Improving Rural Health with Natural Alternatives to Commercialized Soap

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Abstract

Proper handwashing is the easiest and most effective way to prevent the spread of disease. In some rural areas, including San Vito and Coto Brus, Costa Rica, many people cannot afford soap and therefore cannot wash their hands properly. To combat this, the local government-run clinic periodically distributes antibacterial soap and handwashing protocols to the communities; however, this practice is neither practical nor sustainable. This study aims to identify local flora that can be cultivated and used by the community as a substitute for commercialized soap. We identified four local plants — *Phytolacca rivinoides*, PF*, Yucca elephantipes*, and *Clidemia hirta* — which have been shown to contain saponins, the active chemical in soap. Subjects' hands were swabbed prior to and after washing with each treatment. Bacteria were then cultivated and colony-forming units per milliliter (CFU/ml) were calculated. The results showed that treatments with *Yucca elephantipes*, and *Clidemia hirta,* tap water, and Protex© soap significantly decrease the CFU/ml on hands. However, none of the treatments decreased the CFU/ml significantly more than another. Future studies should be conducted to further investigate the potential use of these plants as sustainable substitutes for commercial soap products. Our findings support the importance of proper handwashing techniques with clean water, even when soap is not available.

Introduction

One of the most important and easiest steps that can be taken to prevent the spread of disease and save lives is good hand hygiene [1]. Proper hand washing includes using clean water, soap, washing for at least 20 seconds, and hygienic drying. In a study conducted at the London School of Hygiene and Tropical Medicine, it was shown that if handwashing was not performed after defecation, 44% of samples were found to have bacteria of fecal origin. When water alone was used during hand washing, this number dropped to 23% and when soap was used it dropped even further to 8% of samples [2]. Diarrheal diseases are the second leading causes of death in children worldwide; every day 2,195 children die due to these diseases [3]. Improvements in hand hygiene can reduce gastrointestinal illness by up to 31% and respiratory illness by up to 21% [4].

Within the indigenous communities of San Vito Costa Rica, many people know how to properly wash their hands, especially due to hand hygiene campaigns in 2019. Despite this, they cannot properly carry out these practices because they need to spend their money on food and other necessities [5]. In other rural communities, 50% of people had a barrier preventing them from following the recommended hand washing protocol. These issues included not having access to water and/or soap or not being able to afford commercialized soap [6]. In rural Bangladesh, people used soil and ash as substitutes for soap, which were shown to clean hands comparably to commercialized soap [7]. Past hand washing interventions have employed education on how to properly wash hands and the importance of good handwashing practices [6]. The focus, however, needs to shift towards giving people a sustainable way to practice good hand hygiene, given barriers most rural Costa Ricans face.

In San Vito, Costa Rica, there have been initiatives by the local primary care center to provide soap to those who cannot afford it. This is not a long term or sustainable solution [8, 5]. Dr. Pablo Ortiz stresses the necessity for indigenous communities in the area to have a way to maintain good hygiene without extra cost [5]. Therefore, it is important to find alternative substitutes for expensive commercialized soap.

Saponins, the active chemical in soap, are present in commercialized soaps and can be found throughout the natural world [9]. They are composed of a fatty acid and a salty base [9]. Many lather forming plants contain saponins [10]. Saponins have anti-microbial, anti-mold, and antifungal properties. These properties are also present in plants and may be applicable to human hands [11, 12]. The following saponin containing plants are found in the surrounding area of San Vito, Costa Rica: *Clidemia hirta*, *Phytolacca rivinoides*, and *Yucca elephantipes*.

Clidemia hirta is native to Central America and saponins have been found in its leaves [13]. In Brazil, it has been used to treat skin lesions, has been found to have

antibacterial properties, and has been used as a soap in an indigenous community [14, 13, 8]. *Phytolacca rivinoides* is native to Central America and has been used to treat syphilis and diabetes [15, 16]. It also has antifungal activity that can help protect against pathogenic fungi [17]. *Phytolacca rivinoides* berries are made up of up to 25% saponins, including triterpene saponins [18]. *Yucca elephantipes* is a common plant in Costa Rica and has been used for its anti-inflammatory, antifungal, and antioxidant activity, as well as soap [19, 20]. Steroid saponins are in the stems and leaves [21, 22]. A fourth plant, PF, which is not named in this study due to privacy rights, is also known to contain saponins.

Due to the presence of saponins and their historical uses, these plants have promise for being effective alternatives to commercialized soaps. There is, however, a lack of research on their effectiveness at removing microorganisms from hands. This research is needed due to the necessity of access to an effective way of hygiene in rural or indigenous communities [5].

This study aims to test the effectiveness of these plants in a controlled manner and determine if any of the plants are more effective at removing bacteria. It was predicted that due to the presence of saponins and the historical uses of these four plants, they would be able to remove microorganisms comparably to commercialized soap.

Materials and Methods

This controlled study was conducted at Las Cruces Biological Station in San Vito, Costa Rica from November 13th to 19th, 2015. The researchers conducting the experiment acted as the test subjects. We performed six treatments 15 times each (tap water, Protex© commercial soap, *Phytolacca rivinoides*, *Yucca elephantipes*, PF, and *Clidemia hirta*).

Specimen Collection

All plants were harvested at the beginning of the research week and stored in water until processed for experimentation. *Phytolacca rivinoides*, *Yucca elephantipes*, and *Clidemia hirta* samples were collected from the roadsides surrounding the Las Cruces Biological Station. The *Phytolacca rivinoides* stem containing berries, along with the leafy section of *Yucca elephantipes* and *Clidemia hirta*, were harvested. The trunk of the PF was gathered from an undisclosed location in Costa Rica. Voucher specimens of *Phytolacca rivinoides*, *Yucca elephantipes*, and *Clidemia hirta* are kept for record at the Las Cruces Research Station herbarium.

Specimen Processing

A fresh ration of plants were processed each day before data collection. The *Phytolacca rivinoides* berries and *Clidemia hirta* leaves were removed from the stem and used whole, while the *Yucca elephantipes* leaves were shredded into eighth-inch by four-inch strips. The trunk of PF was divided into four-inch segments and then broken down into thinner strips. For each hand washing trial, approximately 45 *Phytolacca rivinoides* berries, six *Clidemia hirta* leaves, 15 *Yucca elephantipes* eighth inch by four-inch leaf strips, and one four-inch by one-inch strip of PF was used (Appendix 1).

Bacterial Collection and Hand Washing Methodology

A soil solution was composed of six cups of soil and ten cups of Las Cruces Biological Station tap water. A fresh solution was made each day of data collection. The soil for the solution was collected from the residential area northeast of the Las Cruces Laboratory. In order to make the initial amount of bacteria on subjects' hands as similar as possible, the subjects first dipped their hands into one gallon of soil solution for 10 seconds. The subjects were then instructed to rinse and dry their hands thoroughly using a paper towel. However, the exact composition of the soil solution was not analyzed and likely differed somewhat between samples and therefore there is expected to be some variance in initial bacteria.

We swabbed the right hand of the subjects before washing, using a sterile cotton swab moistened with saline solution. The inoculated swab was then placed in an Eppendorf tube containing 1 ml of sterile saline. The subjects then wet their hands, using tap water from the Las Cruces Biological Station, and were given one of the six treatments. The type of treatment was determined by a random number generator [23]. Each subject followed the standard handwashing procedure over the course of 20 seconds, after 10 seconds of lathering with the processed plant material (Appendix 1) [24]. The subjects then rinsed their hands and used paper towels to dry them thoroughly. The subjects' right hands were then swabbed again for remaining bacteria using the same method as previously described. Two samples of each of the plants and controls were also swabbed and plated.

Bacterial Cultivation

We instilled the bacteria for at least 30 minutes prior to dilutions. Blood agar plates were spot plated with five 10

μL spots in each quadrant**;** each quadrant was a different dilution (10^2-10^5) of the initial inoculated sample. The plates were then incubated at 37°C for 24 hours [25]. After the incubation period, the colony-forming units (CFU) were counted, recorded, and converted to CFU/ml.

Statistical Analysis

Dilutions of 10^2 and 10^3 bacteria were counted for both before washing and after washing samples. All statistical testing used the CFU/ml calculated from the 10^3 dilution bacterial counts, as these counts were consistently within the 3-30 range of countable colonies for spot plating. All tests were also done at an alpha level of 0.05. Trials were considered outliers and not included in statistical analysis if the Delta CFU/ml, or CFU/ml Before Washing - CFU/ml After Washing, was 1.5 times the interquartile range greater than the third quartile or less than the first quartile.

In order to determine if a particular treatment was successful in reducing the bacterial load of the test subjects' hands, we ran a paired t-test comparing the CFU/ml Before Washing and the CFU/ml After Washing for each trial within each treatment. We then performed a one-way analysis of variance (ANOVA) to determine if there was a significant difference across the treatment groups in the mean Delta CFU/ml. Three additional two-way ANOVA tests were also run to examine the individual and interaction effects of the treatment, date, and subject identity on the Delta CFU/ml. We used a combination of Microsoft Excel 2011 and Minitab Express 1.3.0 to visualize the data and execute statistical tests.

Ethical Considerations

The OTS Tropical Disease, Environmental Change, and Human Health 2015 program holds a plant-collecting permit from the Costa Rican Government Conservation Areas System (SINAC) SINAC-SE-GCUS-PI-R-107-2015, valid until July 7th, 2016. The four plants collected are not endangered. PF is not named in this study due to privacy rights.

Results

A total of 15 samples for each treatment were included in the study. Of those 15, 1 for *Clidemia hirta*, 2 for *Yucca elephantipes*, 4 for Protex© soap, 2 for tap water, 1 for *Phytolacca rivinoides*, and 0 for PF were considered outliers and not included in the final analysis. Tap water was plated alone and no bacterial growth was observed, confirming that no new bacteria was introduced from the water being used to wash hands.

PF had a before mean CFU/ml of approximately 2700 and a after mean of 2330 CFU/ml with a Delta decrease of 370 CFU/ml. PR had a before mean CFU/ml of approximately 1900 and a after mean of 1633 CFU/ml with a Delta decrease of 267 CFU/ml. CH had a before mean CFU/ml of approximately 2633 and a after mean of 1667 CFU/ml with a Delta decrease of 966 CFU/ml. YE had a before mean CFU/ml of approximately 1467 and a after mean of 740 CFU/ml with a Delta decrease of 724 CFU/ml. Protex had a before mean CFU/ml of approximately 1100 and a after mean of 640 CFU/ml with a Delta decrease of 460 CFU/ml. Water had a before mean CFU/ml of approximately 1766 and a after mean of 640 CFU/ml with a Delta decrease of 1126 CFU/ml.

Figure 1 shows the effectiveness of each treatment at reducing CFU on hands. Treatments of *Clidemia hirta* (n = 14, t = 2.51, P = 0.02), *Yucca elephantipes* (n = 13, t = 2.65, P = 0.02), Protex© soap (n = 11, t = 3.07, P = 0.01), and Tap Water (n = 13, t = 3.99, P = 0.02), showed a significant decrease in the CFU/ml on hands comparing before to after use (Figure 1). Treatments of *Phytolacca rivinoides* (n = 14, t = 0.82, P = .14) and PF (n = 15, t = -0.54, P = 0.599) did not yield significant Delta CFU/ml, there was no significant change in the number of CFU/ml before to after hand washing (Figure 1).

While the Delta CFU/ml was significant in the aforementioned treatments, no one treatment proved to be significantly different from any other in decreasing bacteria. ANOVA: (F = 1.37, P = 0.244), PF (n = 15), *Phytolacca rivinoides* (n = 14), *Clidemia hirta* (n = 14), *Yucca elephantipes* (n = 13), Protex© soap (n = 11), and Tap Water (n = 13) (Figure 2).

The subject, date plated, and treatment or any interaction of these variables did not affect the Delta CFU/ml (Figure 2).

Observationally, there were different types of bacteria present before and after handwashing across treatments. In addition, there appeared to be fungal growth less often in samples after hand washing, though this data was not recorded and not shown. Within all samples, the CFU/ml calculated from the 10^3 dilutions was significantly greater than the CFU/ml of the 10^2 dilutions (results not shown). Fungus did not have a significant effect on CFU/ml, results not shown. There was much less fungus present in 10^3 dilutions and therefore it was hypothesized that fungal presence may have affected the number of CFU/ml; this proved to not be the case.

Discussion and Greater Significance

This study had two aims: to determine which treatments significantly decreased the number of bacteria on hands and to determine which of the treatments was most powerful at reducing bacterial levels. We found that four (*Clidemia hirta, Yucca elephantipes,* Protex©, and tap water) of our six treatments successfully decreased the number of colony-forming units on hands. All proved equally effective, however, contrary to our expectations. Based on previous studies, we expected that the negative control (water) and the positive control (Protex©) would differ in their ability to remove bacteria from hands [2]. For instance, work conducted in Tanzania showed Protex© soap to be one of the leading antibacterial soaps for removing bacteria from hands [26]. Our data does not support this finding. One possible explanation for our results may be the short contact time employed in our experimental protocol. Contact time with each of the treatments in our study was limited to 30 seconds while washing with Protex© occurred for 2 minutes in said Tanzanian study [26]. The duration of contact time is important for the effectiveness of soap. In fact, an increase from 15 to 30 seconds of wash time with antibacterial soap can greatly reduce the number of bacteria removed [27]. A future study that employs a longer contact time with the treatment should be conducted. Considering that in this study the positive control (Protex©) did not perform as expected, changing this variable of contact time could help lead to optimization of the treatments that were proven to work (*Clidemia hirta, Yucca elephantipes,* Protex©, and tap water).

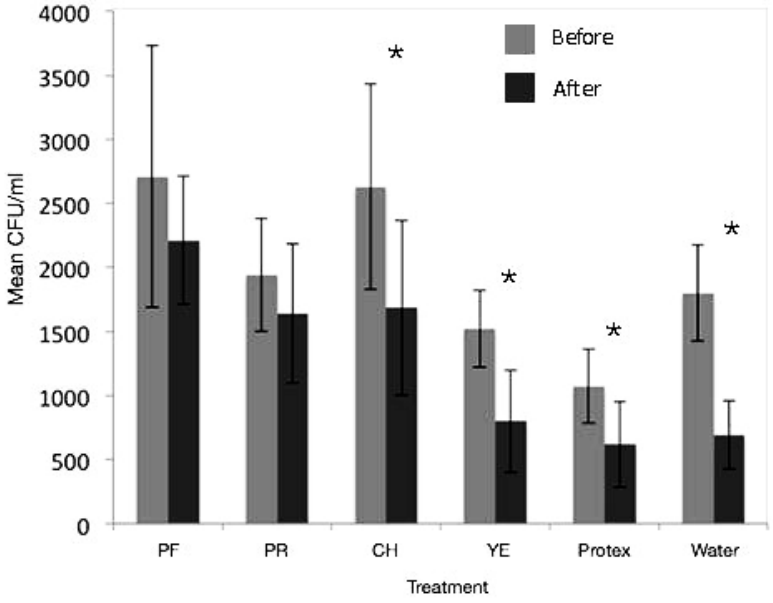
Another important consideration is the type of bacteria present on study subjects' hands before and after hand washing. Although not recorded in this study, we observed what appeared to be different types of bacteria forming colonies before and after hand washing and between treatments. It is possible that certain treatments (e.g. Protex©) remove more pathogenic organisms, including viruses, whereas washing hands with clean water alone may simply remove less harmful bacteria [28]. The same may be true for the mechanical action of washing hands with plants. *Clidemia hirta* and *Yucca elephantipes* decreased colony-forming units on study subjects' hands. Without identifying the bacterial types, however, we cannot be sure that the organisms removed were in fact pathogenic. It is possible that while both tap water and Protex© are removing equal amounts of bacteria, Protex© is removing the more harmful bacteria that tap water is leaving behind, and this could be the reason for the unexpected results of equal effectiveness of tap water and Protex© in removing hand bacteria. While the lack of a difference in effectiveness is surprising, it is not surprising that tap water also significantly reduced bacterial growth. A study conducted in 2010 explored the different particles left on hands after different types of hand washing, both antibacterial soap and only tap water had a large impact when combined with rubbing of the hands together; however, tap water was less effective at removing certain viruses found in stool [28]. Identifying what type of organisms are being removed could be an important next step. While high-tech diagnostic testing is not feasible in this research setting, simply recording the type of bacteria based on observational morphology or diagnostics such as Gram staining to help identify the bacteria would be significant sequential alternatives. This should also be done to record fungal presence and possibly the type of fungus.

It is possible that all of the results we have found are contingent on the fact that only clean water was used during hand washing. We are confident that the tap water used for this study did not introduce new bacteria; water alone was plated, and no bacterial colonies or fungus grew. Results might be different with a larger difference in effectiveness of treatments if dirtier water was used. For example, with different water, Protex© soap and plants may prove to be more effective than just water alone [29]. In addition, unsanitary water is connected with an increase of diarrheal disease, and a decrease in well-being and health [29, 30]. This is especially important due to the target audience of this research: people living in rural settings and indigenous communities who have limited access to hygiene products.

In conclusion, preliminary results suggest that using any of the treatments - *Clidemia hirta*, *Yucca elephantipes*, PF, Protex© soap, and tap water - is equally helpful in removing bacteria when clean water is used during hand washing. Previous studies have shown the importance of clean water, above all other types of interventions, at decreasing diarrheal diseases and therefore more emphasis should be put on finding a way to provide people with access to clean water [30].

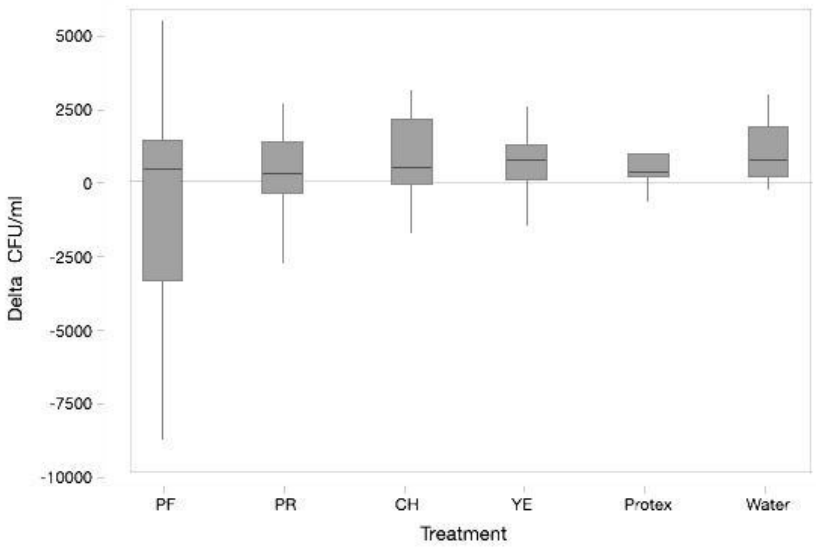
This research has the potential to make a difference in the health of communities with limited access to hygiene products. *Clidemia hirta* and *Yucca elephantipes* show promise and should be followed up with future studies. A larger, more in-depth study that focuses on these treatments, increases contact time with each treatment, investigates the type of organisms present on hands before and after washing with each treatment, and investigates the

effect of each plant when non-clean water is used to wash hands would make this research more applicable to the targeted communities.



PF = PF
PR = *Phytolacca rivinoides*
CH = *Clidemia hirta*
YE = *Yucca elephantipes*

Figure 1: *Effectiveness of each treatment at reducing the number of bacteria on hands. * Denotes a significant difference in mean Delta CFU/ml. Error bars represent one standard error from the mean. A paired t-test was performed comparing the mean CFU/ml Before Washing and the CFU/ml After. PF (n = 15, t = -0.54, P = 0.599) Phytolacca rivinoides (n = 14 t = 0.82 P = .14) Clidemia hirta (n = 14 t = 2.51 P = 0.02), Yucca elephantipes (n = 13 t = 2.65 P = 0.02), Protex soap (n = 11 t = 3.07 P = 0.01), and Tap Water (n = 13 t = 3.99 P = 0.02).*



PF = PF
PR = *Phytolacca rivinoides*
CH = *Clidemia hirta*
YE = *Yucca elephantipes*

Figure 2: * *Denotes a significant difference from other treatment groups. A one-way analysis of variance ANOVA was run to determine if there is a significant difference across the treatment groups in the mean Delta CFU/ml. ANOVA: (F = 1.37, P = 0.244), PF (n = 15), Phytolacca rivinoides (n = 14), Clidemia hirta (n = 14), Yucca elephantipes (n = 13), Protex soap (n = 11), and Tap Water (n = 13).*

Table 1: Two-way ANOVA results. A p-value of 0.05 was considered significant. There were no significant differences based on the subject, date plated, and treatment or any interaction of these variables.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	5	24865724	4973145	1.42	0.2294
Subject	2	8930284	4465142	1.28	0.2865
Treatment*Subject	10	26924811	2692481	0.77	0.6575
Error	62	217046667	3500753		
Total	79	278532969			

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	5	22305643	4461129	1.15	0.3489
Date Plated	4	13780472	3445118	0.88	0.4799
Treatment*Date Plated	20	42813210	2140661	0.55	0.9278
Error	50	194681667	3893633		
Total	79	278532969			

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Subject	2	15035514	7517757	2.35	0.1030
Date Plated	4	27448103	6862026	2.15	0.0847
Subject*Date Plated	8	41756324	5219541	1.63	0.1322
Error	65	207530083	3192771		
Total	79	278532969			

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References

[1] Mathur, P. (2011). Hand hygiene: Back to the basics of infection control. *The Indian Journal of Medical Research*, *134*(5), 611–620. https://doi.org/10.4103/0971-5916.90985

[2] Burton, M., Cobb, E., Donachie, P., Judah, G., Curtis, V., & Schmidt, W.-P. (2011). The Effect of Handwashing with Water or Soap on Bacterial Contamination of Hands. *International Journal of Environmental Research and Public Health*, *8*(1), 97–104. https://doi.org/10.3390/ijerph8010097

[3] *Global Diarrhea Burden | Global Water, Sanitation and Hygiene | Healthy Water | CDC*. (2018, November 9). https://www.cdc.gov/healthywater/global/diarrhea-burden.html

[4] Aiello, A. E., Coulborn, R. M., Perez, V., & Larson, E. L. (2008). Effect of hand hygiene on infectious disease risk in the community setting: A meta-analysis. *American Journal of Public Health*, *98*(8), 1372–1381. https://doi.org/10.2105/AJPH.2007.124610

[5] Ortiz P. *Sistematización buenas prácticas Ngobe.pdf*. (n.d.-b). Retrieved February 4, 2020, from http://www.aecid.es/Centro-Documentacion/Documentos/Publicaciones%20coeditadas%20por%20AECID/Sistematizaci%C3%B3n%20buenas%20pr%C3%A1cticas%20Ngobe.pdf

[6] Jenkins, M. W., Anand, A. R., Revell, G., & Sobsey, M. D. (2013). Opportunities to improve domestic hygiene practices through new enabling products: A study of handwashing practices and equipment in rural Cambodia. *International Health*, *5*(4), 295–301. https://doi.org/10.1093/inthealth/iht026

[7] Hoque, B. (2003). Handwashing practices and challenges in Bangladesh. *International Journal of Environmental Health Research*, *13*(sup1), S81–S87. https://doi.org/10.1080/0960312031000102831

[8] Hector Castaneda. (2015, October 1). *Soap usage in San Vito Costa Rica* [Personal communication].

[9] *Institute for Chemical Technology of Organic Materials*. (n.d.). Retrieved February 4, 2020, from https://www.jku.at/en/institute-for-chemical-technology-of-organic-materials/

[10] Francis, G., Kerem, Z., Makkar, H. P. S., & Becker, K. (2002). The biological action of saponins in animal systems: A review. *The British Journal of Nutrition*, *88*(6), 587–605. https://doi.org/10.1079/BJN2002725

[11] Vincken, J.-P., Heng, L., de Groot, A., & Gruppen, H. (2007). Saponins, classification and occurrence in the plant kingdom. *Phytochemistry*, *68*(3), 275–297. https://doi.org/10.1016/j.phytochem.2006.10.008

[12] Tsuzuki, J. K., Svidzinski, T. I. E., Shinobu, C. S., Silva, L. F. A., Rodrigues-Filho, E., Cortez, D. A. G., & Ferreira, I. C. P. (2007). Antifungal activity of the extracts and saponins from Sapindus saponaria L. *Anais Da Academia Brasileira De Ciencias*, *79*(4), 577–583. https://doi.org/10.1590/s0001-37652007000400002

[13] Musa, N., Musa, N., Ibrahim, W. N., Shariat, M. Z. A., Zamani, A. I., Abdullah, M. R., Wee, T. L., Marip, M., Razak, L. A., & Soh, A. S. A. (2011). Methanolic Activities of Selected Weeds on Bacteria Isolated from Macrobrachium rosenbergii Larvae. *The Thai Journal of Veterinary Medicine*, *41*(4), 535–540.

[14] França, F., Lago, E. L., & Marsden, P. D. (1996). Plants used in the treatment of leishmanial ulcers due to Leishmania (Viannia) braziliensis in an endemic area of Bahia, Brazil. *Revista Da Sociedade Brasileira De Medicina Tropical*, *29*(3), 229–232. https://doi.org/10.1590/s0037-86821996000300002

[15] *FDA Poisonous Plant Database*. (n.d.). Retrieved February 4, 2020, from https://www.cfsanappsexternal.fda.gov/scripts/plantox/detail.cfm?id=770

[16] *Phytolacca rivinoides Kunth & C.D.Bouché | Plants of the World Online | Kew Science*. (n.d.). Plants of the World Online. Retrieved February 4, 2020, from http://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:676397-1

[17] Escalante, A. M., Santecchia, C. B., López, S. N., Gattuso, M. A., Gutiérrez Ravelo, A., Delle Monache, F., Gonzalez Sierra, M., & Zacchino, S. A. (2002). Isolation of antifungal saponins from Phytolacca tetramera, an Argentinean species in critic risk. *Journal of Ethnopharmacology*, *82*(1), 29–34. https://doi.org/10.1016/s0378-8741(02)00145-9

[18] Ravikiran, G., Raju, A. B. & Venugopal Y... *Phytolacca americana: A Review - International Journal of Research ...* Yumpu.Com. Retrieved February 4, 2020, from https://www.yumpu.com/en/document/read/34979276/phytolacca-americana-a-review-international-journal-of-research-

[19] *Price N. Short Course Agroforestry For The Humid Tropics Turrialba Costa Rica March 16-25 1982 Contributions Of Participants*. (n.d.). Bib. Orton IICA / CATIE.

[20] Patel, S. (2012). Yucca: A medicinally significant genus with manifold therapeutic attributes. *Natural Products and Bioprospecting*, *2*(6), 231–234. https://doi.org/10.1007/s13659-012-0090-4

[21] Zhang, Y., Zhang, Y.-J., Jacob, M. R., Li, X.-C., & Yang, C.-R. (2008). Steroidal saponins from the stem of Yucca elephantipes. *Phytochemistry*, *69*(1), 264–270. https://doi.org/10.1016/j.phytochem.2007.06.015

[22] Zhang, Y., Yang, C.-R., & Zhang, Y.-J. (2013). New Steroidal Saponins from the Leaves of Yucca elephantipes. *Helvetica Chimica Acta*, *96*(9), 1807–1813. https://doi.org/10.1002/hlca.201200586

[23] *RANDOM.ORG - Sequence Generator*. (n.d.). Retrieved February 4, 2020, from https://www.random.org/sequences/?min=1&max=90&col=3&format=html&rnd=new

[24] *When and How to Wash Your Hands | Handwashing | CDC*. (2019, December 4). https://www.cdc.gov/handwashing/when-how-handwashing.html

[25] *Schmitt Infection Exposure Questions*. (n.d.). Retrieved February 4, 2020, from https://www.stlouischildrens.org/health-resources/symptom-checker/infection-exposure-questions

[26] Mwambete, K. D., & Lyombe, F. (2011). Antimicrobial Activity of Medicated Soaps Commonly Used By Dar es Salaam Residents in Tanzania. *Indian Journal of Pharmaceutical Sciences*, *73*(1), 92–98. https://doi.org/10.4103/0250-474X.89765

[27] Fuls, J. L., Rodgers, N. D., Fischler, G. E., Howard, J. M., Patel, M., Weidner, P. L., & Duran, M. H. (2008). Alternative hand contamination technique to compare the activities of antimicrobial and nonantimicrobial soaps under different test conditions. *Applied and Environmental Microbiology*, *74*(12), 3739–3744. https://doi.org/10.1128/AEM.02405-07

[28] Liu, P., Yuen, Y., Hsiao, H.-M., Jaykus, L.-A., & Moe, C. (2010). Effectiveness of liquid soap and hand sanitizer against Norwalk virus on contaminated hands. *Applied and Environmental Microbiology*, *76*(2), 394–399. https://doi.org/10.1128/AEM.01729-09

[29] Luby, S. P., Agboatwalla, M., Painter, J., Altaf, A., Billhimer, W., Keswick, B., & Hoekstra, R. M. (2006). Combining drinking water treatment and hand washing for diarrhoea prevention, a cluster randomised controlled trial. *Tropical Medicine & International Health: TM & IH*, *11*(4), 479–489. https://doi.org/10.1111/j.1365-3156.2006.01592.x

[30] Fewtrell, L., Kaufmann, R. B., Kay, D., Enanoria, W., Haller, L., & Colford, J. M. (2005). Water, sanitation, and hygiene interventions to reduce diarrhoea in less developed countries: A systematic review and meta-analysis. *The Lancet. Infectious Diseases*, *5*(1), 42–52. https://doi.org/10.1016/S1473-3099(04)01253-8