HIGH SCHOOL EDITION
Journal of Student Research
Volume 13 Issue 3 (2024)

The Effect of Ayurvedic Medicines On E. coli

Yagnesh Lokesh

Waubonsie Valley High School

ABSTRACT

The issue of inadequate sanitation and water scarcity affects millions globally, particularly in developing regions. This research aims to address these challenges by developing a solution that can kill bacteria and reduce water usage for hygiene. The solution leverages natural ingredients such as honey, lemon juice, neem powder, aloe vera, and turmeric, known for their antibacterial properties. These ingredients are mixed in specific ratios to create an effective, cost-efficient alternative to commercial hand sanitizers. The study hypothesizes that these natural mixtures will prevent E. coli growth, offering a sustainable hygiene solution accessible to impoverished areas. Experimental results indicate varying degrees of antibacterial effectiveness among the mixtures, with some showing promise in maintaining antibacterial properties over time. The findings suggest that further optimization and testing are required to enhance the solution's consistency and efficacy, contributing to global efforts in improving hygiene and public health.

Introduction

Water scarcity and inadequate hygiene are significant global issues affecting millions of people, particularly in developing regions. According to the World Health Organization and UNICEF, approximately 1.8 billion people globally lack access to clean water and proper sanitation, with a notable percentage residing in Sub-Saharan Africa and South Asia (World Health Organization & UNICEF, 2021). This lack of resources leads to severe health problems, including the spread of diarrheal diseases, which are a leading cause of death among children under five (CDC, 2021). These alarming statistics underscore the urgent need for innovative and sustainable solutions to address the dual challenges of water scarcity and inadequate hygiene.

In regions where water is scarce, traditional hygiene practices that rely heavily on water, such as handwashing, become impractical. This exacerbates the spread of infectious diseases, which thrive in environments with poor sanitation. To mitigate these issues, there is a pressing need for hygiene solutions that require minimal water usage and are accessible to economically disadvantaged populations. The development of effective, low-cost antibacterial solutions using natural ingredients offers a promising approach to improving hygiene standards in these regions.

This research aims to develop an antibacterial solution using natural ingredients known for their antimicrobial properties. Specifically, we focus on honey, lemon juice, neem powder, aloe vera, and turmeric. These ingredients have been used traditionally in various cultures for their health benefits and are widely available in many regions affected by water scarcity. By creating a cost-effective and accessible alternative to commercial hand sanitizers, this research aims to improve hygiene in impoverished areas, thereby reducing the spread of diseases and enhancing public health.

Honey has been recognized for its antibacterial properties for centuries, largely due to its high viscosity, low pH, and the presence of hydrogen peroxide (Mandal & Mandal, 2011). Lemon juice, rich in citric acid, creates a hostile environment for bacteria (Khan & Khatoon, 2008). Neem powder is renowned for its antimicrobial properties and has been used in traditional medicine to treat various infections (Biswas et al., 2002). Aloe vera, known for its soothing and healing properties, also exhibits antibacterial activity (Reynolds &

Dweck, 1999). Turmeric contains curcumin, a compound with well-documented antimicrobial effects (Aggarwal et al., 2007).

This study hypothesizes that mixtures of honey with lemon juice, neem powder, aloe vera, or turmeric will effectively prevent the growth of E. coli. The ingredients were selected based on extensive research into their antibacterial properties and their accessibility in various regions. The mixtures were prepared in specific ratios and tested for their antibacterial efficacy, with the goal of developing a sustainable hygiene solution. Experimental results indicate varying degrees of antibacterial effectiveness among the mixtures, with some showing promise in maintaining antibacterial properties over time.

The broader implications of this research extend beyond immediate health benefits. By providing an affordable and effective hygiene solution, this project can contribute to improving the overall quality of life in developing regions. It supports educational and economic activities by reducing illness-related absenteeism and promotes public health initiatives. Moreover, the use of natural and locally available ingredients ensures that the solution is sustainable and culturally acceptable, fostering community engagement and long-term adoption.

Review of Literature

The issue of lack of hygiene throughout the world is a pertinent epidemic affecting millions of people globally. According to Global WASH Fast Facts, throughout the world, over a quarter of the global population (1.8 billion people) are in a dire need of adequate sanitation and 790 million people are without access to a proper improved water supply. The areas where the hygiene issue is greatest are in Sub-Saharan Africa contributing 31%, Southern Asia contributing 33%, and Eastern Asia contributing 65%. Almost 70% of the 1.8 billion people living in places where sanitation is inaccessible were rural inhabitants (*Global WASH Fast Facts | Global Water, Sanitation and Hygiene | Healthy Water | CDC*, 2021).

Additionally, approximately 801,000 children younger than the age of 5 die from diarrhea due to bacteria and inadequate sanitation. This number amounts to 11% of the 7.6 million children under the age of 5 dying meaning 2,200 children are dying every day because of diarrheal diseases. 88% percent of people who die of diarrheal diseases are caused by unsafe drinking water, inadequate availability of hygiene, and lack of access to sanitation. It has been proven that washing hands with soap and water can prevent almost 50 percent of diarrheal infections, hence decreasing the number of overall deaths caused by this disease. Globally, millions of people are infected with neglected tropical diseases (NTDs) which are caused by inadequate sanitation and hygiene. One of the leading and preventable causes of blindness, Trachoma, results from poor hygiene practices that affects almost 41 million people. This disease could be prevented by simply handwashing and purchasing alcohol-based soaps. A large percentage of foodborne illness could have been prevented by proper hand hygiene which could stop the spread of disease among people.

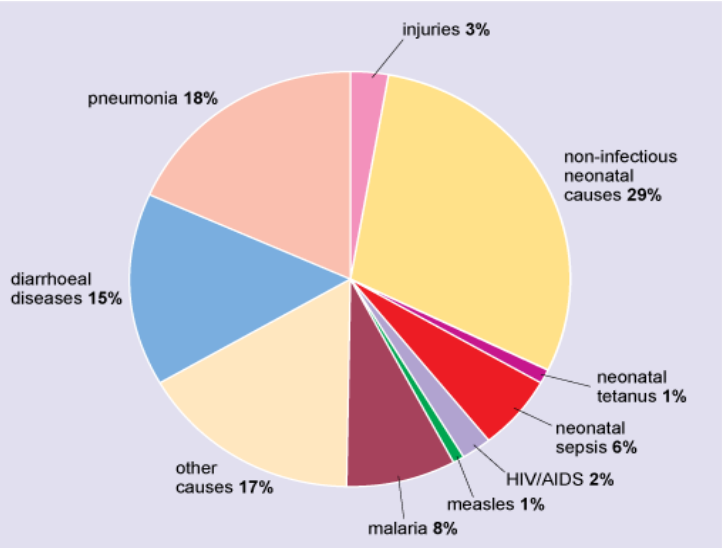


Figure 1. The image above describes the causes of death for children under the age of 5. This image gives perspective on the gravity of diarrheal infections globally. (*Science NetLinks | American Association for the Advancement of Science*, n.d.)

According to healthline.com, although more than half of the global population have access to soap and water, it has been estimated that over 90% of the population wash their hands incorrectly. The CDC suggests that the proper way of washing hands includes lathering your hands with soap and rinsing for 20 seconds. According to Forbes, only 3% of the population were proven to wash their hands for 20 seconds while others had either washed their hands for a significantly less amount of time or did not wash their hands using proper hygiene practices.

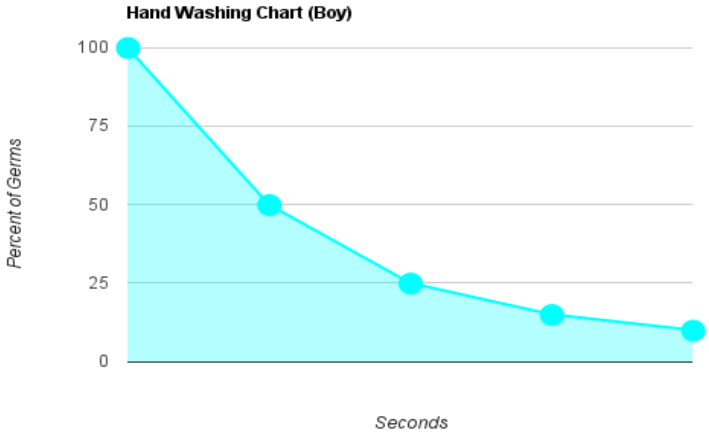


Figure 2. This image describes the amount of germs overtime while washing hands. The graph implies that overtime the number of germs will be washed off as time passes (*9 Dirtiest Places in Your Home*, 2020).

Additionally, those who washed their hands properly and followed CDC guidelines proceeded to dry their hands with used towels or their clothing which contain bacteria and germs. Another study like the one Forbes had conducted showed similar results that were found above. It concluded that only 5% of people properly washed their hands to eliminate all germs, and only 2 in three people use soap when washing their

hands. YouGov claims that almost 40% of those who have the accessibility to wash their hands don't wash their hands after going to the bathroom, and a Healthline article stated that some of the most common areas where bacteria sit in are on bathroom light switches. Those who don't wash their hands after going to the bathroom have the possibility of spreading disease onto food and to others.

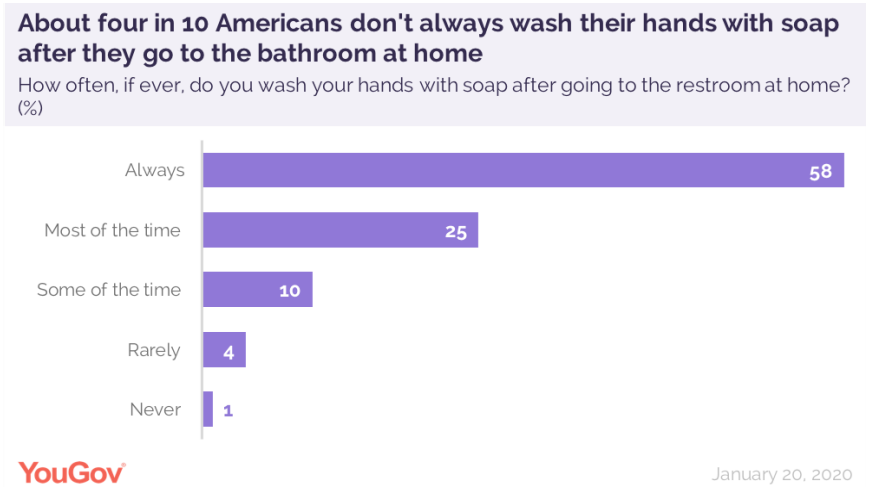


Figure 3. This graph has results from a survey asking Americans how often they wash their hands with soap and water in their homes. This graph indicates that less than 50% of Americans do not always wash their hands in their home (*Many Americans Don't Always Wash Their Hands after Going to the Bathroom | YouGov*, n.d.)

Also, approximately a quarter of Americans don't wash their hands after using the kitchen where Staphylococcus and E. Coli reside. A study proved that more than 75% of kitchen rags and dish sponges contained Salmonella, E. Coli, and fecal matter. Compared to the 9% percent of Salmonella, E. coli and fecal matter, washing hands after using the kitchen will significantly decrease the spread of diseases. The places where bacteria reside are refrigerator handles, stove knobs, and microwave handles. In the bathroom, the moisture from a hot shower is the ideal place for bacteria to grow in. Spots where bacteria growth is the greatest in the bathroom is shower tubs, drains, faucets, floor area around the toilet, bath towels, and toothbrushes. For these reasons it is heavily suggested to air dry your hands rather than towels to prevent the spread of microbes (*Show Me the Science - How to Wash Your Hands | Handwashing | CDC*, n.d.)

The age group that washes their hands the least in their homes are eighteen- to twenty-four-year-olds which, by a survey, suggests that less than half (48%) of the age group washed their hands with soap after going to the bathroom. A quarter of all people in this age group say they wash their hands most of the time while 13% say that they wash their hands sometimes after using the bathroom. Although kids don't wash their hands very often, adults also have overlying issues related to good hygiene practices in the bathroom. About one in seven US adults (14%) say they always use their phones while they are going to the bathroom. In fact, phones are 10 times more germ infested compared to a toilet seat. Another 18% say they use their phones most of the time when they go to the bathroom and 23 percent do so sometimes

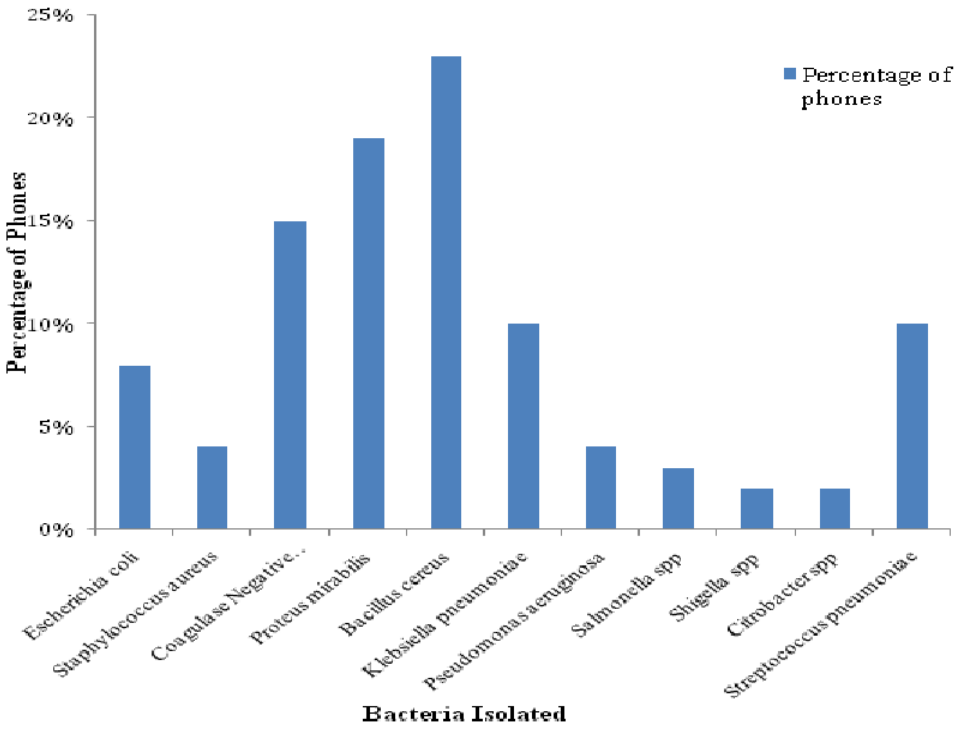


Figure 4. This bar graph shows the percentage of phones that have specific species of bacteria. The bar graph shows Bacillus Cereus is the most common bacteria on phones.
(Potential Sanitation Solutions During an Emergency Response | Global Water, Sanitation and Hygiene | Healthy Water | CDC, 2021)**.**

Although the spread of disease is a constantly increasing problem in the world, water scarcity is another pertinent problem to solve while creating an antibacterial solution. According to Bloomberg.com, it has been proven that Africa is affected the most by water scarcity. According to UNICEF, Eritrea is a country in Africa where 70% of the population doesn't have access to a clean water supply. Almost all the water in the country is either contaminated or has dried up due to droughts. Those in middle eastern countries face similar issues. Gaza is a small area within Palestine where two-thirds of the population don't have access to safe drinking water. Most of the two million people, half of which are under the age of 18, face challenges to access drinking water. According to WASH, although health care facilities expect to have an adequate water supply, globally, 50% lack access to a proper water source. According to usaid.gov, across the world, children must walk an average of 6 kilometers a day while carrying 20 kilograms of water. Some children have to walk 15 hours a week to get access to a water supply. For many families, it is unknown how purified the water is but for almost 50% of families their water supply is contaminated with some type of bacteria or fecal matter.

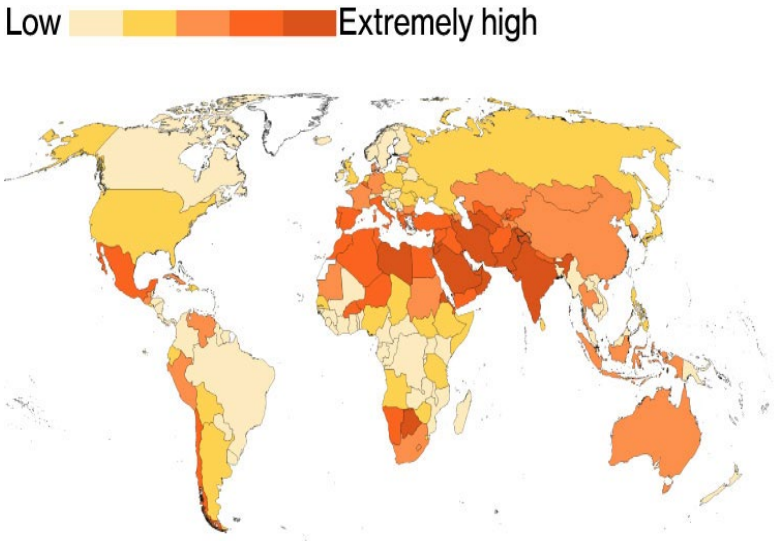


Figure 5. This diagram describes the water scarcity issue in continents and countries. As seen in the diagram, places in Northern Africa, India, and Saudi Arabia, to name a few, are affected the most by this growing issue. (*Water, Sanitation and Hygiene | UNICEF India*, n.d.)

According to medium.com, in Malawi, Africa, the water scarcity issue is becoming a bigger problem daily. In this country, groundwater resources account for 2% of the nationally available water source, but at least 76 percent of the total population and 86 percent of the rural population rely on this water for their day-to-day survival needs. Although Malawi has a freshwater lake, global warming and other droughts have caused the lake to reach an all-time low of water in the past 15 years. This situation is like other countries in the world where their freshwater resource is dried up ("How Water Pollution in Africa Kills Millions," 2020)

Cosmetic Chemist Perry Romanowski described a soap as a "fat of oil, plus an alkali material (most commonly lye, which is made from wood ash), reacted together in a process known as saponification." Another possible classification for soaps is synthetic detergents which are molded together to create syndet bars. According to Slate.com. Dove soap is classified to be a syndet bar and is not environmentally friendly. Although syndet bars are biodegradable, they take an immense amount of time to degrade causing an issue to the environment. Another reason why soaps are harmful to the environment is because the detergents in them break down the surface tension of the water which decreases the oxygen content for small creatures such as fish to use to breathe. Additionally, Dove soaps use surfactants which are also harmful to the environment suffocating small invertebrates. Although their website mentions they use a milder version of surfactants, they are harmful, nonetheless. Research has shown that over 20,000 ounces of water is required for an ounce of soap to be safe for fish to swim in. Mcgill.ca states that soaps in general require heavy amounts of energy to create and to ship, but bar soaps have been proven to require significantly less amount of energy, but still pose a threat to the environment. Liquid soaps have been proven to have a 25% greater carbon footprint than bar soaps.

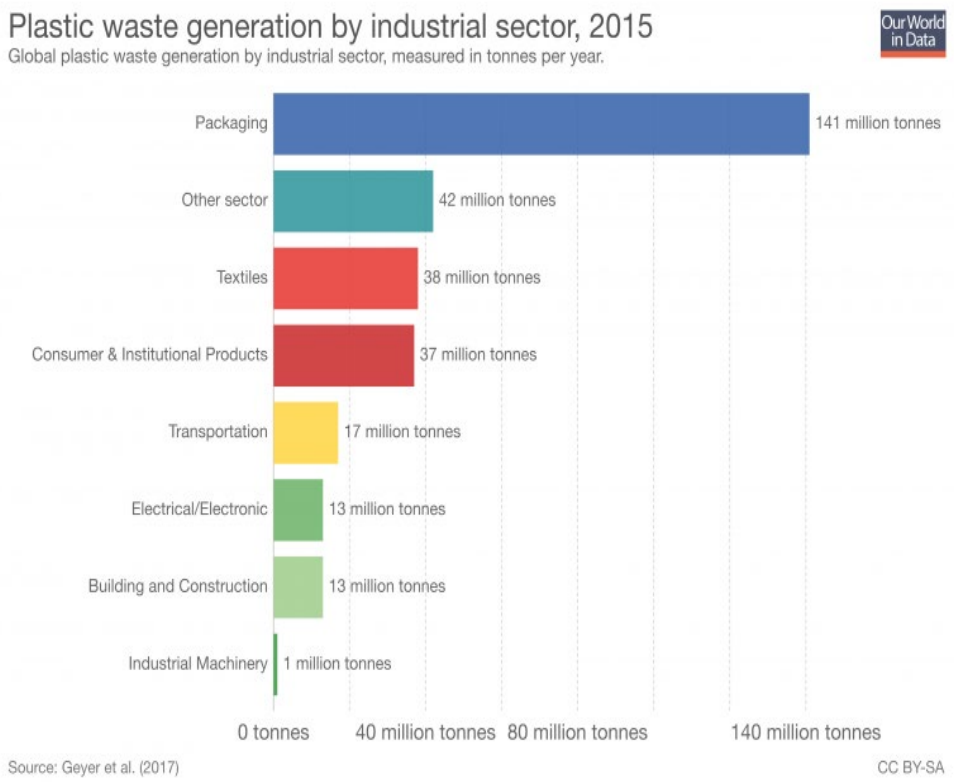


Figure 6. This graph shows the amount of plastic pollution caused by each industrial sector. The chart indicates that more than half the amount of plastic pollution is cause be packaging, which includes the packaging of liquid soaps. (*Liquid or Bar? Soapy Tales | Office for Science and Society - McGill University*, n.d.)

Liquid soap containers are usually made of high density polyethene (HDPE), which requires petroleum or natural gas for mass production. Less than 30% of all HDPE is not recycled creating a big environmental problem. On average, people use 2.3 grams of liquid soaps while people use 0.3 grams of bar soap resulting in higher air pollution and plastic pollution (Soap and the Coronavirus: What You Can Actually Use., n.d.).

To combat this issue, India has utilized ayurvedic medicines to keep themselves clean when access to hygiene is difficult to obtain. According to Johns Hopkins, Ayurveda is a natural system of medicine that originated in India from over 5,000 years ago. There are approximately 600 different types of ayurvedic ingredients and thousands of combinations of ingredients to solve a type of disease. The word ayurveda is derived from the words Ayur(life) and Veda(science or knowledge). It is considered one of the oldest forms of medicine and is the traditional form of medicine in India. Its influence in western countries is slowly growing as almost 240,000 American adults use Ayurvedic medicines daily, with several thousand scholarly papers written about its issues that it combats. A few studies have proven that it may reduce pain and increase function with people suffering from osteoarthritis and manage symptoms in people with Type 2 diabetes. The basis of ayurvedic medicines is that there are doshas, or pillars in every ayurvedic treatment plan: Vatta, Pitta, and Kapha. Many ayurvedic users strive to maintain the balance of the three doshas and believe that the slight imbalance of any dosha will lead to a disease. In order to maintain this balance, ayurvedic users ingest natural ingredients like herbs, plants and mixtures. (*Ayurveda | Johns Hopkins Medicine*, n.d.)

Additionally, many of these ingredients are locally grown in India and mass produced, making it easily accessible in many Indian households and kitchens. Many medicines in India are primarily grown using Ayurvedic medicines and its market is slowly growing in India.

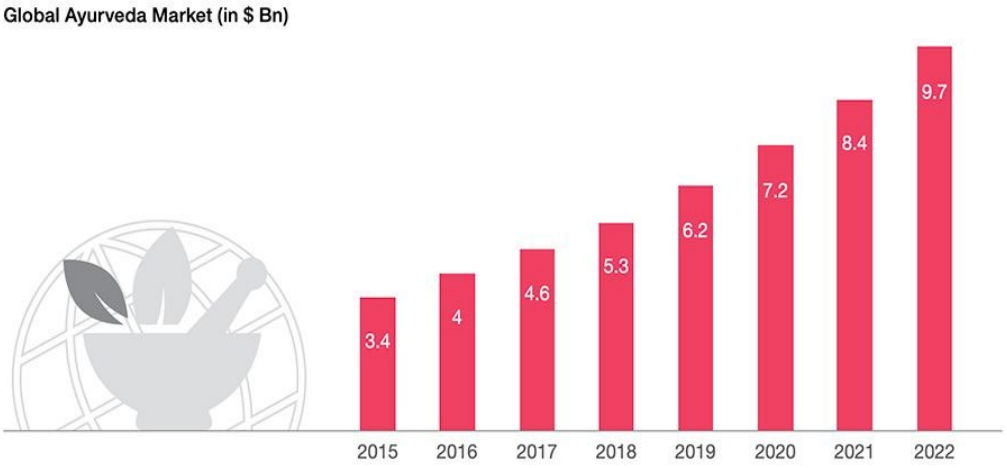


Figure 7. The graph above indicates the growing ayurvedic market globally, showing its growing popularity and worldwide acceptance globally. (*Ayurvedic Medicine Is Regaining Both Relevance and Popularity in India*, 2018)

With approximately 600 different ayurvedic medicines there are a variety to choose from, but there are more common ones than others due some ingredients have more benefits than others. Some of the more common ones include Ashwagandha, turmeric, licorice, cumin, neem and aloe vera. Each of these ingredients have been stated to provide joint and muscle relief, good for weight loss, antioxidants, boost energy levels, and antibacterial and antifungal properties. However, neem, turmeric powder(curcumin), and aloe vera have been noted heavily for its antibacterial properties as its common uses include its placement on top of wounds to prevent bacteria from entering cuts(*Which Are the Most Used Ayurvedic Herbs and Their Benefits?*, n.d.).

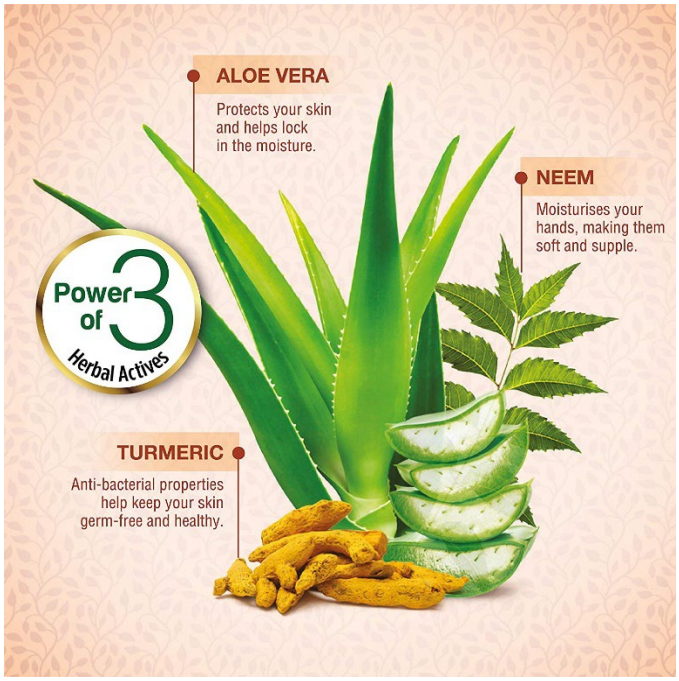


Figure 8. The image above depicts the three more common ayurvedic ingredients and their common uses.

(*Medimix Ayurvedic Hand Wash With Neem Turmeric Aloe Vera, 175 Ml – Govkarts*, n.d.)

Neem is considered one of the most potent ayurvedic medicines in its ability to battle free radicals in the body, flush out toxins, treat bites and cure ulcers. Furthermore, it has powerful antifungal and antibacterial properties, facilitating the treatment of worm infestations, burns, skin disorder and aiding the immune system in its battle against bacteria. Neem powder and other forms of extract have been stated to have strong antimicrobial properties and can prevent infection from insects and bugs as they are the root cause of many health diseases. Neem in its powder or oil form can prevent infectious diseases like malaria, dengue and yellow fever, the more common diseases in places close to the. According to an experiment done to test neem's antifungal properties, it has been found that neem extracts truly have antifungal properties due the chemical constituents of alkaloids, flavoidnis, terpenoids, and tannins, allowing to prevent microbial infections specifically because of its microbial biological activities. For this reason, neem is an active ingredient in many toothpastes and tooth powders. It is commonly used in western medicine for treating gingival problems and alleviation tooth aches(Hamid et al., 2019).

Another experiment done tested neem's antibacterial abilities, and it was concluded that neem has some antibacterial properties. Neem was shown to have antibacterial properties against the pathogen *Vibrio vulnificus* due to its ability to disrupt the cell integrity of the cell membrane. Neem at lower concentration (0.7-1 mg/ml) is found to be nontoxic, however at higher concentrations between (1.2-2mg/mL) it is found to be toxic and is able to disrupt the cell membrane. Neem was also found to show a significant increase in DNA damage when compared to the average human white blood cell cell against *V. vulnificus*. Finally, another study done stated that almost every part of the neem tree has antibacterial properties, mainly the leaf over the stem. Additionally, a water soluble glycolipid, sulfonoquinovosyldiacylglyceride, isolated from the leaves neem have the inhibitory effects against *E. coli , Salmonella,* and *Staphylococcus aureus*, the more common types of bacteria found on hand-held surfaces (Ravva & Korn, 2015). Thus, from the studies done above, it can be proven that neem has strong antibacterial and antifungal and is readily found throughout the world.

Aloe vera is another commonly used ayurvedic medicine commonly used for skin care and skin conditions. Aloe vera gel can only be extracted from the aloe vera plant itself where an incision is necessary. The gel is commonly used to treat burns, improve digestive health, promote oral health, fight bacterial infections, clear acne, and relieve anal fissures. In an experiment done to test aloe vera's effectiveness it was compacted into aloe vera juice and then tested to see if a zone of inhibition would appear. From the experiment, it was found that it has antibacterial, antifungal, and antiviral properties. The juices' antiviral properties have been attributed to the plant's natural anthraquinones. It was also found to be bacteriostatic against *Staphylococcus aureus*, *Streptococcus pyogenes* and also *Salmonella paratyphi,* as seen by the 20-30 mm zone of inhibition formed (Athiban et al., 2012).

The final potent ayurvedic ingredient is turmeric, a commonly used spice in food originally from India. Some common uses of turmeric are used as a treatment for indigestion, colds, cancer, skin conditions, bacterial infections, heart disease, clearing of free radicals. Turmeric is also called curcumin as curcumin is the main ingredient that composes turmeric. Curcumin is the compound that gives turmeric its spice, yellow hue, and anti-microbial properties. Currently scientists have put curcumin to work, creating surfaces primarily out of curcumin.

An experiment was done to prove curcumin's antibacterial against Gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus faecalis)* and Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa),* which represent prominent human pathogens, especially in the hospital setting. Curcumin I, a significant component of commercial curcumin, found that it had strong anti-bacterial properties against both the gram negative and positive bacteria. Through various techniques, it was found that there was a huge membrane leakage in all bacteria on the curcumin I, proving its antibacterial properties against *E.coli (Bactericidal Activity of Curcumin*

I Is Associated with Damaging of Bacterial Membrane, n.d.). Additionally, an experiment performed with scientists found that turmeric and the all-natural manuka honey are one of the strongest combinations of natural ingredients to fight *Staphylococcus aureus* and Infective Endocarditis

Along with ayurvedic medicines other commonly used western ingredients to combat the lack of hygiene is lemon juice and honey. Lemon juice has been proven countless times in western medicine to be effective in the killing of bacteria due to its bioactive compounds such as flavonoid, carotenoids, limonoid, tannin, and terpenoids. Along with these compounds, Vitamin C and citric acid increase the acidity of lemon juice, allowing it to kill bacteria, namely *E.coli.* It has been found to be potent against other strains of *E.coli* and other diarrhea causing bacteria. The zone of inhibition areas were very high, ranging well over 15 mm, indicating that the acidity of the lemon was killing the *E.coli.* It also mentioned the appropriate amount of lemon juice is 900 mg/ml in order to prevent the growth and kill *E.coli* (Ekawati & Darmanto, 2019). Although lemon juice is known to kill types of bacteria, lemon juice can also prevent the growth of bacteria, not necessarily killing it, but stop it from growing further when bacteria come into contact with lemon juice. Also, lemon peels have a similar effect, killing or preventing the growth of various types of bacteria. Similarly to lemon juice's antibacterial properties, honey also has antibacterial effects whether by killing it or preventing its growth. Honey's antibacterial properties stem from its abundance in hydrogen peroxide, which kills bacteria and clean wounds, as well as its ability to starve the bacteria of its food source, high acidity, and its ability to expose the nuclear membrane and DNA of a bacteria, preventing its growth. Honey has been able to kill *E.coli* in various experiments proving its strength in antibacterial properties.

Methods

Materials

1. Filter Paper and Hole Puncher:
    - 225 pieces of 1 cm radius filter paper circles were cut using a hole puncher. Five pieces of filter paper circles were dipped into the solutions and then placed on each agar plate for each trial after the agar plates were swabbed with bacteria.

2. Agar Plates:
    - 20 pre-poured agar plates were used to grow E.coli and test the solution-dipped filter papers.
3. Pipettes:
    - One 100 microliter pipette and one 20 microliter pipette were utilized for applying E.coli on the agar plates and the solution-dipped filter papers onto the plates.

Figure 9. 100 microliter pipette

4. Tips:
    - 33 tips were used; 18 for applying E.coli and 15 for dipping the pipette into the homemade solutions.

Figure 10. Pipette tips.

5. Scrubbers:
    - 18 scrubbers were used to spread E.coli on the agar plates.

6. E.coli HB101:
    - This is the bacteria used for the experiment and scrubbed thoroughly on the agar plates. The individual solutions were tested with this bacteria due to E.coli's frequency on common household items.

Figure 11. The *E.coli* used in this experiment

7. Hand Sanitizer:
    - 5 grams of hand sanitizer was utilized for each trial (3 trials).

8. Natural Ingredients:
    1. Turmeric: 1 gram of neem powder, 4 grams of honey, and 5 mL of water were used.
    2. Aloe Vera Gel: 4 grams of aloe vera gel, 4 grams of honey, and 5 mL of water were used.
    3. Lemon Juice: 4 grams of lemon juice, 4 grams of honey, and 5 mL of water were used.
    4. Neem Leaf Powder: 1 gram of neem powder, 4 grams of honey, and 5 mL of water were used .

Procedure

Safety Precautions:

1. Masks were always worn to prevent COVID-19 spread and contamination of the prototype and testing phase.
2. Gloves were worn and replaced after every solution to prevent contamination.
3. Coats, socks, and goggles were worn to prevent spills, accidents, and eye injuries.

Creation of the Solutions:

1. Turmeric Solution:
    1. Sterilize all equipment.
    2. Mass out 1 gram of neem powder and 4 grams of honey separately using an electronic scale.

3. Pour 1 gram of turmeric powder and 4 grams of honey into a cup.
4. Add 5 mL of water and mix thoroughly for 1 minute until the consistency resembles sanitizer

Figure 12. Turmeric and honey solution.

2. Aloe Vera Solution:
    1. Sterilize all equipment.
    2. Mass out 1 gram of neem powder and 4 grams of honey separately using an electronic scale.
    3. Pour 4 grams of aloe vera gel and 4 grams of honey into a cup.
    4. Add 5 mL of water and mix thoroughly for 30 seconds until the consistency resembles sanitizer.

Figure 13. Aloe Vera and honey solution.

3. Lemon Juice Solution:
    1. Sterilize all equipment.
    2. Mass out 1 gram of neem powder and 4 grams of honey separately using an electronic scale.
    3. Pour 4 grams of lemon juice and 4 grams of honey into a cup.
    4. Add 5 mL of water and mix thoroughly for 30 seconds until the consistency resembles sanitizer.

Figure 14. Lemon juice and honey solution.

4. Neem Leaf Powder Solution:
    1. Sterilize all equipment.
    2. Mass out 1 gram of neem powder and 4 grams of honey separately using an electronic scale.

3. Pour 1 gram of neem powder and 4 grams of honey into a cup.
4. Add 5 mL of water and mix thoroughly for 30 seconds until the consistency resembles sanitizer.

Figure 15. Neem and honey solution.

5. Hand Sanitizer:
    1. Sterilize all equipment.
    2. Mass out 1 gram of neem powder and 4 grams of honey separately using an electronic scale.
    3. Pour 5 grams of hand sanitizer into a cup.

Figure 16. Hand sanitizer.

Set up of Agar Plates:

1. Gather all necessary materials and put on safety gear (goggles, masks, coat, and gloves).
2. Use a 100 microliter pipette to apply E.coli onto the agar plates and scrub thoroughly using the designated swabbing pattern.

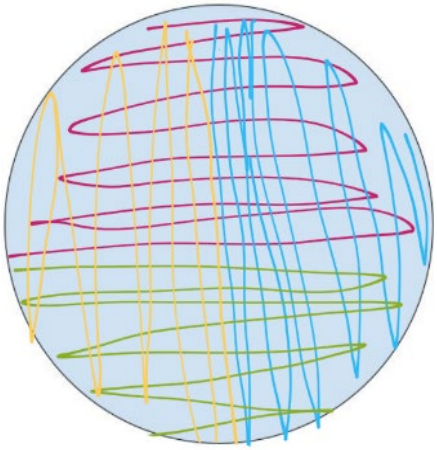


Figure 17. Swabbing pattern model.

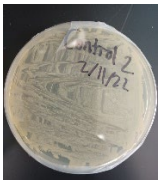
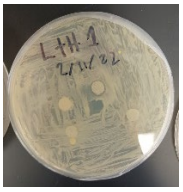
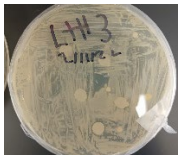
Placement of Solutions into Agar Plates:

1. Gather 15 agar plates filled with E.coli, tweezer, bleach, 20 microliter pipette, tips, hole puncher, filter paper, and parafilm.
2. Hole punch 230 holes of filter paper and place them on a sterilized working area.
3. Use the 20 microliter pipette to dip the filter paper into the solutions and place them onto the agar plates.

Results

To assess the antibacterial properties of various solutions, agar plates were inoculated with E. coli and treated with different antibacterial agents, including turmeric, aloe vera, lemon juice, neem leaf, and hand sanitizer. The plates were incubated and observed over a period of six days to monitor bacterial growth and the effectiveness of each treatment.

Growth Inhibition Zones

Control Bacteria Samples			
Lemon + Honey Samples			

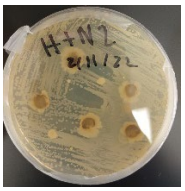
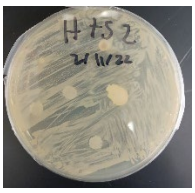
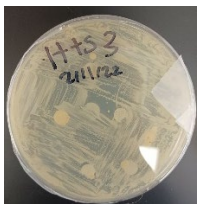
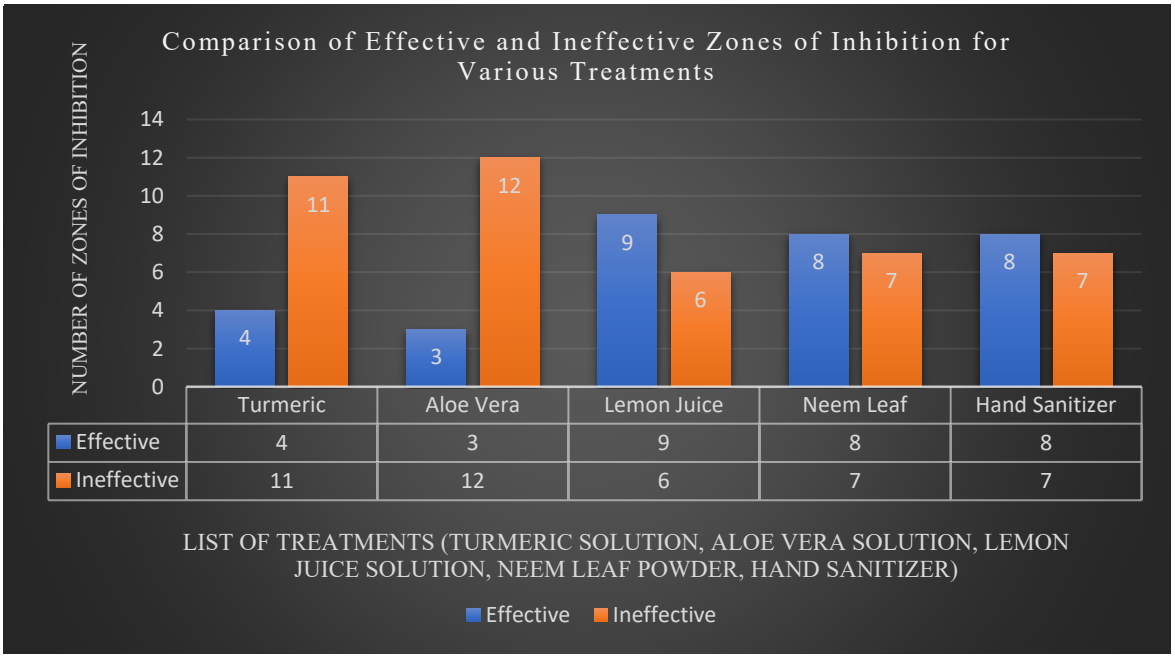
Honey + Aloe Vera samples			
Honey + Neem Samples			
Hand Sanitizer Mixture			
Honey + Turmeric Samples			

Figure 18-35. Mixtures of honey, lemon juice, neem powder, aloe vera, and turmeric prepared in specific ratios for testing antibacterial properties against E. coli.

Quantitative Analysis



Graph 1. The bar graph compares the number of effective (presence of inhibition zone) and ineffective (absence of inhibition zone) results for each treatment over three trials. Each treatment has two bars representing the number of effective and ineffective zones of inhibition.

The bar graph titled "Comparison of Effective and Ineffective Zones of Inhibition for Various Treatments" provides a comparative analysis of the effectiveness of five different treatments: Turmeric, Aloe Vera, Lemon Juice, Neem Leaf, and Hand Sanitizer. The graph reveals that Lemon Juice, with nine effective zones out of 15, exhibits the highest efficacy. Both Neem Leaf Powder and Hand Sanitizer follow closely with eight effective zones each. Turmeric and Aloe Vera have fewer effective zones, four and three respectively, but a higher number of ineffective zones, eleven and twelve respectively. This data suggests that among the treatments tested, Lemon Juice may possess superior inhibitory properties.

Statistical Analysis

To evaluate the effectiveness of different treatments in inhibiting bacterial growth, a chi-squared test was performed. The observed values (number of effective and ineffective zones of inhibition) for each treatment are shown in Table 1. The test compared the observed distribution of effective and ineffective outcomes across the five treatments.

Table 1. Observed and Expected Frequencies for the Effectiveness of Treatments

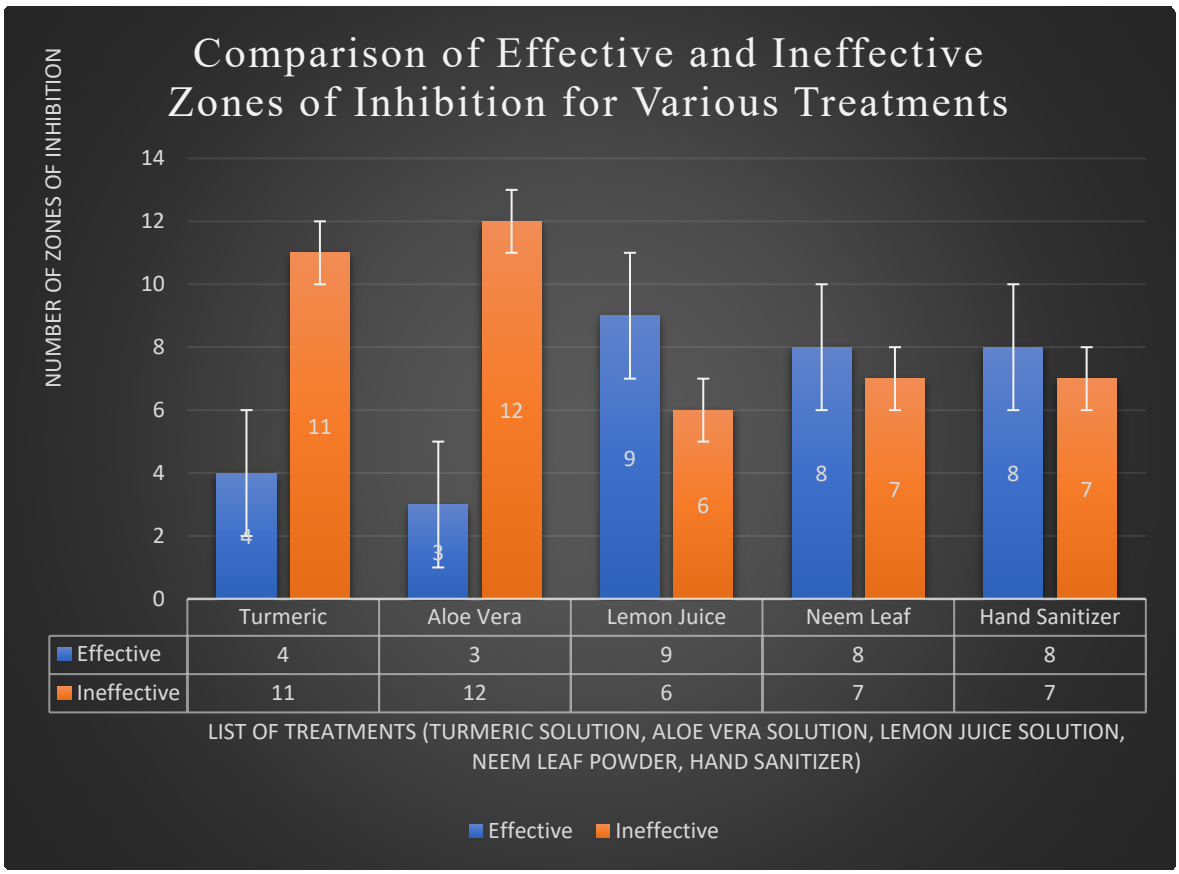
Treatment	**Effective (Observed)**	**Ineffective (Observed)**	**Effective (Expected)**	**Ineffective (Expected)**
Turmeric	4	11	6.4	8.6
Aloe Vera	3	12	6.4	8.6

Lemon Juice	9	6	6.4	8.6
Neem Leaf	8	7	6.4	8.6
Hand Sanitizer	8	7	6.4	8.6

The chi-squared statistic was calculated as follows:

$$\chi^2 = \Sigma ((O - E)^2 / E) = (6.4(4 - 6.4)^2 + 8.6(11 - 8.6)^2 + 6.4(3 - 6.4)^2 + 8.6(12 - 8.6)^2 + 6.4(9 - 6.4)^2 + 8.6(6 - 8.6)^2 + 6.4(8 - 6.4)^2 + 8.6(7 - 8.6)^2) / \Sigma E$$

This calculation resulted in a chi-squared value of 7.97. With 4 degrees of freedom (df = (5 - 1) * (2 - 1)), the critical value at the 0.05 significance level is 9.488. Since the calculated chi-squared value (7.97) is less than the critical value (9.488), the null hypothesis cannot be rejected. Therefore, we conclude that there is no significant difference in the effectiveness of the treatments in inhibiting bacterial growth.



Graph 2. Comparison of Effective and Ineffective Zones of Inhibition for Each Treatment with error bars

To visually compare the effectiveness of each treatment, a bar graph was created (Graph 2). The graph displays the number of effective and ineffective zones of inhibition for each treatment. The error bars represent the range of observed values across the three trials.

Discussion

The chi-squared test results suggest that there is no significant difference in the effectiveness of the different treatments in inhibiting bacterial growth. This finding indicates that all treatments performed similarly under the conditions tested.

Turmeric Solution

The Turmeric solution had 4 effective zones of inhibition and 11 ineffective zones. The calculated chi-squared value for Turmeric indicates that its performance was not significantly different from the other treatments. Despite traditional claims of Turmeric's antimicrobial properties, the results suggest that its effectiveness is comparable to the other substances tested under these experimental conditions.

Aloe Vera Solution

The Aloe Vera solution showed 3 effective zones and 12 ineffective zones. Aloe Vera is widely known for its healing properties and is often cited for its antibacterial effects. However, in this study, Aloe Vera did not demonstrate a statistically significant advantage over the other treatments. This may be due to the specific strains of bacteria tested or the concentration of Aloe Vera used.

Lemon Juice Solution

Lemon juice exhibited 9 effective zones and 6 ineffective zones, making it one of the more effective treatments observed in this study. Lemon juice's acidity and high citric acid content are likely contributors to its antimicrobial effects. The comparatively higher number of effective zones supports the notion that lemon juice can be a potent antibacterial agent. However, variability in the results still leaves it statistically like the other treatments.

Neem Leaf Powder

Neem leaf powder resulted in 8 effective zones and 7 ineffective zones. Neem is traditionally known for its medicinal properties, including antimicrobial activity. The results from this study support these traditional uses to some extent, as Neem leaf powder showed a relatively high number of effective zones. Nonetheless, the chi-squared test suggests that while Neem is effective, its performance is not significantly different from the other treatments.

Hand Sanitizer

The hand sanitizer used as a control had 8 effective zones and 7 ineffective zones. This is consistent with the expected performance of hand sanitizers, which are formulated to kill bacteria and viruses. The results show that the hand sanitizer was effective, but again, not significantly different from the other treatments tested.

Analysis and Implications

The overall chi-squared value of 7.97 with 4 degrees of freedom did not exceed the critical value of 9.488 at the 0.05 significance level, indicating no significant differences between treatments. This lack of statistical significance could be due to several factors, including the variability in experimental conditions, the concentration of solutions used, or the specific bacterial strains tested.

While the results do not show a clear winner among the treatments, they do highlight the potential of natural substances like lemon juice and Neem leaf powder as effective antibacterial agents. These findings are valuable for further research into alternative and complementary antimicrobial treatments. Future studies could explore different concentrations, combinations of treatments, and a wider variety of bacterial strains to build on these findings.

The inclusion of error bars in the results graph (Figure 35) also emphasizes the variability in the data across the three trials. This variability is an important consideration, as it reflects the real-world effectiveness of these treatments, which can be influenced by numerous factors.

Conclusion

At first, it was hypothesized that all combinations of ingredients mixed with honey—such as aloe vera + honey, neem powder + honey, lemon juice + honey, and honey + turmeric—would inhibit the growth of bacteria due to their known antibacterial properties. This hypothesis was only partially supported by the results of the chi-squared test, which indicated no significant difference in the effectiveness of the treatments in inhibiting bacterial growth.

The chi-squared test results showed the following distributions for effective and ineffective zones of inhibition: Turmeric solution (4 effective, 11 ineffective), Aloe Vera solution (3 effective, 12 ineffective), Lemon Juice solution (9 effective, 6 ineffective), Neem Leaf Powder (8 effective, 7 ineffective), and Hand Sanitizer (8 effective, 7 ineffective). The overall chi-squared value of 7.97 with 4 degrees of freedom did not exceed the critical value of 9.488 at the 0.05 significance level, indicating no significant differences between treatments.

Despite the lack of statistically significant differences, some trends were observed. The combination of lemon juice and honey showed the highest number of effective zones, suggesting that it has the most potent antibacterial properties against *E. coli* among the treatments tested. Lemon juice's acidity, combined with honey's natural antibacterial properties, likely contributes to its effectiveness.

Conversely, the Turmeric and Aloe Vera solutions showed the lowest number of effective zones. This might be due to the dilution effect when water is added to these solutions, which decreases the overall acidity and thus the antibacterial potency of honey Additionally, the combination of alkaline aloe vera and acidic honey may neutralize the pH, further diminishing the antibacterial properties.

Hand sanitizer, used as a control, demonstrated consistent results with a low standard deviation and a small difference between the average and the 95% confidence interval. This indicates high reliability and suggests that hand sanitizer would still be a dependable choice for practical applications despite not outperforming the lemon juice and honey combination in terms of effectiveness.

Future Directions and Improvements

To further improve the results of this study, refining swabbing techniques and enhancing sterilization methods to prevent contamination is essential. Future experiments should aim to minimize variables that may affect

bacterial growth and solution effectiveness. The ability to open agar plate lids, which was restricted due to COVID-19 concerns, would allow for more precise observations under a microscope.

Additionally, developing the most effective solutions into a practical hand sanitizer formulation should be pursued. This involves eliminating fewer effective solutions and focusing on those that demonstrated significant antibacterial properties. Collaborating with a professor in a laboratory setting would also provide more controlled conditions and potentially more accurate results.

Further research should also explore the addition of other natural ingredients that complement the antimicrobial properties of honey and lemon juice, maintaining the natural theme of the solution. Testing the solutions against bacteria commonly found on frequently touched surfaces, such as phones, door handles, or human hands, would better simulate the real-world environments of third-world countries. The goal would be to develop a solution that not only targets specific bacterium types but also effectively combats a wide range of bacteria over extended periods, ensuring comprehensive protection.

By addressing these areas, future studies can build on the findings of this research, optimizing the formulation and application of natural antibacterial solutions to improve public health outcomes, especially in resource-limited settings.

Acknowledgments

I would like to thank Ms. Westfall from Waubonsie Valley who made this whole experiment possible through her support, resources, and knowledge. She has guided the experiment from start to finish through the ups and downs, allowing my figment of an idea into an actual experiment. Additionally, I would like to thank Mrs. Hawkins for her constant support in my final paper and her in-depth analysis of my experiment and paper.

References

Aggarwal, B. B., Sundaram, C., Malani, N., & Ichikawa, H. (2007). Curcumin: the Indian solid gold. *Advances in Experimental Medicine and Biology, 595*, 1-75. https://doi.org/10.1007/978-0-387-46401-5_1

Ayurveda | Johns Hopkins Medicine. (n.d.). Retrieved February 19, 2022, from https://www.hopkinsmedicine.org/health/wellness-and-prevention/ayurveda

Ayurvedic medicine is regaining both relevance and popularity in India. (2018, December 24). Retrieved from https://www.consultancy.in/news/1777/ayurvedic-medicine-is-regaining-both-relevance-and-popularity-in-india

Bandyopadhyay, U., Biswas, K., Chatterjee, R., Bandyopadhyay, D., Chattopadhyay, I., Ganguly, C. K., Chakraborty, T., Bhattacharya, K., & Banerjee, R. K. (2002). Gastroprotective effect of Neem (Azadirachta indica) bark extract: possible involvement of H(+)-K(+)-ATPase inhibition and scavenging of hydroxyl radical. *Life Sciences, 71*(24), 2845-2865. https://doi.org/10.1016/s0024-3205(02)02143-4

Centers for Disease Control and Prevention. (2021, December 8). Potential sanitation solutions during an emergency response. Retrieved from https://www.cdc.gov/healthywater/emergency/sanitation.html

Ekawati, E., & Darmanto, W. (2019). Lemon (Citrus limon) juice has antibacterial potential against diarrhea-causing pathogen. *IOP Conference Series: Earth and Environmental Science, 217*, 012023. https://doi.org/10.1088/1755-1315/217/1/012023

Global WASH Fast Facts | Global Water, Sanitation and Hygiene | Healthy Water | CDC. (2021, December 8). Retrieved from https://www.cdc.gov/healthywater/global/wash_statistics.html

Hamid, S. K., Al-Dubayan, A. H., Al-Awami, H., Khan, S. Q., & Gad, M. M. (2019). In vitro assessment of the antifungal effects of neem powder added to polymethyl methacrylate denture base material. *Journal of Clinical and Experimental Dentistry, 11*(2), e170-e178. https://doi.org/10.4317/jced.55458

How Water Pollution in India Kills Millions. (2020, July 14). *BORGEN*. Retrieved from https://www.borgenmagazine.com/water-pollution-in-india/

Khan, M. F., Tang, H., Lyles, J. T., Pineau, R., Mashwani, Z. U., & Quave, C. L. (2018). Antibacterial properties of medicinal plants from Pakistan against multidrug-resistant ESKAPE pathogens. *Frontiers in Pharmacology, 9*, 815. https://doi.org/10.3389/fphar.2018.00815

Liquid or Bar? Soapy Tales | Office for Science and Society—McGill University. (n.d.). Retrieved February 19, 2022, from https://www.mcgill.ca/oss/article/health/liquid-or-bar-soapy-tales

Mandal, M. D., & Mandal, S. (2011). Honey: its medicinal property and antibacterial activity. *Asian Pacific Journal of Tropical Biomedicine, 1*(2), 154-160. https://doi.org/10.1016/S2221-1691(11)60016-6

Many Americans don't always wash their hands after going to the bathroom | YouGov. (n.d.). Retrieved February 19, 2022, from https://today.yougov.com/topics/lifestyle/articles-reports/2020/01/30/hand-washing-soap-poll-survey

Medimix Ayurveda Hand Wash With Neem Turmeric Aloe Vera, 175 ml – govkarts. (n.d.). Retrieved February 19, 2022, from https://www.govkarts.com/product/medimix-ayurveda-hand-wash-with-neem-turmeric-aloe-vera-175-ml-3/

More People in the United States Dying from Antibiotic-Resistant Infections than Previously Estimated | CDC Online Newsroom | CDC. (n.d.). Retrieved February 19, 2022, from https://www.cdc.gov/media/releases/2019/p1113-antibiotic-resistant.html

Ravva, S. V., & Korn, A. (2015). Effect of Neem (Azadirachta indica) on the survival of Escherichia coli O157:H7 in dairy manure. *International Journal of Environmental Research and Public Health, 12*(7), 7794-7803. https://doi.org/10.3390/ijerph120707794

Reynolds, T., & Dweck, A. C. (1999). Aloe vera leaf gel: a review update. *Journal of Ethnopharmacology, 68*(1-3), 3-37. https://doi.org/10.1016/s0378-8741(99)00085-9

Science NetLinks | American Association for the Advancement of Science. (n.d.). Retrieved February 19, 2022, from https://www.aaas.org/programs/science-netlinks

Show Me the Science—How to Wash Your Hands | Handwashing | CDC. (n.d.-a). Retrieved February 19, 2022, from https://www.cdc.gov/handwashing/show-me-the-science-handwashing.html

Spartan Race Inc. Obstacle Course Races | 5 Reasons to Drink Aloe Vera Juice. (n.d.). Retrieved from https://www.spartan.com/blogs/unbreakable-nutrition/5-reasons-to-drink-aloe-vera-juice

Which are the most used Ayurvedic Herbs and their benefits? (n.d.). Retrieved February 19, 2022, from https://www.keralaayurveda.biz/blog/guide-to-the-most-used-ayurvedic-herbs-and-their-benefits

World Health Organization and United Nations Children's Fund (UNICEF). (2021). State of the world's hand hygiene: A global call to action. Retrieved February 19, 2022, from https://cdn.who.int/media/docs/default-source/wash-documents/bls21329-uni-state-of-the-world-s-hand-hygiene_who_web--low.pdf?sfvrsn=f500dcf0_11

Water, sanitation and hygiene | UNICEF India. (n.d.). Retrieved February 19, 2022, from https://www.unicef.org/india/what-we-do/water-sanitation-hygiene