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Formulation of a green hand sanitizer from natural products

BY

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APPROVAL FORM

The undersigned certify that they have supervised, read and recommend to the Bindura University of Science Education for the acceptance of a research dissertation entitled:

Formulation of a green hand sanitiser from natural products

Submitted by Natasha Dzeka

In the partial fulfilment of the requirements for the Bachelor of Science Education Honours Degree in Chemistry.

(Student signature) ……. Date …….

(Supervisor signature) ……... Date……...

(Chairperson signature) ……... Date……...

DECLARATION FORM

I, Natasha Dzeka, do hereby declare to Bindura University of Science Education that this dissertation is my original work and all materials and academic sources of information other have been duly acknowledged. This work has not been submitted to any other academic institution for the purposes of an academic merit.

Signed ……. Date …….

DEDICATION

I dedicate this work to my family and friends who have helped me in every step of the way. My sincere gratitude goes to my colleagues for their support.

ABBREVIATIONS AND ACRONYMS

AIDS ……Acquired immune deficiency syndrome

HIV……...Human immunodeficiency virus

MIC……...Minimum inhibitory concentration

HTST……. High temperature short time

SA…… South Africa

MeOH……... Methanol

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ABSTRACT

This study serves to highlight the production of hand sanitiser and its formulation, using aloe Vera gel, Lippia javanica oil and ethanol. This research is based on the production of hand sanitizer from natural products, hence it allows the use of readily available resources, as a results in reduces the costs of production. This research also focuses on decreasing the spread of covid 19. The Aloe Vera gel was extracted by hand filleting method. The ethanol was produced from the fermentation of corn meal. The lippia javanica essential oil was extracted by solvent extraction method using methanol. This research indicates that *Lippia javanica* contains essential oils that are useful as an antimicrobial agent, Aloe Vera gel is useful because it has moisturising effect (humectant). Ethanol serves as the main reagent that kills presence of bacteria, viruses, fungi through protein denaturation. The ethanol, lippia javanica and Aloe Vera gel extracted was evaluated for their effectives using E coli and staphylococcus bacteria. The results showed that the ethanol, lippia javanica and the formulation of the green sanitizer inhibited the growth of the E coli and staphylococcus bacteria while Aloe Vera gel had the lowest inhibition. There the results showed that the green based hand sanitizer can inhibit the growth of the E coli and staphylococcus bacteria as a result it can be used to decrease the spread of COVID 19.

CHAPTER 1

1.0 INTRODUCTION

De Campos et al. (2011) reported that the genus *Lippia javanica* is made up of almost two hundred types of shrubs, herbs, and not large trees all over central and south as well as tropical Africa. The Lippia javanica is an upright woody herb that grows throughout which is about 4.5 m tall, and when it is crushed it gives off the smell of a lemon like when it is crushed and it has strong aromatic leaves. The stems have got a brownish colour, always erect and also spreading with small glands and small stiff tubercle-based whitish and small glands, and divided with inflorescences in almost every axil. Leaves are at the opposite are opposite or in circular of 3, blades lanceolate to densely and longer pubescent, circular and also wedge shaped at the base, and crenate-serrate on the margins except closer to the leaf bottom. Flowers appear as conical or oblong studs which are purple or not light red in fruit, they turn brown if the get dried. *Lippia javanica* comprises of antioxidants properties, phenolic compounds antiviral characteristics, antibacterial characteristics that do not allow the attack growth of viruses and bacteria's. The species of antioxidants we get from the *Lippia javanica* are excellent at removing out harmful chemical that naturally grow up in the body when it is affected by a fever disease. *Lippia javanica* contains a wide number of phytochemicals that were proven by the scientist and also shows that plant has wides range of pharmacological activities.

Aloe Vera belongs to the liliaceous family which consists of the green leaves that are joined to the stem in a rose like pattern. They are actually produced by the thick epidermis and the cuticle that is around the mesophyll, that is divide into chlorenchyma cells and thinner walled cells that actually result in the creation of the parenchyma of the fillet. Therefore, the parenchyma cells consist of a transparent mucilaginous jelly and this jelly is referred to as the aloe Vera gel. Aloe Vera juice has got a colour that is colourless, transparent water like juice obtained from the aloe leaves that are really fresh. It is said to be tasteless or bitter in taste and odourless as well. Aloe aloes is the name given to aloe Vera latex. Aloe Vera can be consumed in raw form or processed both internally or externally.

1.1 AIM

The study aims to formulate a green hand sanitiser using aloe Vera, *Lippia javanica* and ethanol obtained from fermentation of maize.

1.2 OBJECTIVES

- ✓ To produce ethanol through fermentation of corn
- ✓ To extract *Lippia javanica* essential oils by hydro distillation
- ✓ To extract and Aloe Vera gel
- ✓ To formulate a hand sanitiser from Aloe Vera gel, *Lippia javanica* and ethanol
- ✓ To evaluate antibacterial activity of the formulated sanitizer

1.3 STATEMENT OF PROBLEM

Sanitizers are playing a vital role in mitigating the spread of the virus. Therefore, its production should be cost-effective. This study seeks to produce a green sanitizer using natural resources, utilising readily available resources which would reduce the cost in the production of the sanitizer.

1.4 Significance of study

- ➢ Lippia javanica, corn and Aloe Vera are readily available around Bindura town therefore the raw materials are readily available.
- ➢ Producing hand sanitiser using aloe Vera and lippia javanica is cheap hence there is no need of buying expensive industrial chemicals.
- ➢ this study is of great importance, as it helps to minimise the spread of the virus due to the used of hand sanitiser which can terminate the viruses and bacteria

CHAPTER 2: LITERATURE REVIEW

2.0 INTRODUCTION

The chapter aims on highlighting the assessment of the theory that is required as well as the related work required to put this study together. The chapter also aims at presenting the work that has been previously done by other researchers, their opinions and effective contributions in the synthesis of alcohol based sanitiser in combination with other natural products (lippie javanica, aloe Vera and corn used for fermentation).

2.1 STRUCTURE AND PROPERTIES OF *LIPPIA JAVANICA*

Figure 2.1: *Lippia javanica*

Many phytochemical components and nutritional composition of the lippie javanica were established, these phytochemicals consist of volatile and non-volatile secondary metabolites which include iridoids, amino acids, alkaloids, flavonoids and triterpenes and many other minerals. L. javanica consists so many nutrients that are required in the body, these nutrients include proteins, fats, carbohydrates, vitamins and minerals have a wide variety the leaves of are a good s Lippia javanica are an excellent source of the minerals which include chromium, chromium iron calcium, cadmium, cobalt, zinc, selenium, magnesium, manganese.

Ca and Mg are counted as the most fully sufficient elements in tea plants. These mineral elements are extremely crucial in people's nutrition because L. javanica is consumed as herbal tea and is added to food. Some of the mineral elements that were discovered from L. javanica leaves are really needed by the human body for the restoration of the worn out cell tissues as well as strong bones and also teeth and building of the red blood cells and some other related tissues. L. javanica has got acceptable concentrations of the mineral elements which consist of zinc, magnesium, manganese, calcium and iron, in addition the javanica has got quite higher

phenolic content (Shikanga et al., 2010). The scientist discovered leaf extracts of *L. javanica* contains quite bigger phenolic content of 14.8 mg/g Gallic acid of dry weight than flowers that has got 9.9 mg/g and twigs which consist of 8.3 mg/g. in lippie javanica they are phenolic compounds are good that are antioxidants in exhibiting the medicinal properties for an instance antibiotic, anti-inflammatory, anticancer, and anti-allergic properties. caffeic acid and simple phenolic compounds and its derivative are some of the compounds that are found to be ore sent in lippie javanica. Pharmacological Activities Scientific studied on L. javanica pharmacological activities shows that it has a lot of pharmacological activities and these activities includes anticancer, antidiabetic, antimalarial, antimicrobial antioxidant, antiplasmodial and pesticidal effects and cytotoxicity activities. (Fouche et al.2008). Based on the literature, a number of terpenoid compounds that have been extracted from L. javanica are known to have antitumor properties. For example, linalool is known to have antitumor activity which plays a prevent against hepatotoxicity and the compound was found to have anti-inflammatory activities also. Research done by Zang et al. (2010) showed that limonene has got inhibitory effect on both pancreatic and mammary tumours. In addition, another terpenoid compound, α-pinene, is known to stop translocation of NF-κB or p65 protein into nuclei of LPS-stimulated THP-1 cells. The javanica also acts as an anti-diabetic, therefore the aqueous leaf extracts of L. javanica at the entire dose levels lowered the blood glucose levels in both oral and intraperitoneal routes. The flavonoids present in lippie javanica makes it possible for it to work as an anti-diabetic.

Antimalarial activity is also found in lippia javanica. Suliman et al. (2014) discovered that topical application of L. javanica the alcohol extract resulted in 76.7% protection against mosquitos that is anopheles Arabianises, and it protects for 4 hours. Lippia javanica has been used as a mosquito repellent by the rural communities in Zimbabwe for quite a long period of time. Some of the studies have proved that essential oils obtained from the species have extremely strong and long lasting repellent activity against female A. Arabianises. Research by (onyango et al 2010) portrayed that topical use of 5 mg/cm of L. javanica promotes 100% protection against Anopheles Aegyptus for approximately 8 hours. Mavundza et al. (2014) extracted ethanol and dichloromethane from the leaves of L. javanica for activity against A. Arabianises.

Antimicrobial Activities are also found in Lippia javanica therefore it is used mainly in the treatment of a large number of infectious diseases caused by microorganisms. (Viljoen et

al.2013) observed the antimicrobial properties of L. javanica by making use of the time at which it kills. The essential oil obtained was analysed using the disc diffusion assay on three respiratory pathogens that is Klebsiellosis pneumoniae, Cryptococcus neoformans, and Bacillus cereus. This research highlighted that the killing rate was greatest for K. pneumoniae followed by C. neoformans and very slight decrease of microbial populations was observed for B. cereus. The efficiency of L. javanica oil for K. pneumoniae indicated an execution rate within 30 minutes for the concentrations 0.25, 0.5, 0.75, and 1%, C. neoformans also indicated a killing rate for concentrations 0.5, 0.75, and 1% in a period of an hour, and the least concentration of 0.25% lasted for 8 hours before a bactericidal effect was observed while B. cereus literally indicated some decrease in colonies. The positive antimicrobial activity of L. javanica as shown by the time kill study could be attributed to linalool that averages between 65 and 70% in the yield plus it has got known antimicrobial properties. These research are in agreement or it links with the traditional use of L. javanica as herbal medicine for several range of bacterial and fungal respiratory ailments. Assement has already been done on the essential oil, piperitenone, screened from L. javanica for antibacterial activity on cultures of Bacillus subtilis, Staphylococcus aureus, and Escherichia coli using imipenem, cefazolin, and ampicillin as positive controls. The authors discovered that piperitenone to inhibit S. aureus and E. coli at 1% dilution. Acetone, hexane, and methanol leaf extracts and essential oil isolated from L. javanica really displayed that some activity against fifteen gram-negative and gram-positive bacteria with MIC values ranging from 1.5 to >12 mg/mL. A pure compound piperitenone extracted from L. javanica has got antibacterial activities against Acinetobacter calcoaceticus, Salmonella typhoid, and S. aureus making use of the dimethyl sulphoxide and kanamycin as controls, with MIC values ranging from 12 to 50 μg/Ml. L. javanica methanolic leaf extract has got anti-microbial activity against S. aureus, Enterococcus faecalis, E. coli, and Pseudomonas aeruginosa making use the serial microdilution method with gentamycin (Virbac) and acetone as a positive and also as a negative controls, respectively. Lippia javanica gave antibacterial activities with MIC values ranging from 0.13 to 0.42 mg/mL against the whole four pathogens. The obtained minimum inhibitory concentrations are positive, because the natural products with MIC values below 1 mg/mL are really regarded to be not proper. Lippi lactone was obtained from the ethyl acetate extract of aerial parts of L. javanica showed some activity against the S. aureus and E. coli at a concentration of 10 mg/mL. (Lekganyane et al.,2010) reported that the antibacterial activity of L. javanica acetone leaf extracts against E. coli, E. faecalis, P. aeruginosa, and S. aureus with MIC values ranging from 0.32 to 0.64 mg/mL and activity of the same species ranging from 127 to 253 mg/Ml altogether. When the Methanol

and water was extracted from the leaf was found that L. javanica exhibited some antiproteus activity against Proteus mirabilis and Proteus vulgaris with MIC values 7.5 mg/mL. (katerere et al.,2012) discovered and investigated that, the antifungal activity of aqueous and organic extracts of L. javanica utilizing a serial microdilution assay.

(Mujovo et al., 2010) evaluated that L. javanica compounds against a drug-sensitive strain of Mycobacterium tuberculosis utilizing the radiometric respiratory methods. Of all the screened compounds, jus only one triterpenoid carboxylic acid, euscaphic acid, exhibited ant mycobacterial activity with MIC value of 50 μg mL−1 against this strain. In the same research the leaf extract of L. javanica exhibited ant mycobacterial activity against M. smegmatis in a determination which actually utilised microdilution assay and rifampicin as control, in addition Acetone extract was the most extractable with MIC value of 0.47 mg/mL; it actually drawn out the antibacterial agents which was shown by the least MIC value.

(Mujovo et al., 2010) discovered that the tuberculosis can be treated using the L. javanica and also other respiratory ailments. Antiviral Activity. (Mujovo et al.,2010) discovered that the lippia javanica inhibited the HIV-1 reverse transcriptase enzyme due to piperitone. No enough information is known about the HIV RT activity of L. javanica extracts or compounds, but flavonoids are known to be active against viral RT and also as good inhibitors of the cellular alpha and beta DNA polymerase.

2.1 STRUCTURE AND CHEMICAL CONSTITUENTS OF ALOE VERA

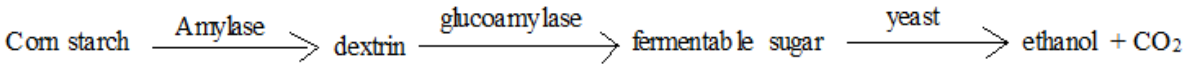
Figure 2.2: Aloe vera leaves

Joshi et al. (1998) had given the chemical constituents of aloe Vera barbadensis. The plant consists of 99% water with average pH of 4.5, the remaining solid material contains 75 different ingredients including vitamins, minerals, enzymes, sugars, anthraquionones or phenolic compounds, lignin, saponins, sterols, amino acids and salicylic acid. The main constituents of

aloe Vera leaves are anthraquinone complex, methanol-precipitable solids, polysaccharides glycoprotein, and salts of organic acids (Berghichen et al., 2008).

2.3 ETHANOL PRODUCTION FROM MAIZE MEAL

Maize known also as corn is graminaceous annual plant which is originated from America. Maize is a good source of energy however it has got poor protein content. It is the staple food of Zimbabwe hence it is locally and readily available. Fermentation is a process of converting simple sugars produced to ethanol and carbon dioxide under anaerobic conditions that is without oxygen. In addition, the enzymes added from certain strains of yeast which acts as a catalyst and in combination with the absence of oxygen are key ingredients for the disintegration of sugar and the production of ethanol and carbon dioxide. The distillation process is used after the fermentation is done. Distillation is used to separate ethanol from other components after fermentation, at a temperature of 78 ºC which is the boiling point of ethanol. The equation of fermentation is:



2.4 PROPERTIES OF HAND SANITISER

Hand based sanitizer typically contains ethanol, isopropyl alcohol, or n-propanol with percentages of 60% and 95% alcohol as the most effective. Sanitizer kills various types of microorganism but certainly not pores. In addition, compounds such as glycerol can be added to prevent drying of the skin. Some other versions of hand sanitiser contains fragrances, but they are not really urged as it has risk of allergic reactions (Napoli et al., 2009). There are also non-alcohol based hand sanitizers which contains benzalkonium chloride or triclosan, but are less effective than sanitizers with alcohol.

The alcohol destroys germs through denaturisation. This process occurs when alcohol molecules bond with the fat membrane covering a virus or a bacteria cell. As the fat is disintegrated, the inside of the cell consisting all its essential components becomes exposed as a result it begins to dissolve and immediately dies. Alcohol based hand sanitizer can eliminate bacteria such as E. coli, salmonella, and staphylococcus aureus, enterococcus faecalis. It has been proven that alcohol can kill viruses such as herpes, hepatitis B, HIV, influenza, rhinoviruses and coronaviruses. (Merkel et al., 2012).

CHAPTER 3: METHODOLOGY

3.0 REAGENTS AND APPARATUS USED FOR *LIPPIA JAVANICA* TREATMENT

Table 3.1: Reagents and apparatus

Reagents	**Apparatus**
❖ Distilled water ❖ Lippia javanica ❖ Methanol	❖ Distillation unit ❖ Measuring cylinder ❖ Thermometer ❖ Blender ❖ Watch glass ❖ Oven ❖ Heating mantle ❖ Clock ❖ Shaker ❖ Filtering cloth ❖ Beaker

3.1 *LIPPIA JAVANICA* COLLECTION AND PREPARATION

Fresh *L. javanica* leaves were harvested around the Bemberero High School Mashonaland Central Province. The leaves were removed from their branches and then washed with distilled water, the initial weight of the leaves before drying were measured and the mass was 65 g. After washing the leaves were spread over a clean tray and were spread evenly. The tray was placed in a closed room where sunlight do not penetrate. The leaves were dried at room temperature in which temperature ranged from 22 ^{0}C to 25 ^{0}C for 2 days. After 2 days of drying the mass of the uncrushed leaves was measured and was 55g, the leaves where grinded into fine smaller pieces using a blender. After crushing the leaves weighed 50 g.

50 g of the dried *L. javanica* leaves were soaked in 150 ml of methanol and placed on a shaker, shaking for 48 hours. After soaking the mixture was filtered into a 250 ml beaker using a cloth the distillation was done for about 2 hr in an all-glass Clevenger apparatus, with heat supplied to the heating mantle at 60 ^{0}C the essential oil was extracted with a volume of 150 ml of methanol, after 1 hr the distillation was stopped until no further essential oil was obtained. After distillation the methanol extracts was evaporated at room temperature in the fume hood for 48 hr. The yield of the *Lippia javanica* essential oil was determined using the following equation

$$Yield = \frac{weight\ of\ dried\ leaves\ before\ extraction\ -\ Weight\ of\ the\ dried\ essential\ oil}{weight\ dried\ leaves\ before\ extraction}$$

3.3 REAGENTS AND APPARATUS USED FOR ALOE VERA TREATMENT

Table 3.2: Reagents and Apparatus

Reagents	**Apparatus**
Citric acid Aloe Vera leaves Distilled water	Thermometer Heating mantle PH meter Reagent bottle Measuring cylinder Balance Conical flask Beaker Filters Filtering paper

3.4 SAMPLE COLLECTION AND TREATMENT OF ALOE VERA

The aloe Vera leaves were collected from ZAOGA Mountain in Bindura near the Cathedral. The aloe Vera leaves were undamaged and were not rotten and the leaves were mature. The leaves were treated according to the reaction scheme in Figure 3.3. The leaves were washed with distilled water to remove the surface dirty. The outer layer of about 800 g of the aloe Vera leaves was removed by peeling off. The leaves where squeezed to obtain the gel into a 500 ml

plastic clean beaker. Due to the reaction of enzymatic browning, the longer the time taken to gather the fresh gel from the leaves the more the browning index in Aloe Vera gel juice. Therefore, this process should be shortened within 10-20 min in order to avoid the enzymatic browning reaction of Aloe Vera gel.

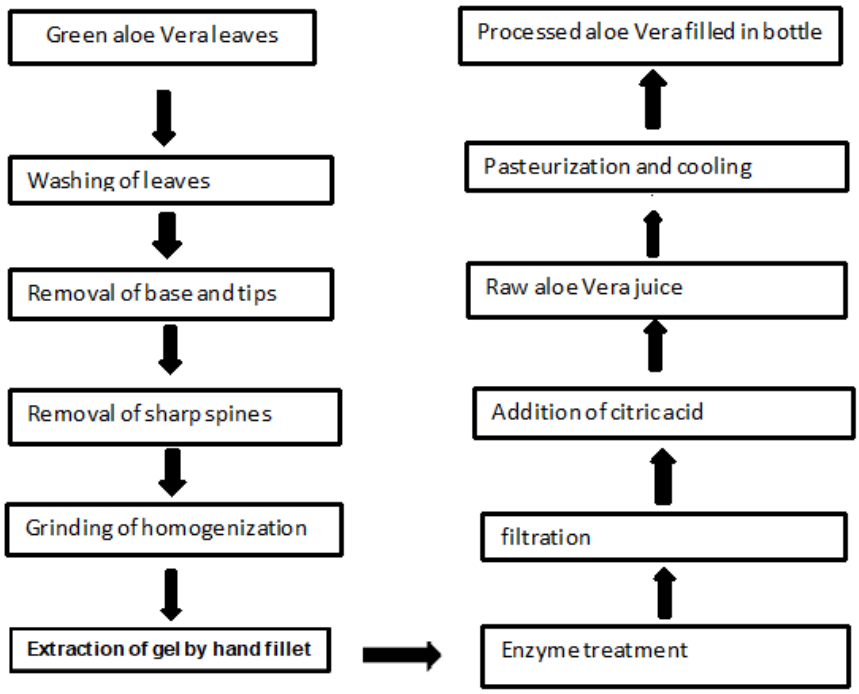


Figure 3.3: Aloe Vera gel extraction

The raw juice of the Aloe Vera obtained above was filtered using filtering paper. The sedimentation of particles was observed which resulted in the lowering of the filtration process. After filtration was completed the volume of aloe Vera gel obtained was 250 ml and its initial pH was measured using a pH meter. About 100 ml of lemon juice as a source of citric acid was measured in a measuring cylinder. The pH of the citric acid was measured before being added to the aloe Vera gel. After the citric acid has been added to the Aloe Vera gel the final pH of the mixture was measured using the pH meter. The pH of aloe gel juice was adjusted between 3.0 and 3.5 by adding of citric acid. The aloe Vera juice was de-aeration to prevent the oxidation of citric acid. This stage improved the shelf life of *Lippia javanica*.

The 250 ml aloe Vera juice in citric acid was pasteurized by heating in a 250 ml conical flask to a temperature of 85-95 ºC on a heating mantle for 1-2 min

After pasteurization was carried out the juice was flash cooled to 5 °C or below within 10-15 sec and then stored in a sterilized glass container and refrigerated.

3.5 REAGENTS AND APPARATUS USED MAIZE FERMENTATION

Table 3.3: Reagents and Apparatus used

Reagents	**Apparatus**
❖ 2 kg Mealie meal ❖ Distilled water ❖ GC-358 Alpha amylase ❖ Urea ❖ Yeast ❖ G-enzyme 480 glucose amylase ❖ HCl ❖ NaOH ❖ Ampicillin ❖ Yeast nutrient	❖ Thermometer ❖ Pot ❖ Measuring cylinder ❖ Balance ❖ Stirrer ❖ Clock ❖ Filter paper ❖ Funnel ❖ Hot plate ❖ PH meter

3.6 FERMENTATION OF MAIZE MEAL

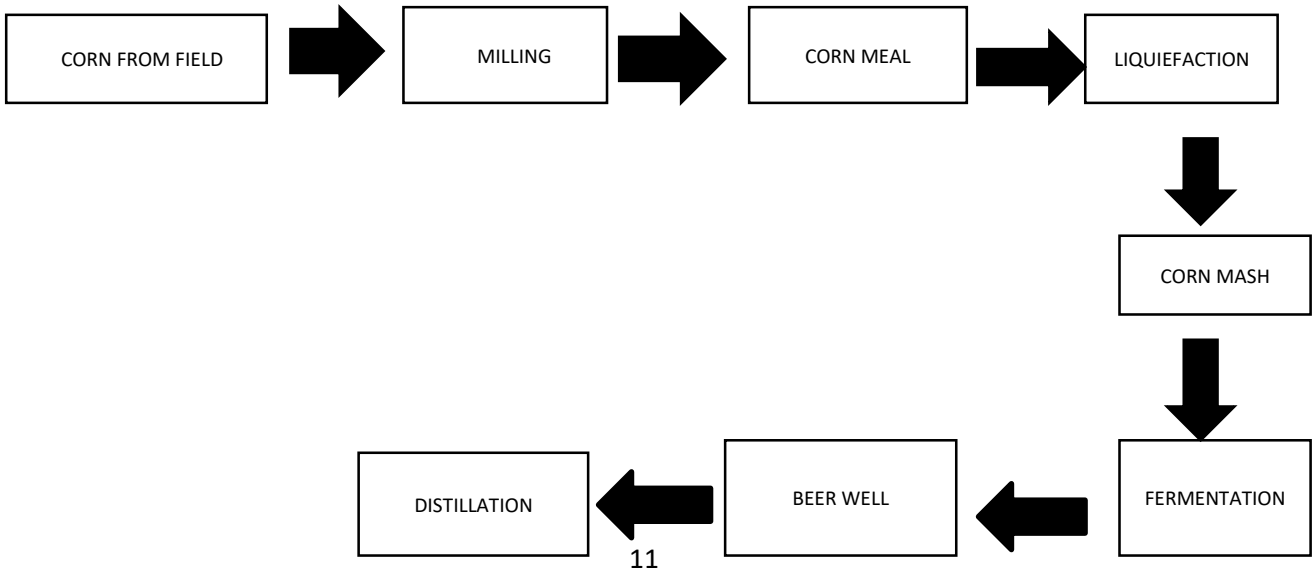


Table 3.4: Stages in fermentation of maize meal

Stage	**Description**
Grinding	A hammer mill is utilized to do the grinding. The grinding allows the reduction of tough outer coating of the corn kernel so as to increase the surface area of the starch.
Cooking or liquefaction	Gelatinization is another name given to this stage. The starch in the corn interact with the water when temperature is above 60 ºC and a viscous suspension is formed. The liquefaction is also known to be a partial hydrolysis that reduces the viscosity. It very crucial in breaking down the longer starch chain into smaller starch chains. At this very stage the alpha amylase enzyme is introduced which plays as the internal α- (1,4) -glyosidic bonds to yield dextrin and maltose
Saccharification	It is a process of making ethanol and also of further hydrolysis to glucose, monomers and glucoamylase enzyme is used as a result it splits both α-(1,4)-glyosidic bonds and α- (1,6) -glyosidic bonds from dextrin ends to form ethanol.

Fermentation	This the final stage in the synthesis of ethanol from the starch. The reaction of fermentation is 1 mole of glucose yields 2 moles of ethanol and 2 moles of carbon dioxide. Yeast was added that is saccharomyces cerevisiae which is a unicellular fungus that is needed during the fermentation process. Ammonium sulphate was added as a source of nitrogen, to convert proteins to amino acids to add an additional yeast nutrient. Then ampicillin was added as an antibiotic
Distillation	This is separation method which is done to increase the concentration of ethanol.

The equation of fermentation is:

$C_2H_{12}O_6 \rightarrow 2\ C_2H_6OH + 2CO_2$

2 kg of mealie meal was measured and placed in a plastic container. 15 L of de-ionized water was measured using a measuring cylinder. To a huge clean pot 15 L of the measured water was added and heated to 90.6 ^{o}C. The hot water was added into the measured mealie meal slowly and then mixed until it is completely mixed. The mixture of mealie meal and hot water is now called corn mash. When temperature of corn mash was between 75-85 degrees 2000 µL of GC-358 alpha amylase enzyme was added. The corn mash was transfer into a pot and placed on a hot plate and was heated at 83.3-85 ºC for 15 minutes. After 15 minutes the pot was removed from the heat and allowed to cool at room temperature till the next day.

Before getting ready to do the fermentation, a small sample (25 mL) of corn mash was taken to measure pH of sample before fermentation. If the PH of the corn mash is higher or below 5 diluted NaOH and sulphuric acid was used to maintain the PH. 20 mL of 0.5% (w/v) ampicillin was added to the corn mash ready for fermentation to minimize bacterial contamination of the corn mash. To the corn mash 4 g of Urea (Nitrogen source) was added and 2000 µL of yeast nutrient was also added. After addition of urea to the corn mash 40 g of dry yeast was added also to the corn mash followed by 10 ml of G-enzyme 480 (**Glucoamylase**) (10% solution). After the addition of these reagent the top of the container was sealed completely. Fermentation was done for 72 hours at room temperature. Figure 3.4 shows a sample of corn mash during fermentation, distillation of the obtained solution and distilled ethanol

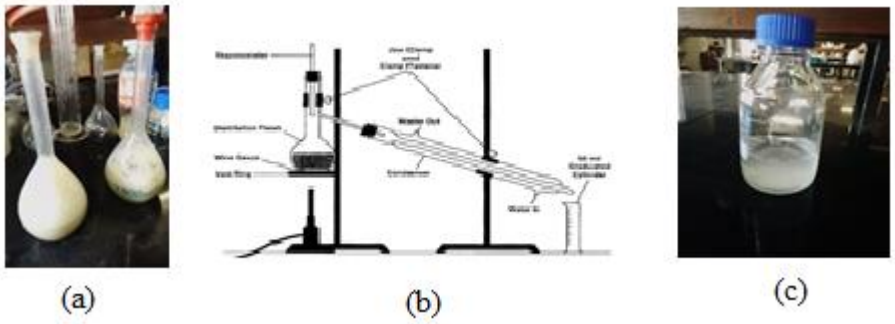


Figure 3.4: Corn marsh during fermentation (a), distillation (b) and distilled ethanol (c**)**

3.7 ANTIBACTERIAL ACTIVITY OF FORMULATED GREEN SANITISER

Reagents and apparatus

Mannitol salt, Nutrient agar, Nutrient broth, conical flask, burner, test tube, incubator, sterile filter paper disc, sterile distilled water, samples to be tested (Aloe Vera juice, WHO sanitizer, lippia javanica, fermented ethanol and green sanitizer), forceps, alcohol in a beaker covered with foil, incubator, petri dish, tongs

Preparation of culture

3.2g of nutrient broth was measured in a 250ml conical flask. Also 7g of nutrient agar was measured in a 250ml conical flask and 30g of mannitol salt agar was also measured in a 25o ml conical flask. All the three conical flask were filled with distilled water to top up to the 250ml mark. All the flask was stirred to allow complete dissolution of the reagents and there were placed on a flame while stirring for complete dissolution. After dissolution the dissolved nutrient broth, nutrient agar, and the mannitol salt agar they were placed in labeled test tubes,

about 9 ml of each reagent was placed in each test tube until all the reagents have been distributed into the test tubes and they were placed into an autoclave for sterilization at 121 degrees for about 15 minutes. The disc to be used were created by punching a filter paper and they were placed in a foil paper and placed in an autoclave for sterilization as well. The other equipment to used were also placed in an autoclave for sterilization.

After sterilization the sterile molten agar was held to the right hand side and the cap was then removed with the little left finger and neck was also flamed. The lid of the petri dish was lifted slightly and the molten agar was then poured in the petri dish and the lid was replaced. The dish was gently rotated to make sure the medium covers all the bottom of the petri dish and the plate was allowed to solidify. The media was stored at room temperature away for the direct sunlight.

Streak plate

The loop was used to prepare a streak plate, which includes the dilution of an inoculum of bacteria over the surface of the solidified agar medium in a petri dish that allows the maximum growth of the colonies separated from each other. The loop was inserted into the culture broth and was withdrawal. The lid of petri dish containing the solid medium was lifted slightly and the inoculation was smeared backwards and forward across a small area of the medium and the loop was flamed and allow to cool and the dish was turned through 90^0anticlokwise.

Inoculating the petri dish

1m1 of the inoculum was pipetted using sterile pipette into t the petri dish and the inoculum was gentle released into the center of the petri dish and the petri dish was closed. And the used pipette was placed in a discharge jar

Four sections on the base of the petri dish, were labeled. Sterile forceps were used the disc were dipped into the samples needed to be tested that is lippia javanica, Aloe Vera, WHO recommended sanitizer, fermented ethanol, and green hand sanitizer, the disc were drained on the side of the container and placed on firmly on the required section for each of the seeded agar plate. The forceps were rinsed and sterilized between each sample and to open the plate

for the minimum possible time. And the plate was sealed and also placed in an incubator at 25^0-30^0 for 48 hours

The results were collected after 48 hours, which were shown by the inhibition zones of each sample. The diameter of the inhibition zones was measured in mm and recorded.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 EXTRACTION AND YIELD OF *LIPPIA JAVANICA*

Table 4.1: Results of *Lippia javanica* extraction

Mass of *Lippia javanica* + beaker (g)	113.60
Mass of beaker (g)	108.98
Mass of lippia javanica extract	4.62

Yield of *Lippia javanica* was 9.24%. Figure 4.1 shows Lippia javanica before extraction, residue left after extraction and the extract.

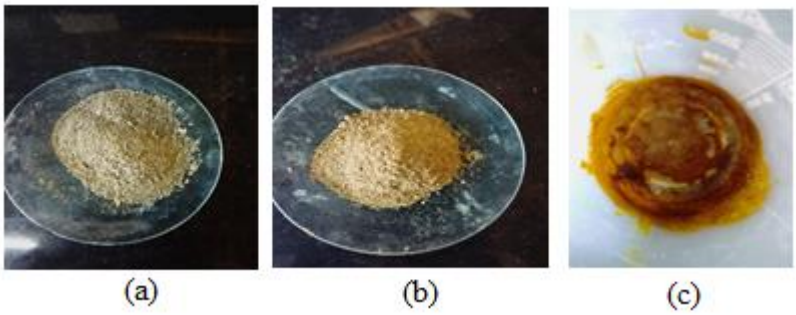


Figure 4.1: *Lippia javanica* powdered leaves before extraction (a), the residue left after extraction (b) and extract produced

Before extraction the crushed dried *Lippia javanica* had a green colour. The residue left after extraction was brownish in colour. The *Lippia javanica* oil extract obtained had a yellow-brownish colour. The intense of colour change increases as follows. Lippia javanica before extraction < residue left after extraction < the oil produced after extraction.

L. javanica is known for its essential oils that are volatile which are actually utilised for their antimicrobial properties. *Lippia javanica* essential oil was extracted using solvent extraction method, the leaves were dried at room temperature, to prevent the decomposition of essential components. In the extraction, methanol was used, to screen for the polar compounds which includes flavonoids, diterpenoids also triterpenoids. Triterpenoids are known to be tetracyclic alcoholic compounds, which actually consists of 30 carbons which are distributed in certain plants. Lippa species contain harmful or toxic triterpenoids in low concentrations, however, using them in huge doses for a quite long time can have harmful effects according to Wky et al. (2013). Diterpenoids are products that possess round 20 carbon atoms. Diterpenoids has

anti-microbial and anti-bacterial properties, however they are toxic when used in large concentrations.

The plant consists of terpenes which can decompose at high temperatures such as 60 ºC. Therefore, the leaves can be oven dried at lower temperatures than 60 ºC. The percentage yield of *Lippia javanica* extract was 9.24%. The theoretical percentage yield of the essential oil when using MeOH extraction determined by a student at university of Johannesburg (2006) was 10.1%. The 9.24% was different from this value probably due to the fact that the samples were from different geographical environments with different weather conditions. The moisture content of the *Lippia javanica* leaves was calculated to be 0.15%.

4.2 EXTRACTION AND PROPERTIES OF ALOE VERA GEL

Amount of Aloe Vera juice obtained from about 800 g of the leaves was 250 ml with an initial pH of 5.47. 100 ml citric acid with an initial pH 2.33 was added to the Aloe Vera gel, to prevent it from decomposition and the final pH 4.20.

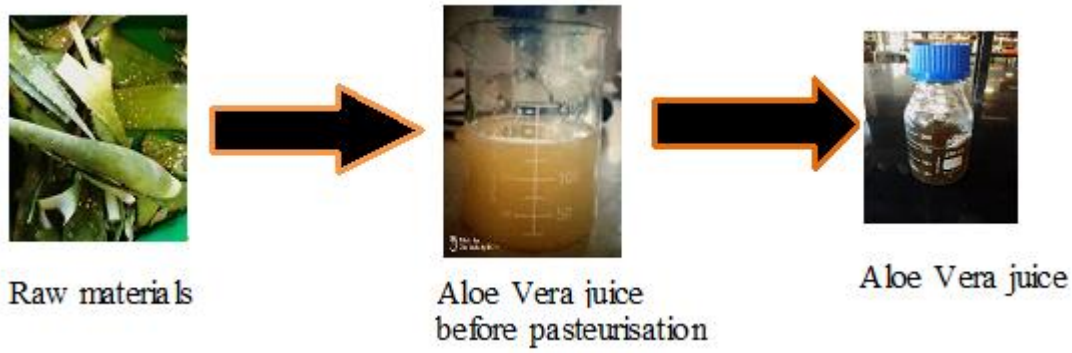


Figure 4.2: Extraction of Aloe Vera gel from the leaves

The aloe Vera gel was produced from the fresh aloe Vera leaves (Figure 4.2). The leaves were undamaged and were not rot so as to keep the active ingredients in full concentration. The temperatures of the environment were supposed to be below 25 ºC so as to prevent the Aloe Vera gel from browning. The citric acid was added mainly to stabilize the juice so as to prevent it from decomposing, pasteurization was also done so as to prevent the loss the biological activity of the Aloe Vera gel and to mask off the bad flavour. Then flash cooling was done to preserve the biological activity.

The gel obtained from the leaves was colourless but as the gel was exposed to air during processing the gel was slightly changing colour from colourless to off white and cream like.

The Aloe Vera gel was preserved using citric acid, from lemon juice because it has high concentration of citric acid among other fruits. Citric acid also inhibits the growth of bacteria. The pH of the aloe Vera gel decrease after addition of citric acid because the citric acid was too acid. After the pasteurisation at 85 ºC the colour of aloe Vera gel changed to brown. After the aloe Vera gel was produced it was stored in the refrigerator at 10 ºC as a way of preserving it.

4.3 ETHANOL PRODUCTION FROM MAIZE MEAL

Table 4.2 presents pH changes of the fermentation broth over the 3-day period.

Table 4.2: pH changes of the fermentation broth over the 3-day period

Day	**pH value**
1	5.05
2	4.34
3	4.21

The weight of the ethanol produced from 2 kg of maize meal was 644.79 g. This was a yield of approximately 32.2%.

Soluble sugars present in the fibre of corn mash are glucose, maltose, low concentration of monomeric sugars in the fibre because alpha amylase cannot hydrolyse starch to monomeric sugars in the fibre. The corn mash preparation for the fermentation process had an effect on starch and protein. They were hydrolysed that is starch into glucose and peptides into amino acids.

As the fermentation proceeds the pH of the fermented maize decreased as the number of days of fermentation increases. The pH change is as a result of the ethanol produced which is acidic. The fermentation process was further extended with 3 days due to lower room temperatures in the laboratory due to the winter season. During the experiment bubbles were formed as a result of carbon dioxide produced, in addition, the fermentation was completely sealed so as to promote anaerobic conditions. Presumptive test for the presence of alcohol was done on the fermented product before distillation, using potassium dichromate and followed by the addition of dilute sulphuric acid the changes from orange to green showing the presence of ethanol. The distillation was carried out at 78 ºC because the boiling point of ethanol. The ethanol produced after distillation was colourless and had a beer like smell which is shocking when inhaled.

Large amount of yeast that is saccharomyces cerevisiae was used to help accelerate the rate of fermentation of the corn sample. The volume of ethanol produced was 500 ml and the percentage purity of the alcohol produced was tested using a hydrometer at the lab which showed 75% of ethanol was produced. Therefore, this result suggested that the ethanol produced was highly pure as a result ethanol manufactured from corn is of high quality. The temperature of ethanol recorded was found to be 27degrees and its boiling was 78.37^0 as a result this showed that ethanol is partially volatile. The physical state of ethanol proved that ethanol is a liquid at room temperature. This process of fermentation of corn mash allows the detoxification through microbial binding or biotransformation of mycotoxins that is they will be made less toxic. Strains of lactic acid bacteria as well as yeast demonstrates detoxifying characteristics and their ability to remove toxins have been reported (Abuja et al., 2017). This is achieved by non-covalent bonding of mycotoxins by fraction of cell wall skeleton of lactic acid bacteria and yeast, and the pH and temperature influence the binding (pH 4, temperature 37 ºC).

4.4 GREEN SANITIZER FORMULATION

Formulation of a hand sanitizer based on WHO regulations requires 96% ethanol (8333 ml), 3% hydrogen peroxide (417 ml) and 98% glycerol (145 ml). The green hand sanitizer was formulated from the ethanol obtained by fermentation, Aloe Vera gel and *Lippia javanica* extract according to the scheme in Figure 4.3. According to literature the minimum inhibitory concentration (MIC) of terpenes in *Lippia javanica* is 64 mg/ml.

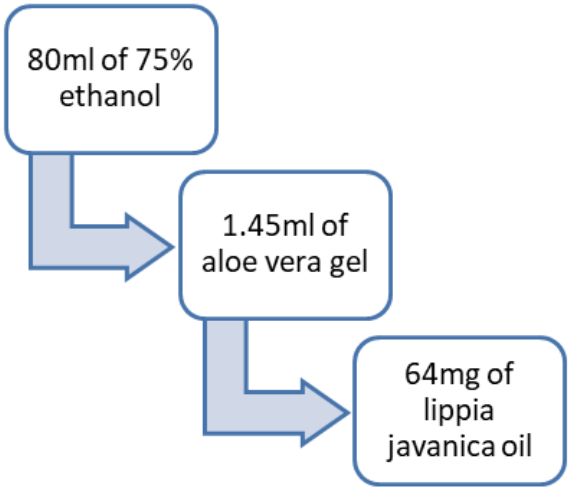


Figure 4.3: Formulation of a green hand sanitiser

The effectiveness of the formulated green hand sanitizer in killing microorganisms was tested on *Escherichia coli* and *Staphylococcus aureus.* The WHO recommended hand sanitizer was used as a reference. Figure 4.4 shows the sequence of steps towards testing for antibacterial activity.

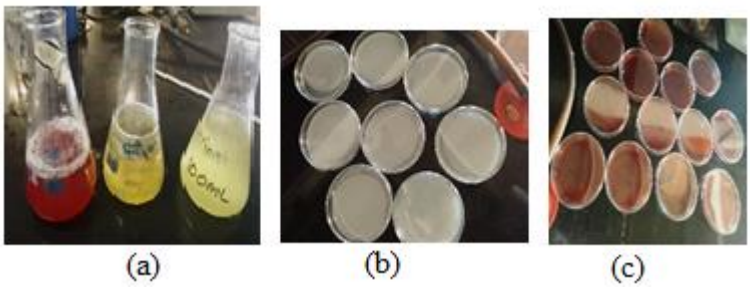


Figure 4.4: Samples (a) to be tested on *E. coli* (b) and *S. aureus* (c)

The agar diffusion procedure is used in many areas in the industry such as sensitivity of microorganism to disinfectants, antibiotic and antiseptics etc. the method involves preparing a spread plate of microorganism and adding little amount of test sample to the agar medium or a paper disc which is placed on agar surface. After incubation an inhibitory effect on the test organism is therefore indicated by the clear zone around the test sample and microbial growth is visible to the naked eye in areas of the plate that are not affected.

Escherichia coli bacteria lives in the intestines of the human body mostly. Most of the types of E coli are quite not harmful or cause slight diarrhoea. However, a few strains which includes E coli 0157:H7, can lead to stomach cramps also leads to severe diarrhoea, vomiting and nausea. Figure 4.5 and Table 4.3 shows the inhibition zones of *E. coli* by the different samples.

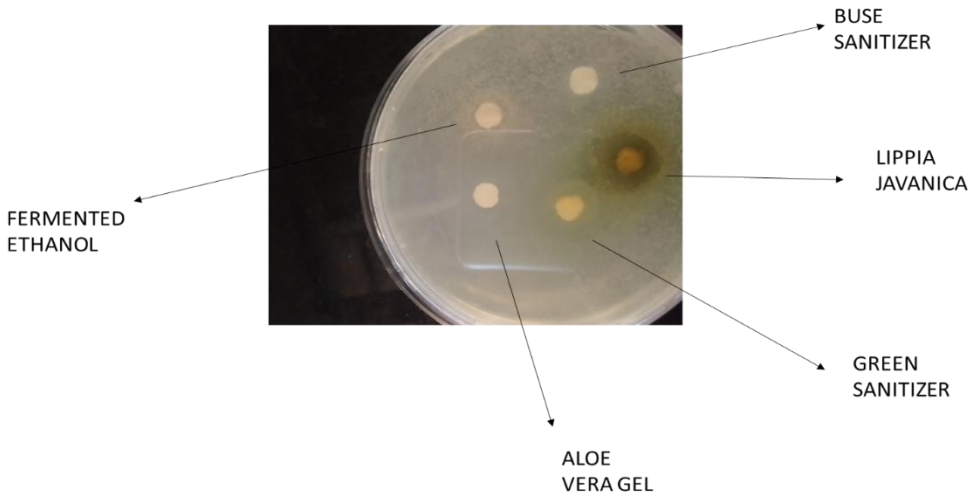


Figure 4.5: Inhibition of *E. coli* by different samples

Table 4.3: Inhibition zones of different samples against *E. coli*

Sample	**Diameter of the inhibition zone (mm)**
Lippia javanica	6.10
Aloe Vera	0.80
Fermented ethanol	8.00
Green sanitizer	9.10
WHO recommended sanitizer	13.00

Staphylococcus aureus is the deadliest among other staphylococcal bacteria. They are gram positive and spherical. They mostly cause skin infections and cause pneumonia, heart valve infections as well as bone infections. This type of bacteria can be spread by direct contact with the infected person using a contaminated object. Breathing in the infected droplets dispersed by coughing or sneezing. Figure 4.6 and Table 4.4 shows the inhibition of *S. aureus* by the different samples.

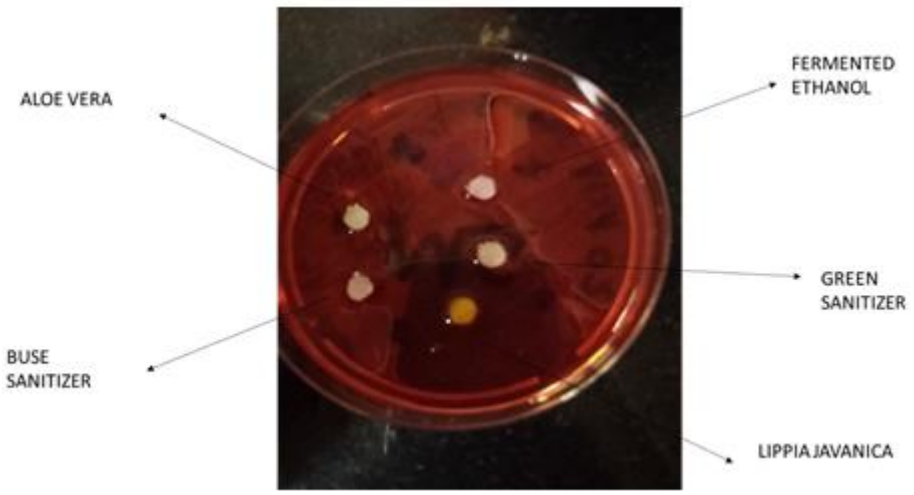


Figure 4.6: Inhibition of *S. aureus* by different samples

Table 4.4: Inhibition zones for different samples against *S. aureus*

Sample	**Diameter of the inhibition zone (mm)**
Lippia javanica	5.0
Aloe Vera	0.02
Fermented ethanol	6.0
Green sanitizer	7.0
WHO recommended sanitizer	12.0

The green sanitizer was evaluated using the biological microorganism so as to determine its effectiveness against *E. coli* and *S. aureus* bacteria. The samples showed that its inhibition on E. coli was greater than that from S. aureus as shown by the inhibition zone diameters above. The method used was disc diffusion method. The effectiveness was measured by identifying the inhibition zones of the samples that were tested for anti-microbial properties. Each disc was soaked in the sample for analysis so as to suck up sufficient amount of sample for analysis. The discs were placed on the bacteria that is *E. coli* an *S. aureus* and were labelled respective to each sample and placed in the incubator and results were collected after 24 hrs the results showed that the WHO recommended sanitizer was more effective followed by the formulated green sanitizer, fermented ethanol, *Lippia javanica* extract, then lastly the Aloe Vera gel. the distance in the inhibition zones where measure in mm and were plotted on the graph. The WHO recommended hand sanitizer was the most effective may be because it has 80 % ethanol and it contains 3.5 % hydrogen peroxide which is a disinfectant and hence more effective against bacteria. The green

hand sanitizer work by dissolving the lipid membranes of microbes, as a result the microbes will be inactivated.

CHAPTER 5: CONCLUSIONS AND RECOMMENDATION

5.1 CONCLUSIONS

The research was successful as the green hand based sanitiser was produced the aim of the experiment and the objectives were met. Adequate hygiene is among the important control strategies of lowering the direct or indirect transmission of the bacteria, therefore the use of hand sanitisers is becoming common due to their immediate action and efficiency in eliminating bacteria. The samples being investigated showed effectiveness on the *E. coli* and *S. aureus* bacteria. Conclusively the research proved that ethanol can be produced from food that we eat. The Aloe Vera gel was produced and preserved in citric acid and the essential oil of the lippia javanica was extracted using solvent extraction method, by utilising the readily available resources this research has proved that the sanitizer can be produced at lower costs. The green sanitizer was tolerated by the skin, as it increased the hydration of stratum corneum, due to the addition of the natural emollient (Aloe Vera) with the required ethanol that is good at acting as an anti-microbial.

RECOMMENDATIONS

The toxicity of lippia javanica needs to be investigated further, the green sanitiser viscosity can be increased. For production of higher ethanol percentage yield sugarcane bagasse or sugarcane juice can be used for fermentation, and during fermentation it is very crucial to note the PH, temperature, and amount of the corn meal. The PH of the substrate must be within the limits of the fermenter enzymes or else it will cause the destruction of the enzymes or it might reduce the ethanol that may be fermented, the temperature of the substrate should be between 30-45 degrees for the optimum yield. The aloe Vera gel is quite unstable therefore much care is needed during extraction. In addition, further findings are required to be implemented on this present strategy in industrial process, and economic demand. Further studies are needed on the testing of the green hand sanitizer on other types of microorganisms.

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