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Authors: Butron, William, and Gaikwad, Joel

Source: BIOS, 80(2) : 66-75

Published By: Beta Beta Beta Biological Society

URL: https://doi.org/10.1893/011.080.0203

Research Article

An *in vitro* analysis of the efficacy of selected bar soaps as antibacterial agents

William Butron and Joel Gaikwad

Department of Biology, Oral Roberts University, Tulsa, OK 74177

Abstract. The disturbing prevalence and increasing incidence of nosocomial infections and community-acquired infections, compounded with bacterial resistance to antimicrobial agents, dictates the need for effective and varied means with which to cleanse hands and work surfaces. Lye Soap, Dial Antibacterial, Cinthol and Dettol bar soaps were evaluated *in vitro* for their effectiveness as antimicrobial agents against eleven selected genera of bacteria that have been implicated in nosocomial infections and are part of the resident or transient flora of the skin. Trichlorocarban, the active ingredient in Dial Antibacterial, Cinthol and Dettol soaps, and Lye soap were assessed for their antibacterial activity. The null hypothesis was that there would not be a significant difference in antimicrobial efficacy when compared with the non-antibacterial soap Irish Spring used as a control based on zone of inhibition analysis. The alternative hypothesis, which was accepted for all of the soaps except Lye soap, stated that the rejection of the null hypothesis is indicative of significant bacterial inhibition ($p<0.001$). Statistical analysis of the data by the Kruskal Wallis test demonstrated that there was a significant difference between the inhibitory capacity of all of the soaps tested, excluding Lye soap, when compared with the Irish Spring control ($p<0.001$ for each) for three of the four tested concentrations validating the rejection of null hypothesis. It was also determined that there was no significant inhibition of Gram negative bacteria by any of the tested soaps ($p<0.001$).

Introduction

The importance of proper hygiene is a concept that has only become well understood in what can be considered recent history. Hand hygiene in particular is one of the single greatest means by which the spread of diseases, especially of nosocomial infections, can be prevented (Gillespie, 1961; Kampf et al., 2004a).

Correspondence to: Joel Gaikwad, Department of Biology, Oral Roberts University, 7777 S. Lewis Ave, Tulsa, OK 74177; phone: (918) 495-6944; fax: 495-6297; e-mail: jgaikwad@oru.edu

Hygienic conditions are necessary for maintaining good health in homes, communities, businesses (especially those that are food related) and in health care settings. The recent outbreak of *E. coli* in the community as a result of a contaminated spinach crop (FDA, 2006) is a poignant reminder that sanitary conditions in industry and the community are of great importance.

Appropriate testing of products is also a significant concern. Many naturopathic products, such as Lye Soap (Remwood Products Co., USA) make a variety of claims about their effectiveness, but have not been appropriately tested to confirm these claims. Even products that have been tested, however, are not always as effective

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as they are claimed to be. If too few tests are performed the results that are obtained may be misleading and may result in undesirable outcomes as a result of a lack of product effectiveness in certain situations.

Public health concerns

Nosocomial infections and person to person transmission of pathogenic organisms has become a problem that is costing health care facilities and patients millions of dollars every year (Kampf et al., 2004a). The increasing prevalence of bacteria that are resistant to antimicrobial agents and the inadequacy of certain antimicrobials in eliminating bacteria have resulted in a need for the development of a wider variety of more effective hand cleansers and has become a significant concern in maintaining good public health (Stickler and Thomas, 1976; Haley, 1985; Aiello, 2004). Bar soap has traditionally been one of the most commonly used means of hand sanitation, particularly since the advent of antimicrobial hand soaps. It has been demonstrated that soaps that do not contain agents that are anti-microbial in nature are seldom effective at decreasing the bacterial population present on the skin (Kampf et al., 2004a). If it can be determined that the soaps in question, Lye Soap, Dial Antibacterial (Armour-Dial, USA), Dettol (Reckitt Benckiser, United Kingdom) and Cinthol (Godrej Consumer Products Ltd., India), are effective as antimicrobials in *in vitro* testing it would provide a justification for *in vivo* testing, which is necessary to determine the actual effectiveness of these products (Voss, 1975). The tested soaps are being compared with a control soap, Irish Spring (Colgate Palmolive, USA) bar soap, to determine if there is a significant difference in antibacterial activity because it does not contain any antibacterial agents. Demonstrating the effectiveness of these soaps as inhibitors of the bacterial genera that compose the transient and resident bacteria of the skin would provide both clinicians and the general populace with more tools for combating the transmission of pathogens and decreasing the incidence of nosocomial infections and community disease (Kampf et al., 2004a). This is particularly important in developing nations where simple soaps may be the only affordable or available means of maintaining good hygiene. If these soaps are not effective at inhibiting the transient and resident flora of the skin then altering the concentration of the antibacterial agents that are incorporated in these products may be necessary (McBride, 1984).

Mode of bactericidal activity

The selected soaps were chosen based on their characteristics as antimicrobial agents. Lye Soap contains alkaline compounds which may be effective in facilitating bacterial inhibition (Madden et al., 1973). Lye soap is composed of animal lard and lye. The lye used for making lye soap is made by mixing wood ash with water and obtaining the extremely caustic liquid lye that is formed (Brown et al., 1942; Environmental Protection Agency, 1995). Lye is a strong alkali primarily composed of either sodium or potassium hydroxide which is why it is such a caustic compound (Brown et al., 1942). Lye has been used traditionally as a cleansing product is recognized as an effective cleansing agent (Brown et al., 1942; Champion et al., 1918). Lye Soap needs to be evaluated because the lye content is low enough so as to ensure that the soap's alkalinity does not result in skin irritation or toxicity (Brown et al., 1942), which may affect its antibacterial properties. In addition to the potential bactericidal effect that the lye contained in the soap may have there is also a possibility that some of the many compounds contained in the oil and fat component (lard) of the soap may have a bactericidal effect.

Dial Antibacterial, Dettol and Cinthol contain the active ingredient Trichlorocarban (TCC) which is an anilide compound (McDonnell et al., 1999; Voss, 1975). Anilide compounds, and specifically TCC, act as antibacterial agents through the disruption of cellular membranes; the membrane is disrupted through the uncoupling of proton-translocators (Hamilton and Jeacock, 1972; Hamill 1984; McDonnell et al., 1999). The concentrations of Trichlorocarban in these soaps are unknown; necessitating comparison with a control and the determination of their efficacy as antimicrobials. Trichlorocarban has been reported to be an effective antibacterial; however, its

efficacy when used on the skin is questionable (McDonnell et al., 1999). Uncertainties about the antimicrobial capacity of these soaps require that they be tested, especially because of the increasing resistance of bacteria to antibacterial agents (Aiello et al., 2004, McDonnell et al., 1999).

Gram positive and gram negative susceptibility

Gram negative organisms are traditionally more difficult to eradicate and to treat in infections (Kampf et al., 2004a; McDonnell et al., 1999). Gram negative organisms are also very commonly implicated in nosocomial infections with up to 64% being caused by gram negative organisms (Kampf et al., 2004b). The lipopolysaccharide sheath encasing gram negative bacteria is an extremely effective barrier against many antimicrobial agents and in conjunction with the outer membrane of the bacteria serves to prevent the entrance and action of hydrophobic compounds. In addition to this, the inner membrane has been thought to serve as a barrier to hydrophilic molecules giving the gram negative bacteria a very effective defense against a wide range of bactericidal agents (McDonnell et al., 1999). Another factor that may be of importance for disinfection but is unlikely to relate to skin cleansing is the increased tolerance for antiseptics through the formation of biofilms by gram negative genera (McDonnell et al., 1999). Gram positive organisms are generally much more susceptible to antiseptic treatments because the peptidoglycan composing their cell walls is able to be disrupted more easily than the polysaccharide sheath encapsulating gram negative bacteria (McDonnell et al., 1999; Kampf et al., 2004a). Trichlorocarban is especially effective against gram positive organisms and also eliminates gram negative organisms but the minimum inhibitory concentrations required for the eradication of gram negative genera is much greater than for the gram positive organisms (McDonnell et al., 1999; Voss, 1975).

Further research

This study is designed to be a preliminary justification for an *in vivo* experiment to determine the true efficacy of these bar soaps as topical skin antimicrobial agents. If these soaps prove to be effective they may represent an inexpensive alternative to other means of disinfection. This is beneficial, especially in the case of Lye Soap because of the difficulty that bacteria will have in developing resistance to this product because it has not been used extensively as an antibacterial and because it provides another alternative to products that bacteria may become resistant to. The results that are obtained *in vitro* often vary widely from the actual *in vivo* activity necessitating a supplementary *in vivo* evaluation if the *in vitro* test results demonstrate significant inhibitory capability (Marples et al., 1974). The null hypothesis is that there will not be a significant difference between the zone of inhibition size of the test soaps, Dial Antibacterial, Cinthol, Dettol, and Lye Soap and the Irish Spring control soap ($p = 0.05$). The alternative hypothesis is that the rejection of the null hypothesis is demonstrative of significant bacterial inhibition.

Materials and Methods

Media

Mueller-Hinton agar plates were used (Hamill et al., 1984; Burns et al., 2000) for testing the antimicrobial capacity of the soaps analyzed. The Mueller Hinton plates were prepared using 17g of agar (Fisher Scientific) and 22g of Mueller-Hinton Broth powder (BBL) per liter of deionized water according to the instructions in the Difco Manual (1998a) and were in 9 cm sterile plastic plates to a depth of approximately 4 mm (Deacon, 1976). For the standard plate counts nutrient agar plates (Difco Manual, 1998b) were used. All cultures were grown and the appropriate dilutions were performed using TSB media (Difco Manual, 1998c).

Bacteria

Eleven genera of bacteria were tested in this study, all of which are found in the normal resident flora of the skin and which have been implicated in nosocomial infections and are members of the transient skin flora. The organisms included for testing were obtained from Presque Isle Cultures. The Gram positive organisms were

Staphylococcus aureus, Staphylococcus epidermidis, Micrococcus luteus, Streptococcus mitis, Streptococcus salivarius, Bacillus cereus, and *Enterococcus faecalis*. The gram negative organisms were *Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa and Serratia marcescens*.

Inoculum

In order to standardize the inoculum size serial dilutions were performed with *E. coli, S. marcescens* and *S. aureus* after the organisms were inoculated in TSB and allowed to grow for 18 hours at 37°C and adjusted to a specific turbidity to ensure an appropriate and consistent bacterial population. A McFarland 0.5 (0.05ml 0.048M $BaCl_2$ in 9.95ml 0.36N H_2SO_4) standard was used to adjust the turbidity of the broth tubes after 18 hours of growth (Kerr and McHale, 2007). Following the adjustment of turbidity with the McFarland 0.5 standard the optical density of the cultures was determined using a Bausch & Laumb Spectronic 20 spectrophotometer. Serial dilutions to 10^{-8} were performed in conjunction with a standard plate count to determine the approximate number of viable cells in the culture adjusted to the McFarland 0.5 standard. An optical density between 0.10 and 0.15 at 540nm in a culture (corresponding to a turbidity McFarland 0.5 standard) contains approximately $1x10^{-7}$ CFU, which is desirable for performing antimicrobial susceptibility tests. When performing spread plating 0.1ml of culture was plated using a glass spreader after the culture was adjusted to a McFarland 0.5 standard and verified spectrophotometrically to have an optical density between 0.10 and 0.15 at a 540nm wavelength.

Soaps and concentrations

The five soaps; Lye Soap, Dial Antibacterial, Dettol, Cinthol and the non-antibacterial Irish Spring control were tested for antimicrobial capacity by agar well diffusion (Deacon, 1976; Rojas et al., 2006) on Mueller-Hinton agar (Difco labs) of approximately 4mm in depth. Four concentrations of each soap were prepared in sterile water; 0.1g/ml, 0.01g/ml, 0.001g/ml and 0.0001g/ml. The solutions were prepared by serial dilution from a 0.1g/ml stock concentration. The 0.1g/ml solution was prepared by addition of 10.0 g of soap in 100ml of warm, sterile, de-ionized water.

Agar well diffusion

After inoculation and spread plating was performed, each plate had 5 wells 9mm in diameter (Deacon, 1976) punched using a sterile cork hole borer. To four wells on each plate 0.1ml of soap solution was added. The fifth well was used as a control and had 0.1ml of sterile saline added. The soap solutions were warmed in a 55°C incubator and thoroughly mixed before addition to the wells to ensure that a uniform concentration was obtained. If necessary the soap solutions were warmed in a boiling water bath to facilitate obtaining a uniform concentration. The plates were incubated at 37°C for 24 hours after which the zones of inhibition were determined. The zones of inhibition were measured to the nearest 0.5mm (Deacon, 1976).

Replicates

The testing was conducted three times with one replicate in each test group. The same soap solutions were stored and used in each test set and replicate to ensure that a large enough sample size was obtained for analysis. For every replicate fresh bacterial sample were prepared for inoculation. The McFarland Standard used to adjust the CFU count in the inoculum was prepared no more than half an hour prior to standardization.

Determination of pH

The pH of each soap solution at each concentration was performed twice using a Beckman Expendomatic IV pH meter. Each soap solution was thoroughly mixed (with warming if necessary) to ensure a uniform soap concentration and pH level. The pH meter was calibrated before use and after temperature adjustment for the warmed solutions, (55°C) using buffers of pH 3, 5, 7, 9 and 11.

Statistical analysis

The statistical analysis of the data was performed using Minitab® 14 Statistical Software.

The software was used to evaluate the data and to produce box plots to assess normality and determine whether parametric or non-parametric analysis should be used (LeBlanc, 2006). The Kruskal Wallis test was selected for a comparison of medians because the data were significantly skewed and the data sets contained outliers (LeBlanc, 2006).

Results

Bacterial inhibition

The inhibition of bacteria was most pronounced with the highest soap concentration. The diffusion of the soap solutions into the media was clearly visible as a ring that formed around the well into which the solution had been pipetted (Figure 1). Clear zones of inhibition were formed surrounding the wells on plates cultured with the organisms that were susceptible to the antibacterials contained in the soaps (Figure 1). Lye Soap demonstrated infrequent and random inhibition. On some of the plates the growth of bacteria surrounding the wells containing Lye Soap had reduced, but not completely inhibited, growth.

Comparison of soaps

The inhibitory effect of the soaps was determined utilizing the non-parametric Kruskal Wallis test because of the skew of the data and the presence of outliers in the sample groups, as demonstrated in Figure 2. Analysis with Kruskal Wallis demonstrated that the inhibitory effect of Lye Soap was significantly different from Dial Antibacterial, Cinthol and Dettol at concentrations of 0.1g/ml ($p<0.001$), 0.01g/ml ($p<0.001$) and 0.001g/ml ($p<0.001$), respectively (Figure 2). Dial Antibacterial, Cinthol and Dettol soaps, when analyzed, were not be significantly different from each other because the p-value was too high to discount the null hypothesis. At concentrations of 0.1g/ml, 0.01g/ml and 0.001g/ml the Kruskal Wallis values were $p = 0.661$, $p = 0.513$ and $p = 0.948$ demonstrating that these soaps did not have significantly different inhibitory activity. Although soap concentrations of 0.0001g/ml were prepared and tested, they demonstrated no inhibitory effect on bacterial growth and thus were not subjected to statistical analysis.

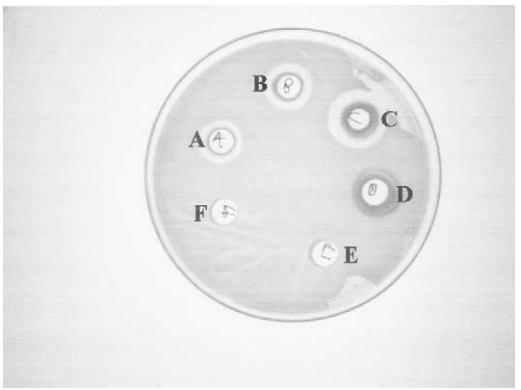

Figure 1. Plate demonstrating zones of inhibition of *Staphylococcus aureus* for a 0.1g/ml soap Concentration. Well A is cinthol, B is dettol, C is Dial antibacterial, D is lye soap and E is a saline control.

It is also shown in Figure 2 that as the soap concentration decreased so did the diameter of the zones of inhibition. As previously stated, the 0.0001g/ml soap concentrations were not effective for inhibitory purposes suggesting that this concentration is below the minimum inhibitory concentration for the antibacterial compounds contained in each of the soaps included in the study.

The inhibitory effect of Lye Soap when compared with that of the Irish Spring control was not determined to be different because every instance of inhibition in Lye Soap was considered to be an outlier point (Figure 3). The instances of bactericidal activity in Lye Soap were limited to Gram positive bacteria.

Gram positive vs. gram negative genera

The soaps that were tested were compared for efficacy against gram positive versus gram negative organisms (Figure 4) in order to determine the relative susceptibility of each category to Lye Soap and to the unknown TCC concentration in each antibacterial soap. The Gram positive organisms were *S. aureus, S. epidermidis, M. luteus, S. mitis, S. salivarius, B. cereus,* and *E. faecalis.* The gram negative organisms are *E. coli, P. vulgaris, P. aeruginosa* and *S. marcescens.* This test was performed after observing that most of the gram negative organisms were not inhibited by any of the soaps at any of the

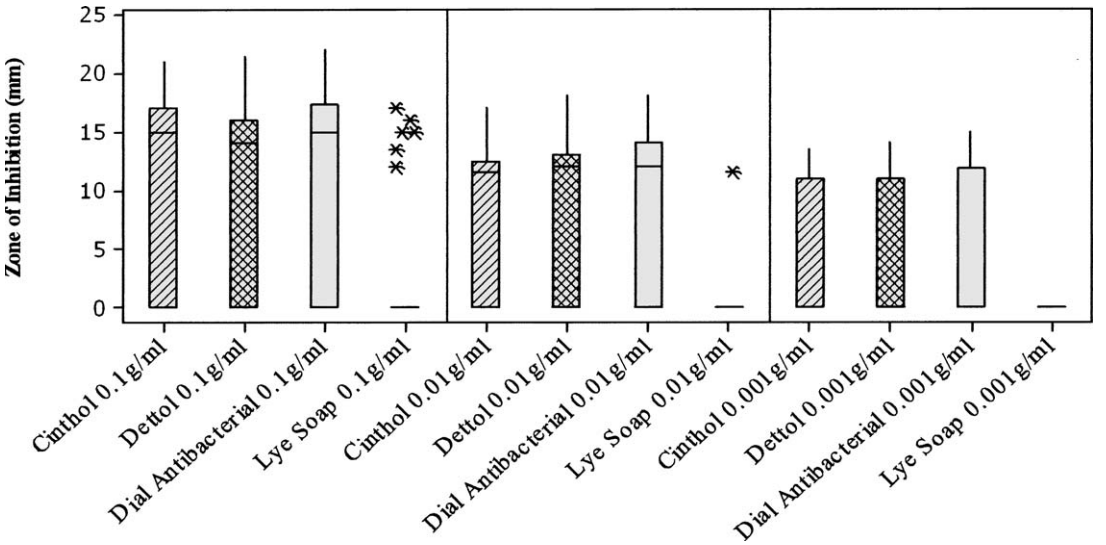

Figure 2. Zone of inhibition comparison of tested soaps for all test concentrations by Kruskal Wallis analysis. The box plots of the zones of inhibition from each soap with suspected antibacterial capacity are ordered from the strongest concentration (0.1g/ml) to weakest concentration (0.001g/ml) that showed antibacterial activity.

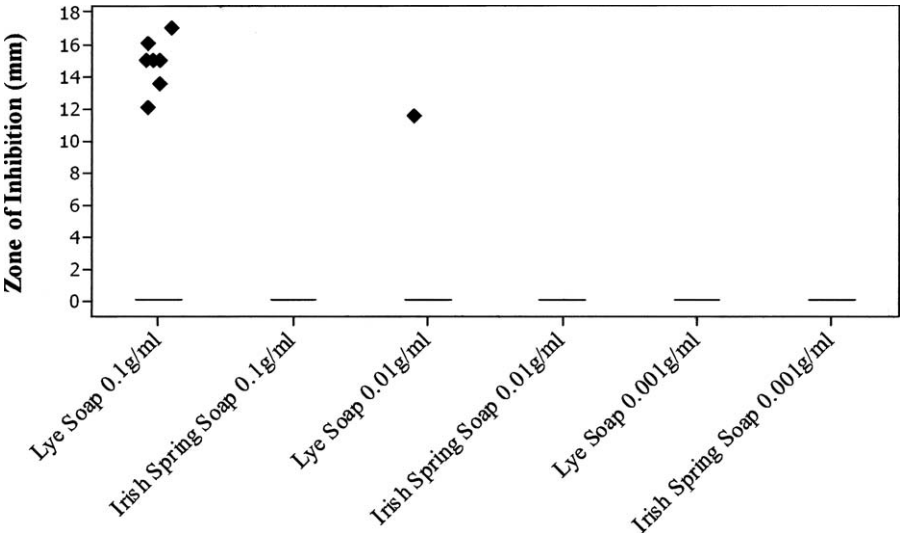

Figure 3. Zone of inhibition comparison of lye soap and the Irish Spring control by Kruskal Wallis analysis. The diamond shapes indicate outlier data points.

tested concentrations. The data was analyzed using the Kruskal Wallis test and indicated definitively that there was a significant difference ($p<0.001$) between the inhibition of gram positive and gram negative organisms at each of the effective concentrations of the soaps that were tested (0.1g/ml, 0.01g/ml and 0.001g/ml). As anticipated the overall inhibition of bacteria decreased with decreasing concentration of soap; the trend of soaps being more effective against gram positive organisms is also clearly evident from the data (Figure 4). It was demonstrated that there was a significant difference in inhibition between gram positive and gram negative organisms at concentrations of 0.1g/ml ($p<0.001$), 0.01g/ml ($p<0.001$) and 0.001g/ml ($p<0.001$).

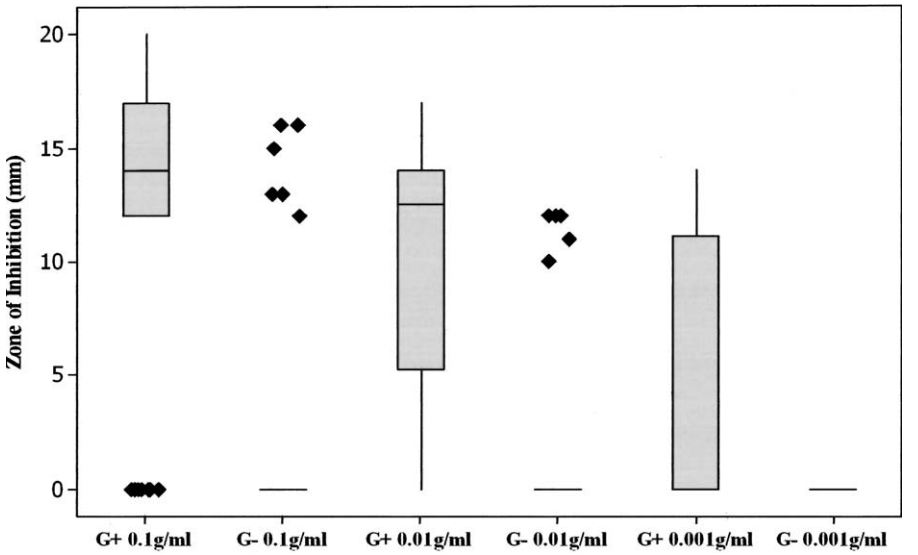

Figure 4. Comparison of zone of inhibition in Gram positive and Gram negative organisms by Kruskal Wallis analysis. The diamond shapes indicate outlier data points.

pH

Comparisons of the pH levels of each type and concentration of soap were performed graphically because determining whether the differences in pH were significant statistically does not necessarily correspond to significance with regard to inhibitory effect. The pH values were all very similar for the soaps, and in only one instance was the pH of Lye Soap greater than that of Irish Spring (at 0.1g/ml concentration) indicating that any inhibitory effect of Lye Soap would be due to a mode of action other than antagonistic pH levels, although it is possible that pH does play a role in its minimal inhibitory capacity (statistically insignificant). Irish Spring soap demonstrated no inhibition whatsoever, matching the control saline wells, and because it had very similar pH levels to those of the other soaps at each concentration, as is shown in Figure 5, it can be concluded that pH is not likely to be, in itself, a significant factor in bacterial inhibition by bar soaps.

Discussion

The use of naturopathic products such as the Lye Soap tested in this study has become common because of the claims that are made by the companies producing the products about the potential benefits that the product may impart to the consumer. The label on the Lye Soap tested in this study was attributed with six health benefits such as being an effective treatment for psoriasis and eczema. In the interests of developing more and better health care products; the testing of naturopathic products for actual efficacy is important and potentially beneficial. There was some inhibition of bacteria demonstrated by the Lye Soap; however these data points are all outliers demonstrating the overall inefficacy of the soap as an antimicrobial. In addition to this, when Lye Soap was an effective inhibitor of bacterial growth, it was not as effective as the other antibacterial soaps that were included in the study. The similarity between the Irish Spring soap and the Lye Soap is indicative of a lack of antimicrobial capacity, however because Lye Soap did demonstrate some antibacterial activity (statistically insignificant) there may be an antibacterial agent present in the soap in low concentrations.

One of the most surprising and perhaps the most significant finding of the study was the difference in efficacy of antimicrobial soaps against gram negative versus gram positive organisms. The active ingredient in the antibacterial soaps,

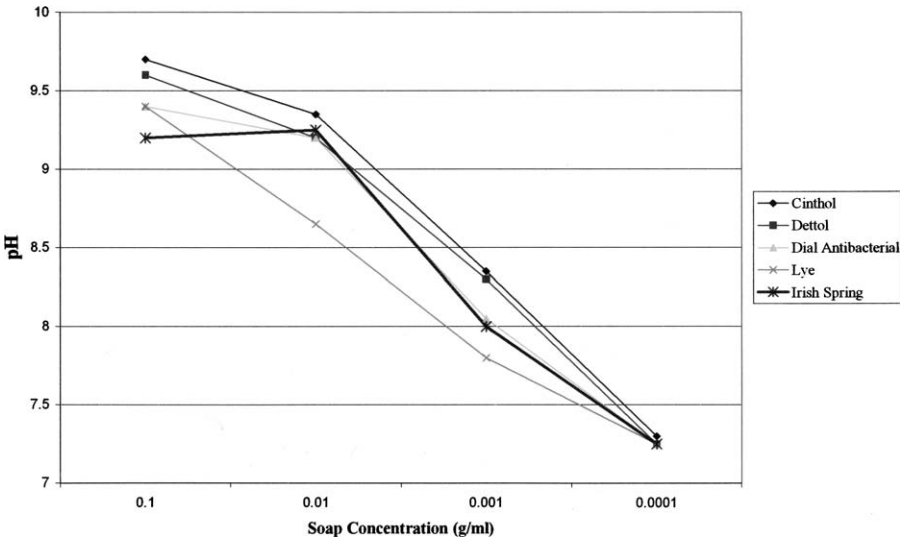

Figure 5. Comparison of pH of soap solutions utilized. The different soaps all display similar pH levels, and the control soap, Irish Spring, has a pH that very closely matches Dial antibacterial, which was the most effective at inhibiting the organisms tested.

TCC, is an anilide compound (McDonnell et al., 1999). The mode of action of TCC is as a membrane disruptor (Kampf et al., 2004a) which results in lysis of the bacteria and death. As was previously described the lipopolysaccharide layer and inner and outer membranes of the gram negative organisms seem to be responsible for much of their resistance to bactericidal agents (McDonnell et al., 1999) and is a significant factor in the pronounced lack of inhibition of gram negative organisms by TCC. It is still surprising, however, that there was no significant inhibition of gram negative organisms by the soaps containing TCC because it has been demonstrated to have, in appropriate concentrations, a bactericidal effect against both gram negative and gram positive organisms (McDonnell et al., 1999). This seems to suggest that either new novel antimicrobial agents need to be developed for incorporation in antibacterial soaps so that they will be effective against gram negative organisms or increased concentrations of TCC may be necessary for a significant gram negative antimicrobial effect to be present. Merely increasing the concentration of this antibacterial may not be an effective solution because in high enough concentrations it may cause irritation to the skin (McDonnell et al., 1999). In light of the recent *E. coli* outbreak (FDA, 2006) and other concerns with regard to nosocomial and community acquired infections (Kampf et al., 2004a; Kampf et al., 2004b; McDonnell et al., 1999; Aiello et al., 2004) antimicrobial soaps that are effective inhibitors of gram negative organisms need to be developed in order to promote better hygiene and facilitate better health.

Although pH does not seem to be a significant factor based upon the trends that were observed in the soaps when compared to Irish Spring there is a possibility that at higher concentrations of 0.1g/ml or higher the pH could be significant. The similarity of the pH levels was considered to be sufficient to demonstrate the pH was not a significant factor with regard to bacterial inhibition by bar soaps. The primary support for this conclusion is that the pH of Irish Spring soap, which demonstrated no bacterial inhibition, was roughly equivalent to that of Dial Antibacterial soap which demonstrated the overall best inhibition of the bacteria tested.

Lye Soap was not demonstrated to be an effective antimicrobial agent ($p<0.001$). The fact that the control soap demonstrated no inhibition in any case seems to indicate that Lye Soap may have some antibacterial capacity despite the fact that the inhibition that was observed was determined to be insignificant. This is indicative that naturopathic products must be subjected to more

rigorous testing because their advertisements and labeling can be misleading or even contain incorrect information. There are some naturopathic products that have been demonstrated to be very beneficial but the implementation of rigorous testing is necessary to determine which products are actually effective and which ones are not. In the case of Lye Soap, there may be some minor health benefits, the *in vitro* analysis indicated that this is unlikely, but *in vivo* testing is necessary to determine whether there are any real health benefits from using this product.

Gram negative organisms are not susceptible to antibacterial bar soaps for the concentrations tested while gram positive organisms were ($p<0.001$) indicating that there is a need for the development of more effective antimicrobial agents and perhaps a more rigorous means of testing these soap products by including a larger selection of bacterial genera when analysis of antibacterial capacity is performed. The pH of the soap solutions does not seem to be a significant factor in bacteria; however, pH may act synergistically with the antibacterials to inhibit bacterial growth. Especially in light of the recent problems that have been encountered with gram negative contaminations and the importance of gram negative bacteria in nosocomial infections; the development of antimicrobial soaps which can be used daily by the general public for cleansing is necessary.

Acknowledgements. I would like to thank Rachel Budavich and Okafor Ebuka for their help with media preparation and moral support. This research was supported by the Department of Biology, Oral Roberts University. I would also like to thank God for giving me the endurance and guidance to be able to complete this project.

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Received 20 December 2007; accepted 8 April 2008.