of Amish burn wound ointment dry ingredient (DI) extract on culture human epidermal keratinocytes. **A**. Cell growth vs incubation days. Graph demonstrates that the DI extract inhibited keratinocyte growth at the highest concentration (0.1% wt/vol) after addition at incubation day 2, plus 3 days of exposure, compared with controls ($P < .05$). **B**. Photomicrograph of control keratinocytes with 0.0% DI extract. Note normal density and cellular structure. **C**. Photomicrograph of keratinocytes with DI extract at 0.1% (wt/vol). Note the decreased keratinocyte density and altered structure after 3 days of exposure to the DI extract. Scale bars, 0.1 mm.

Antimicrobial Activity. Neither the DI extract nor the BL extract demonstrated antimicrobial activity against any of the strains of microorganism tested (Table 1). Honey used in the ointment

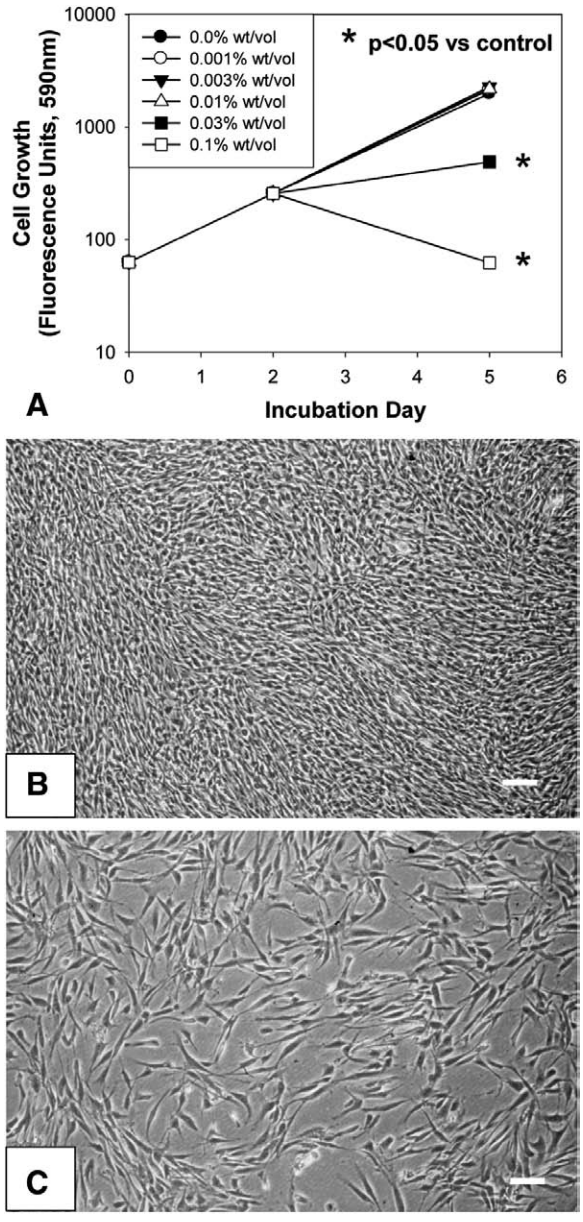

Figure 2. Cytotoxicity testing of Amish burn wound ointment dry ingredient (DI) extract on culture human dermal fibroblasts. **A**. Cell growth vs incubation days. Graph demonstrates that the DI extract inhibited fibroblast growth at the highest concentration (0.1% wt/vol) after addition at incubation day 2, plus 3 days of exposure, compared with controls ($P < .05$). **B**. Photomicrograph of control fibroblasts with 0.0% DI extract. Note normal density and cellular structure. **C**. Photomicrograph of fibroblasts with DI extract at 0.1% (wt/vol). Note the decreased fibroblast density and altered structure after 3 days of exposure to the DI extract. Scale bars, 0.1 mm.

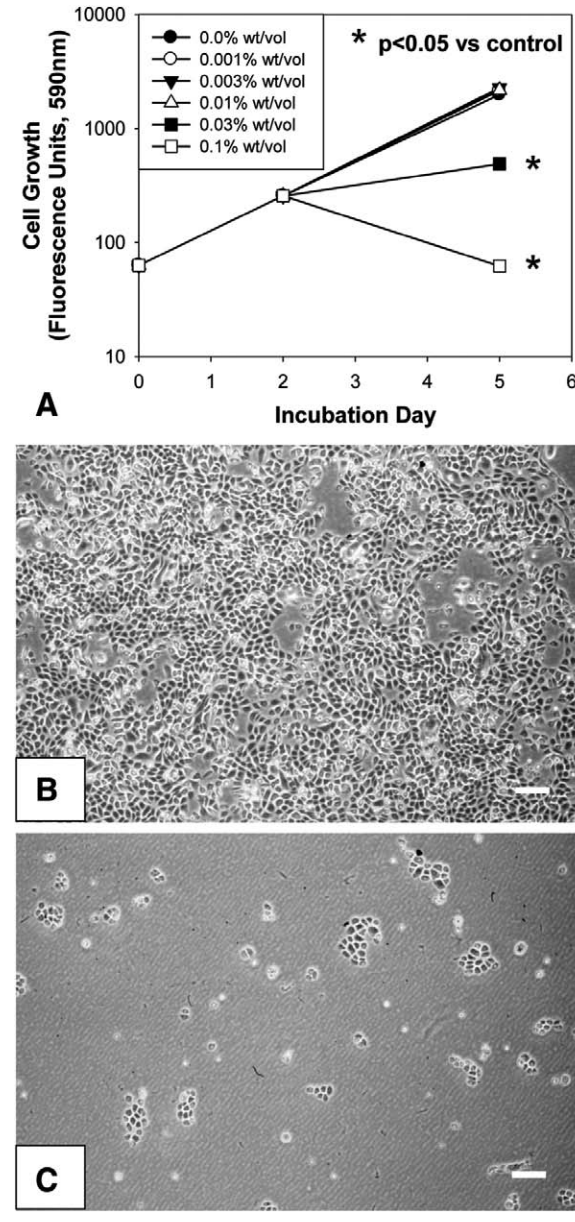

Figure 3. Cytotoxicity testing of Amish burn wound ointment burdock leaf (BL) extract on culture human epidermal keratinocytes. **A**. Cell growth vs incubation days. Graph demonstrates that the BL extract inhibited keratinocyte growth at the highest concentration (0.1% wt/vol) after addition at incubation day 2, plus 3 days of exposure, compared with controls ($P < .05$). **B**. Photomicrograph of control keratinocytes with 0.0% BL extract. Note normal density and cellular structure. **C**. Photomicrograph of keratinocytes with BL extract at 0.1% (wt/vol). Note the decreased keratinocyte density and altered structure after 3 days of exposure to the BL extract. Scale bars, 0.1 mm.

had minimal antimicrobial activity against one bacterial strain tested. Although there was no clear zone of antibacterial inhibition, there was a ring of bacterial suppression observed against the methicillin-resistant *S. aureus*, possibly representing a bacteriostatic effect. The antimicrobial effect of 5% Sulfamylon® solution (Mylan Bertek Pharmaceuticals, Canonsburg, PA) against the same panel

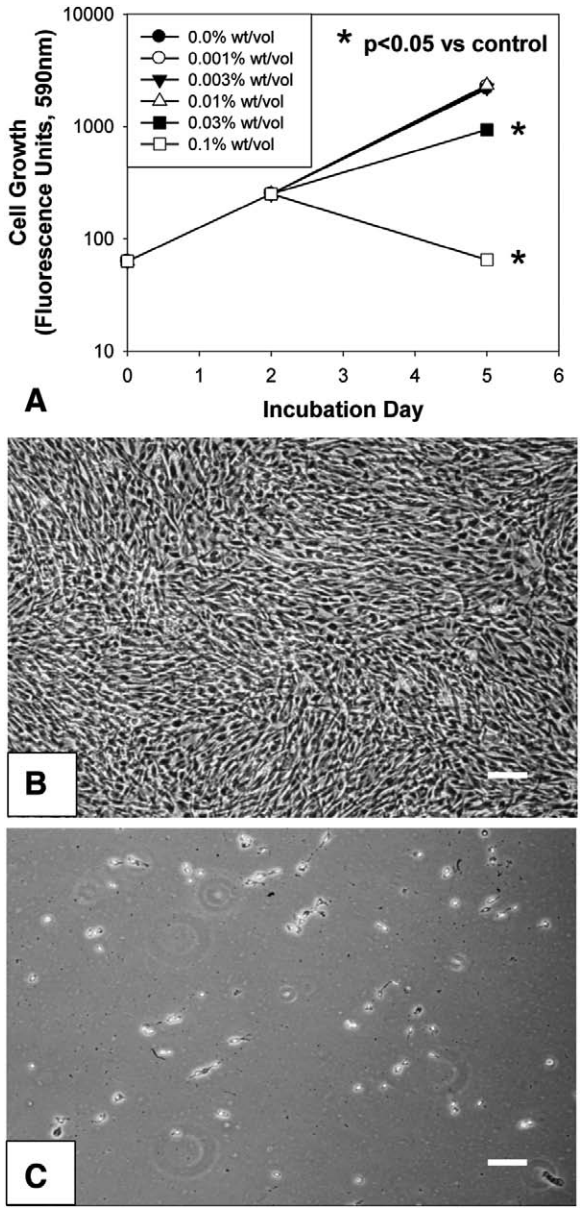

Figure 4. Cytotoxicity testing of Amish burn wound ointment burdock leaf (BL) extract on culture human dermal fibroblasts. **A**. Cell growth vs incubation days. Graph demonstrates that the BL extract inhibited fibroblast growth at the highest concentration (0.1% wt/vol) after addition at incubation day 2, plus 3 days of exposure, compared with controls ($P < .05$). **B**. Photomicrograph of control fibroblasts with 0.0% BL extract. Note normal density and cellular structure. **C**. Photomicrograph of fibroblasts with BL extract at 0.1% (wt/vol). Note the decreased fibroblast density and altered structure after 3 days of exposure to the BL extract. Scale bars, 0.1 mm.

of microorganisms is provided only for method comparison purposes.

Cytotoxicity Assays. The DI extract inhibited keratinocyte and fibroblast growth at the highest (0.1% wt/vol) concentration. This inhibition was statistically significant compared with the control and other lower concentrations of the DI extract ($P < .05$). The inhibition of the DI extract on keratinocyte and fibroblast cell growth at day 5 is depicted in the graphs and photomicrographs given in Figures 1A–C and 2A–C.

The BL extract inhibited keratinocyte and fibroblast growth at the two highest (0.1–0.03% wt/vol) concentrations. This inhibition was also statistically significant compared with the control and other lower concentrations of the BL extract ($P < .05$). The inhibitory activity of the BL extract on keratinocyte and fibroblast cell growth at day 5 is depicted in the graphs and photomicrographs in Figures 3A–C and 4A–C. The inhibitory activity of the BL extract on growth of both cell types was greater than that of the DI at the same concentrations.

DISCUSSION

When seeking health care from medical professionals, the Amish often request ABO with BL dressings to be used as part of their wound treatment instead of Food and Drug Administration–approved topical agents. Despite the lack of scientific evidence for the safety and effectiveness of the ABO, it has become a traditional practice among the Amish. This often poses ethical issues for burn care specialists providing care to Amish people with burn injuries.

This study explored the properties of this traditional treatment in the interest of both cultural sensitivity and patient safety. The Amish prefer to "take care of their own" in a cost-effective manner, but will seek advanced care from medical professionals under certain circumstances. Access to care outside of their community is dictated by their culture and requires permission of local church leaders. Their primary preference for natural healing is for holistic and faith-based reasons, but they also avoid costly medical bills by choosing culturally acceptable options.

Wound treatment with ABO and BL has been reported by the Amish to heal wounds painlessly and without infection. Yet, other case reports published by the Amish describe treatment challenges such as reactions to the ointment (prompting use of another topical salve on alternating days) or to the BL (prompting substitution with plantain leaves, cabbage leaves, or sprouts that are less irritating).[5,6,17]

The present investigation examined the antimicrobial and cytotoxic activities of the components of the ABO and BL as these characteristics have not been studied previously. As the ointment itself is manufactured using a nonaqueous base, it was not possible to

Table 1. Antimicrobial activity of components of Amish burn ointment ingredients and burdock leaf extracts

Organism	Burn Ointment Dry Ingredients	Burdock Leaf Extract	Burn Ointment Honey	Sulfamylon 5% Solution (Control)
Enterobacter cloacae	No zone	No zone	No zone	No zone*
Enterococcus faecalis	No zone	No zone	No zone	22 mm
MRSA	No zone	No zone	No zone	23 mm
MSSA	No zone	No zone	No zone*	15 mm
Pseudomonas aeruginosa	No zone	No zone	No zone	27 mm
Escherichia coli	No zone	No zone	No zone	No zone*
Proteus vulgaris	No zone	No zone	No zone	No zone
Proteus mirabilis	Not tested	Not tested	No zone	12 mm
Klebsiella pneumonia	No zone	No zone	No zone	19 mm
Candida albicans	No zone	No zone	No zone	No zone

MRSA, methicillin-resistant *Staphylococcus aureus*; *MSSA*, methicillin-sensitive *S. aureus*.
*indicates a ring of bacterial suppression, although there was no clear zone of antibacterial inhibition.

test the DI in the at-use product; therefore, the DI were tested as supplied by the manufacturer.

A temperature range of 210 to 225°F was used by the ABO manufacturers for preparing the DI extract. This temperature range was approximately the same as that for the extraction technique we used (180°F; 82.2°C). It is unclear whether this 30 to 45°F temperature difference might have influenced our results.

One very important aspect of this study was that the assays of the extracts used in this study were prepared in an aqueous solvent; therefore, it is likely that the profile of extracted compounds tested was not identical to that of the plant extracts in the ABO ointment, which is manufactured using an organic (oil-based) solvent. Furthermore, because the molecular composition of the active agents in the extract used for the ABO ointment is not known, the chemical similarity of the aqueous extract to the organic extract could not be determined.

Last, it is possible that the chemical stability of any active agents in the ABO ointment may be different from the stability of the aqueous extract used in this study. Although a detailed chemical analysis was beyond the scope of this study, further purification and testing of possible active agents may be considered for future investigations.

There is the possibility that some lipid-soluble components and small hydrophobic molecules in both the DI and BL may have been removed or altered during the aqueous phase extraction process. It is unknown whether these agents actually inhibit microbial growth and/or mitigate the growth-inhibitory properties of the ABO. Although we have demonstrated that the aqueous phase components of the DI inhibit cell growth at defined concentrations, we were unable to determine which, if any, components integrate with the aqueous wound transudate from the lipid-based ABO formulation. Furthermore, we are unable to estimate what concentration of the DI (water- or fat-soluble) is actually presented to the surface of the burn wound, as the scope of this study focused on testing the antimicrobial and cytotoxic activities components of the DI and BL in vitro.

Additionally, the scope of this study did not include testing of the potential activities of the combination of the DI and the BL together. Although it is possible that combination of the plant extracts may result in increases or decreases of cytotoxic activities, the results of this testing did not suggest that combination of the extracts would provide either antimicrobial activity or stimulation of cell growth.

As the ABO is not manufactured under sterile conditions, these initial studies were focused on the potential for contamination of the product as it is distributed. The at-use sample of the ABO was not contaminated, as evidenced by negative microbial cultures. The honey used in the ABO demonstrated negligible bacteriostatic activity. Extracts of the DI and of the BL dressing material did not demonstrate any antimicrobial activity. Both extracts inhibited the growth of hK and hF in vitro at the highest concentration tested.

The results of this initial investigation do not provide sufficient evidence to support controlled clinical trials, but without accurate case studies and scientific investigations we have only testimonials of the Amish healers and their community burn teams. The conventional health care provider is reliant upon current best practice that is evidenced-based and standard of care whereas the Amish are bound by religious belief, tradition, and their church community. Denied their wound treatment, the Amish may dangerously exclude conventional medical care to the detriment and even the demise of a burned victim because of

this cultural dilemma. To this end, we are hopeful that this study will result in collaboration with the Amish manufacturers to determine a scientific basis for the effect of their preferred burn and wound treatment. For those in specialized burn care centers, the decision as to which topical antimicrobial agents and dressing materials to be used must be based on sound medical and manufacturing practices. At our institution, we remain open-minded about the use of the Amish therapy (when requested) for small, superficial wounds but have not modified our protocols for the management of deeper or more extensive burns where the risk for a less-than-optimal outcome exists.

CONCLUSION

These results suggest caution in the use of the ABO and BL wound treatment. Although minor, superficial burns might be managed successfully with ABO treatment in the outpatient setting, monitoring and oversight by experienced burn specialists is warranted particularly when there is more than a minimal risk of complications from the severity of the burn injury and/or the effects or limitations of the topical treatment used. Considering the number of ingredients, their varied sources of origin, and the rudimentary conditions under which the product is prepared, there is reason to be concerned for adverse (eg, allergic, infectious, or cytotoxic) activities or ineffectiveness of the ABO on wound healing.

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