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Security And Health Implications Of Covid-19; Prevention And Control Using Lime And Lemon Hand Sanitizer

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Abstract

Problem: The covid-19 pandemic has heavily impacted countries across the globe, including Nigeria. Apart from the obvious health implications, the pandemic has also brought about security concerns and implications for the Nigerian population. Several countries including Nigeria depend on importation of hand sanitizer which makes the products scarce and expensive. Therefore, the need to formulate a homemade hand sanitizer was necessary to bridge the gaps. In the formulation of these hand sanitizers, the antibacterial activity of Lemon and Lime juice on mesophilic bacterial isolates was evaluated. **Approach**: Freshly collected lime and lemon were adequately washed and blended into syrup. 100 ml of isopropyl alcohol was added to 100 ml of Lemon juice to create the lemon (LE 100) hand sanitizer. 100 ml of isopropyl alcohol was also added to 100 ml of Lime juice to create the lime (LI 100) hand sanitizer, and 100 ml of isopropyl alcohol was mixed with 50ml of lemon and 50 ml of lime juice to form the (LE/LI 50/50) hand sanitizer. The antibacterial sensitivity test was carried out using the disc method, which was socked in the hand sanitizer. **Findings:** *Micrococcus* sp. was more sensitive than other Gram-positive bacteria to LE 100 with a 29 mm zone of inhibition. *Streptococcus* sp. was more sensitive to LI 100 with a 31 mm zone of inhibition and *Bacillus* sp., was more sensitive to LE/LI 50/50 with a 25 mm zone of inhibition. *Escherichia coli* was more sensitive than other Gram-negative bacteria to LE 100 and LE/LI 50/50 with 22 mm and 20 mm zone of inhibition respectively. *Pseudomonas aeruginosa* was more sensitive to LI 100 with 18 mm zone of inhibition. Lemon and lime extracts have shown effectiveness as antimicrobial agents against these bacterial isolates. They are natural, affordable, available and safe. It has shown the ability to inhibit the growth of most Gram-positive and Gram-negative bacteria, and thus can be used in the control and prevention of Covid-19.

Keywords: Lime, Lemon, Covid-19, Hand Sanitizer, Anti Bacterial sensitivity

Introduction

Severe acute respiratory syndrome corona virus 2 or SARS-CoV-2, more commonly known for causing corona virus disease 2019 which has been declared a global pandemic by the World Health Organization in early 2020. Since its discovery in December 2019 in Wuhan, there have been millions of confirmed cases worldwide. [1, 2] With cases increasing exponentially around the world, it has caused a significant burden on all aspects of society despite aggressive isolation approach to prevent the spread of the virus. [3] The covid-19 pandemic has heavily impacted countries across the globe,

including Nigeria. Apart from the obvious health implications, the pandemic has also brought about security concerns and implications for the Nigerian population. It has lead to an increase in infection and deaths in Nigeria. The healthcare system has been under immense pressure to provide adequate care and treatment to those affected by the virus. There have been challenges with testing, tracing, and accessing healthcare facilities, particularly in remote and rural areas. This has led to a strain on the healthcare infrastructure and has highlighted the need for better investment in healthcare services and infrastructure. The pandemic has had severe economic consequences on the Nigerian population, particularly for those in vulnerable and low-income groups. Many people have lost their jobs or experienced reduced income, leading to financial hardships and food insecurity. This has not only affected their overall well-being but has also created a conducive environment for crime. From a security perspective, the covid-9 lockdown measures and restrictions have resulted in increased crime rates in Nigeria. With the loss of jobs and income, people are resorting to desperate measures to survive, including theft, burglary, and looting which was witnessed in Nigeria during the "END-SARS" protest. Reports of armed robberies and attacks have surged during the pandemic, posing a threat to the safety and security of the Nigerian population.

Following the face-mask approach, appropriate hand hygiene is of utmost importance as hands may be contaminated from direct contact with patients' respiratory droplets from coughs and sneezes or indirect contact via surfaces, which may then facilitate the transmission and spreading of the disease. [4] One of the many ways implemented to prevent the spread of this virus, as with previous contagious pathogens, is frequent and effective hand washing. In both healthcare and community settings, alcohol-based hand sanitizers have become a popular alternative to traditional hand washing with soap and water. Alcohol-based hand sanitizers have been utilized as an effective alternative to hand washing to prevent bacterial and viral infections, making it one of the essential protocols in decreasing the healthcare burden. [5] To get the maximum benefit from hand sanitizers, the Centers for Disease Control and Prevention (CDC) recommends that people use a product that contains at least 60% alcohol, cover all surfaces of their hands with the product, and rub them together until dry. A range of hand sanitizers is available with various combinations of ingredients and modes of delivery. Given the popularity of hand sanitizers during this pandemic, it is essential to understand which hand sanitizers work best against this novel virus. [6] The World Health Organisation recommends alcohol-based hand sanitizer products with 62%–95% of alcohol as it is capable of denaturing the proteins of microbes and inactivating viruses. [7, 8, 9, 10] There are a few challenges and concerns about this formulation in terms of fire hazards and skin toxicity due to high alcohol content. [11]

Improving the efficiency of antimicrobial compounds requires a proper understanding of their mode of action. For example, gamma-corona virus pre-treated with elderberry extract damaged the viral molecular structure, causing elimination of Vero cell cytotoxicity. The mechanism of elderberry extract efficacy was attributed to altered virion envelopes and membrane vesicles. [12] Tangerine, a polymethoxylated flavones found in citrus fruits peels such as limes and lemons inhibits viral entry into cells by blocking viral fusion. Citrus extracts are active against avian influenza virus, Newcastle virus, and many other viruses in different environments. [13] The use of Lime and lemon hand sanitizers is a priority in eradicating Covid-19 due to the natural content of lime and lemon extract.[14] Natural antimicrobials, including fruits and plant extract, or organic acids, are currently tested to inhibit

viruses and prevent their pathogenic impact on the host.[12] Studies revealed that lemon fruit extract contains a high level of phytochemicals such as flavonoids, phenols, alkaloids, and quinines, which are termed antiviral molecules against Herpes simplex virus type 1, Hepatitis A virus and many other viral microbes. [13] Chemical raw materials for hand sanitizer are increasingly scarce in the market and can cause fire hazards and skin toxicity due to high alcohol content. Therefore, there is a need to use natural ingredients that are available, affordable, environmentally friendly, safe, and without allergies.

Methodology

Freshly collected lime and lemon fruits were adequately washed and blended into syrup. The paste was then sieved using a big plastic sieve to remove the chaff. This was done separately for both lime and lemon. Hundred (100 ml) of lemon and lime were measured out separately using a glass measuring cylinder, a corresponding volume (100 ml) of 95 percent isopropyl alcohol was added to lemon and lime respectively. 100 ml of isopropyl was added to 100 ml of Lemon juice to create the lemon (LE 100) hand sanitizer. 100 ml of isopropyl was also added to 100 ml of Lime juice to create the lime (LI 100) hand sanitizer, and 100 ml of isopropyl was mixed with 50ml of lemon and 50 ml of lime juice to form the (LE/LI 50/50) hand sanitizer. The solution was further filtered using a membrane filter to remove residues and provide sterility. After this was done, a very small quantity of alcohol was added to check for evaporation. The mixtures were transferred into sterile well-labeled spray bottles.

Table 1: Hand Sanitizer formulation and concentration

Hand Sanitizer	Juice concentration (ml)	Isopropyl alcohol concentration (ml)
Lemon Hand Sanitizer (LE)	100	100
Lime Hand Sanitizer (LI)	100	100
Lemon/Lime Hand Sanitizer	100 (LE 50 +LI 50)	100

Collection of bacterial isolates

Five Gram-negative and Gram-positive bacterial isolates were obtained from the Specialist hospital Lokoja, Kogi State. The Gram-positive isolates were *Staphylococcus* sp., *Streptococcus* sp., *Micrococcus* sp., *Streptobacillus* sp., and *Bacillus* sp. The Gram-negative isolates are *Pseudomonas aeruginosa, Samonella typhimurium, Escherichia coli, Samonella typhi,* and *Klebsiella* sp.

Determination of the Inhibitory and Bactericidal Potential of the Hand Sanitizers

To determine the inhibitory and bactericidal potential of the hand sanitizer, the hand sanitizer sensitivity disc was designed using a filter paper which was perforated to a size of 0.7cm in diameter.

The filter paper disc was soaked in each of the individual sanitizers for 30 minutes. The Mueller-Hinton Agar was prepared according to the manufacturer's instruction and poured into 25 plates. Each bacterial isolate was streaked on five plates respectively. The discs were placed on the streaked plates for a particular bacterial isolate. This was done for all the isolates and incubated at 37^{o}C for 24 hours. The clearance zone was determined by measuring the diameter of the cleared area around the disc with a meter rule. [15, 16]

Results

Table 2: Antimicrobial activity of Lemon and lime hand Sanitizers against Gram-positive bacteria

Isolates	LE 100	LI 100	LE 50/ LI 50
Staphlococcus spp.	S	S	M
Streptococcus spp.	R	S	MS
Micrococcus spp.	S	S	S
Streptobacillus spp.	R	MS	R
Bacillus spp.	S	S	S

Key: R=Resistance, S=Sensitive, MS=Moderately sensitive

Table 3: Zone of inhibition of Lemon and Lime hand Sanitizers against Gram-positive bacteria

Isolates	LE 100 (mm)	LI 100 (mm)	LE 50/ LI 50 (mm)
Staphlococcus spp.	21.0	17.0	14.0
Streptococcus spp.	0.0	31.0	19.0
Micrococcus spp.	29.0	26.0	17.0
Streptobacillus spp.	0.0	17.0	0.0
Bacillus spp.	22.0	19.0	25.0

Key: mm = Millimeter (diameter of zone of inhibition).

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Plate 1: Lime Hand Sanitizer (LI 100)
Plate 2: Lemon Hand Sanitizer (LE 100)
Plate 3: Lime/Lemon Hand Sanitizer (LI 50/LE50)

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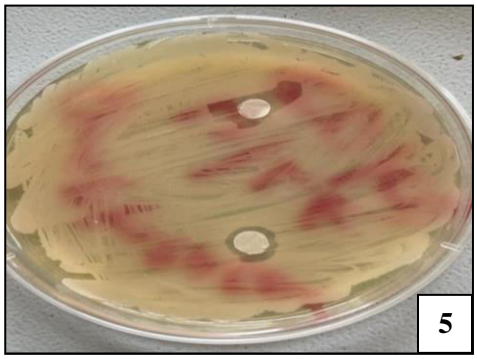

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Plate 4: Inhibitory effect of LE 50 hand Sanitizer on *Micrococcus* sp
Plate 5: Inhibitory effect of LE 50/LI50 hand Sanitizer on *Bacillus* sp
Plate 6: Inhibitory effect of LI100 hand sanitizer on *Streptococcus* sp

Table 4: Antimicrobial activity of Lemon and lime hand sanitizers against Gram-negative bacteria

Isolates	LE 100	LI 100	LE 50/ LI 50
Pseudomonas aeruginosa	R	S	R
Samonella typhimurium	S	R	S
Escherichia coli	S	MS	S
Samonella typhi	S	S	S
Klebsiella spp.	R	R	R

Key: R=Resistance, S=Sensitive, MS=Moderately sensitive

Table 5: Zone of inhibition of Lemon and Lime hand sanitizers against Gram-negative bacteria

Isolates	LE 100 (mm)	LI 100 (mm)	LE 50/ LI 50 (mm)
Pseudomonas aeruginosa	0.0	18.0S	0.0
Samonella typhimurium	21.0	0.0	16.0
Escherichia coli	22.0	12.0	20.0
Samonella typhi	21.0	16.0	19.0
Klebsiella spp.	0.0	0.0	0.0

Key: MM = Millimeter (diameter of zone of inhibition).

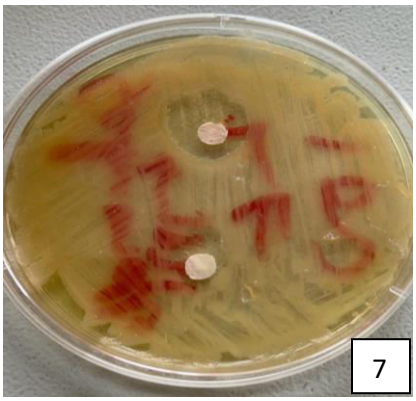

Plate 7: Inhibitory effect of LE100 hand sanitizer on *E. coli*
Plate 8: Inhibitory effect of LI 50/LE50 hand sanitizer on *E. coli*
Plate 9: Inhibitory effect of LI100 hand sanitizer on *Pseudomonas aeruginosa*

Discussion

The lemon, lime, and lemon/lime 100% alcohol-based hand sanitizers were determined for their antimicrobial activity against clinical bacterial isolates, which comprised of five Gram-positive and Gram-negative bacteria. The Gram-positive bacteria includes *Staphylococcus* sp., *Streptococcus* sp., *Micrococcus* sp., *Streptobacillus* sp. and *Bacillus* sp. while the Gram-negative bacteria are *Pseudomonas aeruginosa, Samonella typhimurium, Escherichia coli, Samonella typhi,* and *Klebsiella* sp. The evaluation of antimicrobial activity was conducted in triplicate using disc diffusion agar method.[15] The degree of inhibition of the hand sanitizers against the bacterial isolates is shown in Table 2 and 4. The results indicates that the lemon, lime and lemon/lime hand sanitizers, exhibited an antibacterial activities towards the Gram-positive and negative bacteria, but with variability relating to the bacterial genus and species. The reason for the different sensitivity of the Gram-negative bacteria compared to that of Gram-positive bacteria (Table 2 and 4) could be due to differences in their cell wall composition. Gram-positive bacteria contain an outer peptidoglycan layer, an effective permeability barrier, whereas Gram-negative bacteria have an outer phospholipid membrane.[17] Hayes and Markovic [18], investigated the antimicrobial properties of lime and lemon. They found that both citrus fruits possess significant antimicrobial activity against *S. aureus, Escherichia coli, Samonella typhi, Streptobacillus* sp., *Bacillus* sp., and *Streptococcus* sp.

Nevertheless, *Klebsiella* sp., and *Pseudomonas aeruginosa* showed complete and slight resistance respectively (Table 4). This could be due to the physiological, biological, and genetic makeup of the organisms or the acidic pH of this citrus that affected the charges of the amino acids that constitute the peptidoglycan, and it may affect the active sites of enzymes leading to defects in their activity. [19, 20] Infections caused by *P. aeruginosa*, especially those with multidrug resistance, are among the most difficult to treat with conventional antibiotics. [21, 22, 23]

In this study, the growth of *Streptococcus* sp. and *P. aeruginosa* was slightly inhibited by the hand sanitizers. Such results are exciting, and with expectation, increasing the concentration of the extracts may produce more inhibition of this bacterium (Table 3 and 5). They may inhibit bacteria by a different mechanism and may have therapeutic value as an antibacterial agent against multi-drug resistant bacterial strains. [24, 25] The potency of the above-mentioned hand sanitizers against other mentioned isolates in (Tables 2 and 4) might be a result of the essential oil present in the Citrus extracts, which are limonene and γ-terpinene that can disrupt the bacterial membrane and inhibit the respiration and ion transport processes. Therefore, when the permeability of the membrane increases, essential oils will pass through the cell wall and the cytoplasmic membrane may also thicken in the cytoplasm and damage the lipids and proteins layers. Flavonoid which is a phytochemical constituent in citrus has an antibacterial, antimicrobial, antioxidant, and anti-inflammatory activities, with proven ability to inhibit specific enzymes, to stimulate some hormones and neurotransmitters, and scavenge free radicals. The bacterial isolates used in this study are mesophilic bacteria with an optimum temperature of 20^{o}C to 45^{o}C and structurally can adapt and thrive in the tropics compared to SARS-CoV-2 which as an envelope virus cannot thrive in temperature range of 20^{o}C to 45^{o}C and its envelope proteins are vulnerable to high temperatures and chemical agents like antiseptics and disinfectants. The effectiveness of these hand sanitizers on these mesophilic bacteria is a strong evidence and proves that it can be use as a tool in the control and prevention of Covid-19.

Conclusion

Lemon and lime extracts have an essential role as antimicrobial agents against microorganisms. They are natural, affordable, and safe. Lime and Lemon hand sanitizer are locally made, and the materials are available in our environment. It has shown the ability to inhibit the growth of most Gram-positive and Gram-negative bacteria, and thus can be used in the control, prevention and management of Covid-19. The use of locally made hand sanitizers should be encouraged because the ingredients needed for the formulation are safe, cheap, easily accessible, affordable, and without causing any adverse effect on the user.

Reference

1. He, J., Deng, F.Y., and Li, W. (2020). "Corona virus disease 2019 : What we know?" *Journal of Medical Virology.***1**:1–7.

2. Han, M.S., Choi, E.H., Chang, S.H., Jin, B.L., Lee, E.J., and Kim, B.N. (2020). Clinical Characteristics and Viral RNA Detection in Children with Coronavirus Disease 2019 in the Republic of Korea. *Journal of American Medical Association Pediatrics*. **28**: 45-52.

3. World Health Organization. Transmission of SARS-CoV-2: implications for infection prevention precautions, July 9, 2020.

4. Li, H., Liu, S., Yu, X., Tang, S., and Tang, C. (2020). "Corona virus disease 2019 (COVID-19): current status and future perspectives." *International Journal of Antimicrobial Agents*. **10**:51-59.

5. Pittet, D., Allegranzi, B., and Boyce, J. (2009). The World Health Organization guidelines on hand hygiene in health care and their consensus recommendations. *Journal of Infections Control, Hospital and Epidemiology*. **30**:611–622.

6. Boyce, J.M., and Pittet, D. (2002). Healthcare infection control practices advisory committee. society for healthcare epidemiology of America. Association for professionals in infection control. Infectious diseases society of America. Hand hygiene task force guideline for hand hygiene in health-care settings: recommendations of the healthcare infection control practices advisory committee and the HICPAC/SHEA/APIC/IDSA hand hygiene task force. *Infection Control Hospital Epidemiology*. **23**(12):33–40

7. Kampf, G., and Kramer, A. (2004). Epidemiological background of hand hygiene and evaluation of the most important agents for scrubs and rubs. *Journal of Clinical Microbiology*. **17**:863–893.
8. Kampf G. (2012). Efficacy of ethanol against viruses in hand disinfection. *Journal of Hospital Infection*. **98**:331–338.
9. Kassem, I., and Jaafar, H. (2020). The potential impact of water quality on the spread and control ofCOVID-19 in Syrian refugee camps in Lebanon. *Water International*. **12**:27-36.

10. Dixit, A., Pandey, P., Mahajan, R., and Dc, D. (2014). "Alcohol-based hand sanitizers : assurance and apprehensions revisited." *Journal of Pharmaceutical, Biological and Chemical Science.* **5** (558): 558–63, 2014.

11. Eva, G., Gladys, B. and Bandar, W. (2014). "The antibacterial effectiveness of selected commercial hand sanitizers against *Escherichia coli* and *Staphylococcus aureus*," *Advancing Medical Technology Research*. **2**: 106–130.

12. Zhai, J.P., Ding, Y., Wu, X., Long, J., Zhong,Y., and Li, Y. (2020). "The epidemiology, diagnosis and treatment of COVID-19." *International Journal of Antimicrobial` Agents,* **10**:36-52.

13. Hayat, J.A., and Munnawar, F. (2017). "Antibacterial effectiveness of commercially available hand antibacterial effectiveness of commercially available hand." *International Journal of Biology and Biotechnology*. **13**(3): 427–31.

14. Acharya, S.B., Ghosh, S., Yadav, G., Sharma, K, and Ghosh, S. (2020). "Formulation, evaluation and antibacterial efficiency of water-based herbal." Vol. 1. p.1–16.

15. Udochukwu, U, F.I. Omeje, I. S. Uloma, F. D. Oseiwe (2015). Phytochemical analysis of *Vernonia amygdalina* and *Ocimum gratissimum* extracts and their antibacterial activity on some drug resistant bacteria. *American Journal of Research Communication*, 3(5): 2325-4076.

16. Udochukwu, U, Igweze Austine, O.C.Udinyiwe and P. N. Ijeoma (2014): Effect of crude oil pollution on orange (Citrus) leaves, *International journal of current microbiology and applied sciences.* 3(10): pp.58-64

17. Samarakoon, K., Senevirathne, M., Lee, W., Kim, Y., Kim, J., Cheo, M., and Jeon, Y. (2012). Antibacterial effect of citrus press-cakes dried by high speed and far-infrared radiation drying methods. *Nutritional Restrictions Practices*. **6**(3):187-194.

18. Hayes, A.S., and Markovic, B. (2002). Toxicity of Beak housie citrodora (Lemon Myrthle). Anti-microbial and in vitro cytotoxicity. *Journal of Food Chemical and Toxicology*. **40** (4): 535-543

19. Aronoff, S.C., Hall, A., and Del Vecchio, M.T.(2020). The natural history of SARS-Cov-2 related multisystem inflammatory syndrome in children (MIS-C): *Journal of Paediatric Infectious Diseases Society*. **6**:56-67.

20. Abu-sharab. B., Adwan G, Abu-safiya D, Jarrar N and Adwan, K (2004). Antibacterial activities of some plant extracts utilized in popular medicine in palestine. *Turkish Journal of Biology*. **28**: 99-102.

21. CDC. NNIS System (1999). National nosocomial infections surveillance (NNIS) system report, data summary from January 1990-May 1999. *America Journal of Infectious Contro*l. **27**: 520-532

22. CDC. Corona virus Disease 2019: Scientific brief: SARS-CoV-2 and potential airborne transmission. Centre for disease control and prevention. centre for disease control and prevention.

23. CDC. (2021). Underlying medical conditions associated with high risk for severe COVID-19: Information for healthcare providers. Center for Disease Control and Prevention.

24. Kavathekar, M., Bharadwaj, R., and Kolhapur, S.A. (2004). Evaluation of clinical efficacy and safety of pure hands in hand hygiene. *Journal of Medicine,* **12**(3): 49-55.

25. Ravikumar, K., Pratibha, L., and Kolhapure, S.A.(2005). Evaluation of the antimicrobial efficacy and safety of puerhands as a hand sanitizer: A prospective, double-blind, randomized and placebo-controlled phase III clinical trial. *Indian Journal of clinical practice*. **5**(10): 19- 27.