

ANTIBACTERIAL ACTIVITY OF EXTRACTS OF SOAP COMPONENTS AND TOILET DISINFECTANTS AGAINST DIFFERENT BACTERIAL STRAINS



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Submitted to

Mahatma Gandhi University, Kottayam

In partial fulfilment of requirement for the degree of

Bachelor of Science in Zoology

2023-24

**ANTIBACTERIAL ACTIVITY OF EXTRACTS OF SOAP
COMPONENTS AND TOILET DISINFECTANTS AGAINST
DIFFERENT BACTERIAL STRAINS**

CERTIFICATE

This is to certify that the project report entitled “ANTIBACTERIAL ACTIVITY OF EXTRACTS OF SOAP COMPONENTS AND VARIOUS TOILET DISINFECTANTS AGAINST DIFFERENT BACTERIAL STRAINS” submitted by Ms. Thrisha Antony, Reg No: AB21ZOO027 in partial fulfilment of the requirement of Bachelor of Science of Mahatma Gandhi University, Kottayam, is a bonafide work under my guidance and supervision and to my best knowledge, this is her original effort.

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EXAMINERS

1)

2)

DECLARATION

I, hereby declare that this project work entitled “**ANTIBACTERIAL ACTIVITY OF EXTRACTS OF SOAP COMPONENTS AND TOILET DISINFECTANTS AGAINST DIFFERENT BACTERIAL STRAINS**” is submitted to St. Teresa’s College (Autonomous), Ernakulam affiliated to Mahatma Gandhi University, Kottayam in partial fulfilment of the requirement of Bachelor of Science degree in Zoology. This work has not been undertaken or submitted elsewhere in connection with any other academic course and the opinions furnished in this report are entirely my own.

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ACKNOWLEDGEMENT

I would like to express my gratitude and appreciation to all those who have contributed to this project. First and foremost, I would like to thank my project guide Dr. Helvin Vincent for the invaluable guidance, support, and encouragement. Thank you for guiding us along to build a good project work, by providing all the necessary information and the best available resources.

I thank all the teachers of the department of Zoology, St. Teresa's college, Ernakulam who were always willing to encourage and help us in all our efforts. I also thank our non-teaching staffs for their whole-hearted support and help.

My project work would not have been a success without the constant encouragement from my parents and my friends. Their support has kept me motivated and focused on completing this project successfully.

Above all I would like to thank God Almighty for showering his blessings upon me abundantly and for giving me the strength, knowledge, ability, and opportunity to undertake and complete this project work.

THRISHA ANTONY

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ABSTRACT

The incidence of toilet infections has become common. Toilet hygiene and personal hygiene plays a major role in prevention of many infections including diarrhea and food poisoning. The effectiveness of toilet disinfectants used to clean the toilet as well as our personal hygiene supplements like soap helps in the prevention of these infections. This study aimed at demonstrating and assessing the antibacterial activity of different extracts of soap components (Honey, Amla and Lime) and Toilet disinfectants (Bleaching powder, Domex, Florex Phenoyl, Homemade disinfectant, Sanifresh and Texma) against 2 different bacterial strains (*Escherichia coli* and *Staphylococcus aureus*). The study was performed using Kirby Bauer disc diffusion method and well diffusion method. Extracts of soap components showed a zone of inhibition ranging from 1-2.1cm against *Escherichia coli* and 1.7-3.2cm against *Staphylococcus aureus*. The highest zone of inhibition was shown by Honey and lowest was shown by lime against *Escherichia coli*. In case of *Staphylococcus aureus*, the highest zone of inhibition was shown by Amla and lowest was shown by Lime. On the other hand, Toilet disinfectants exhibited zone of inhibition ranging from 0.6-3.6cm against *Escherichia coli* and 0.6- 4cm against *Staphylococcus aureus*. The highest zone of inhibition was shown by Sanifresh and lowest was shown by homemade by

Escherichia coli. In case of *Staphylococcus aureus*, the highest zone of inhibition was shown by Domex and Texma and lowest by homemade disinfectant.

INTRODUCTION

In this era of prevailing diseases, food poisoning is found to be a day-to-day menace. It is an illness caused by bacteria or other toxins in food, typically with vomiting and diarrhea. Not having a personal hygiene can cause germs and bacteria, such as *E. coli*, to stay on the skin and toilets play a major role in this exposure of microbes. Therefore, infections such as food poisoning may peak at high level if an affected user handles food with contaminated hands. The main types of bacteria found in food are *Salmonella*, *E. coli*, *Shigella*, *Bacillus cereus*, *Listeria* and *Campylobacter*. These bacteria are more likely to affect children, senior adults and those with compromised immunity and is the major cause for food poisoning. The main symptoms of food poisoning is diarrhea. These contaminations are mainly due to the poor personal as well as bathroom hygiene. Diarrheal disease is the second leading cause of death in children under five years old, and is responsible for killing around 525000 children every year. In India, Diarrhea is the third most common cause of death in

under-five children, responsible for 13% deaths in this age-group, killing an estimated 300,000 children each year. Flushing the toilet with the lid up can spray diarrhea-causing bacteria into the air and surrounding regions such as hand flush, toilet seat, floor etc. According to NHS germs like *Salmonella*, *E. coli* can survive in the washroom surfaces for as long as four hours. So, when these regions are touched by a person could cause spreading. Bacteria remain active on the skin for up to 2 hours.

E coli, *Salmonella* and *Staphylococcus aureus* are three of the biggest bathroom culprits. These germs and bacteria are found across bathroom surfaces like toilets, bathroom floors and shower curtains. On average, a toilet bowl contains 3.2 million bacteria per square inch, including germs in toilet water. This is in addition to the bacteria found on the other parts that may have come into contact with, such as the flush handle covered in as much as 83 bacteria per sq. This is where the relevance of toilet disinfectants and soaps (personal hygiene) comes into play. Cleaning agents or hard-surface cleaners are substances used to remove dirt, including dust, stains, foul odors, and clutter on surfaces as well as microorganisms. Cleaning agents with antimicrobial agents play a major role in the killing of disease-causing microorganisms hence reducing the rate of infection as well as prevents spreading. Using any cleaning agent is not the solution but rather usage of an efficient one which has antibacterial and antimicrobial one is the key. Prevention of such microbial diseases not only require cleaning of the space but also require cleansing of our body especially hands and this leads to the importance of soaps for the same and the effectiveness of these are also to be considered. So, study is required in order to identify the most effective cleaning agents (commonly used in households) available in the market.

REVIEW OF LITERATURE

The circulation of infectious diseases in the community settings in urban and rural areas remains to be a Hectic problem. One of the sources of microbial diseases is toilets. Improper use of the toilets, inadequate cleanliness of the toilets facilitates can transmit bacteria from the toilets to the Household living rooms. Contaminated hands of toilet users can transmit the bacteria from their hands to the flushing handles, door handles and faucets of the toilets as well as household door

handles and equipment. Toilet seats and lids, the surrounding floors, and the nearby surfaces can be contaminated by toilet flush aerosols which are produced in substantial quantities during flushing (Barker and Jones, 2005). Bacteria from public restroom are of public health importance when they enter the body through hand to mouth contact or hand to food contact leading to diseases (Sabra, 2013). The diseases which can be contracted through the use of restrooms include; boil and food borne diseases (*Staphylococcus aureus* and *Escherichia coli*), Urinary tract Infections (UTI) and diarrhea (*Escherichia coli*, *Pseudomonas aeruginosa*) and sore throat (*Streptococcus pyogenes*) (Peleg and Hooper 2010; Schmidt and Brubaker, 2004). To reduce the risk of bacterial infection from the toilets, regular hand washing, thorough daily washing and cleaning of public restrooms with disinfectants (at least twice daily) is particularly recommended for infection control programs (Boyce, 2007). The ability of the pathogen deposited to survive on the different surfaces in the toilets poses a great risk of infection to the toilet users (Boone and Gerba, 2007). The time of survival depends on the type of pathogen, majority including *Shigella* species, *Escherichia* species, *Clostridium* species, severe acute respiratory syndrome (SARS) coronavirus, and norovirus which can survive on surfaces for weeks or even months (Kramer *et al.*, 2006).

Skin bacterial flora consisting of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* and *Pseudomonas aeruginosa* can frequently cause either skin or systemic infection including the bacterial transmission in healthcare units and household (Pilanthana *et al.*, 2014).

Disinfection of contaminated surfaces in the home both reduces the overall level of contamination and prevents the redistribution of bacteria to other surfaces via contaminated cloths or sponges or cutting boards. Antibiotic-resistant pathogens have emerged in recent years as a major problem among both community and health care-associated infections. (William *et al.*, 2023).

Lime in its natural state is widely used in West Africa, particularly in Nigeria. From information gathered from market study in the southwest part of Nigeria, lime is very much employed in herbal medicine (Ibukun *et al.*, 2007).

Honey is a substance produced by bees to store as a sugar food source, collected as nectar from flowers (and occasionally from the sap of plants). Since the earliest recorded times, humans have taken honey for use not only as a food product, but also as a medicine, especially for wound care (Zumla and Lulat, 1989). The bees concentrate the dilute sugar solutions they collect from the plants by evaporating off most of the water (honey is typically 17% water and 80% sugars). They also add enzymes, so that as the honey ripens in the comb, its composition changes and it becomes impossible for microbes to grow in it and spoil the stored food. One of these enzymes converts sucrose, the major sugar in nectar and sap, into a more soluble mixture of glucose and fructose. The sugar molecules in solution bind up water molecules, thereby denying microbes the water that is essential for their survival. Another enzyme added is glucose oxidase, this converts some of the glucose to gluconic acid, making honey too acidic for microbes to grow (honey has a pH of about 3.5), and as a by-product of this reaction, forms hydrogen peroxide. This is a sporicidal antiseptic that sterilizes the honey that is sealed in the comb (when subsequently extracted from the comb, Honey can be contaminated with microbial spores as the enzyme that produces hydrogen peroxide is inactive at that time because there is insufficient free water, these can survive the acidity and high sugar content). These factors, which ensure the preservation of honey in the comb, are also

useful in suppressing microbial growth when honey is applied to wound (Jeremiah and Saaondo, 2023).

Emblica officinalis Gaertn. Or *Phyllanthus emblica* Linn., popularly called as Indian gooseberry or Amla, is among main herbal plant in Indian traditional medicine. Different parts of *E. officinalis* are beneficial for curing various ailments but the fruits especially show tremendous pharmacological and medicinal applications. Phytochemical analysis revealed important bioactive chemical compounds such as tannins, alkaloids, polyphenols, gallic acid, ellagic acid, emblicanin A and B, phyllembein, Quercetin, ascorbic acids, vitamins and minerals. Different extracts of amla possess potent antimicrobial activities to counter different bacterial pathogens. Amla phytochemicals also possess antioxidant, Anti-inflammatory, hepatoprotective, cardioprotective, immunomodulatory, hypolipemic, memory enhancing, anticancer, antidiabetic, antidepressant, anti-ulcerogenic, insecticidal, larvicidal, and wound healing activities. All of these well-established activities can have broad applications in managing most of the diseases affecting human and animal patients (Sandip *et al.*, 2019)

Antibiotics represent one of the most important drug groups used in the management of bacterial infections in humans and animals. Due to the increasing problem of antibiotic resistance, assurance of the antibacterial effectiveness of these substances has moved into the focus of public health. (Voigt *et al.*, 2019)

METHODOLOGY

SAMPLES

Honey,Amla, Lemon, Domex, Sanifresh, Texma , Homemade disinfectant, Bleaching powder, Florex and phenoyl.

BACTERIAL STRAINS

Escherichia coli and *Staphylococcus aureus*

PREPARATION OF NUTRIENT AGAR FOR STERILIZATION

First the medium for the growth of bacteria is prepared. 200ml Nutrient agar medium is prepared by adding 2.6 g of nutrient broth and 4 g of agar to 200 ml of water taken in the conical flask. After mixing well it is plugged using cotton and covered using newspaper.

STERILIZATION OF MATERIALS

10 Petri plates required for the experiment is also covered using newspaper. Filter paper disc was prepared by punching the filter paper which was taken in a beaker and covered using newspaper. Buds were also taken in the same way. Nutrient Agar medium, Petri plates, filter paper discs, buds, etc were kept for sterilization in the autoclave at 15 lbs pressure and 121 degrees Celsius for 15 minutes. After the complete procedure of 45 minutes of sterilization it is cooled to the room temperature.

METHOD

Rest of the procedures was done inside the laminar to avoid the contamination. Before using laminar air flow, it was wiped completely using spirit and UV lamp was turned on for 15 minutes before the work. After hands were made germ free using spirit and on the presence of flaming Bunsen's burner Nutrient Agar medium was poured into plates. On each time of pouring the mouth of the conical flask is flamed in order to avoid contamination and mixed well before pouring. After pouring the plates was not completely closed it is kept in half closed state. Then the lid of the laminar was closed and UV lamp was turned on for 15 minutes which allows the setting of the medium. The plates were kept overnight in hot air oven for incubation. Samples of cleaning agents and the extracts of the certain materials were taken in the sample bottles.

Sample of *Escherichia coli* and *Staphylococcus aureus* was prepared a day before. The cleaning agents were added and tested using disc diffusion method under aseptic condition. The bacteria from the samples in the test tube is taken using the sterilized bud and swabbed in the petri plates. The mouth of the test tube is flamed before and after taking the bacterial sample from the test tube. Swabbing is done neatly by rotating the plates to ensure complete growth of bacteria.

DISC DIFFUSION METHOD (Bauer *et al.*, 1959)

Sample names and the bacteria used was labelled on the petri plates. By using a sterilized forcep, the filter paper disc was taken and soaked in the appropriate sample and placed in their position on the plates. Then the plates were covered using cling film and kept for incubation at 37°C for 24 hours.

WELL DIFFUSION METHOD (S Magaldi *et al.*, 2004)

The inoculation of the samples of extracts of soap components was done using the gel puncture method. Here after swabbing the bacteria in the plate, wells are made by using a gel puncture and by using the pipette and 2 µl of sample is taken and the well was filled using sterilized tip. The new tip was used for each sample. All the plates were wrapped using cling film and kept for incubation in the hot air oven for 24 hours.

EXAMINATION FOR ZONE OF INHIBITION

The next day the results are noted by taking the diameter in centimeter of the zone of inhibition. It is done by measuring the diameter using a scale.

All the materials used for the experiment were decontaminated in the autoclave and dried.

RESULT

OBSERVATIONS

EXTRACTS OF INGREDIENTS OF SOAP

Table 1: Table showing the diameter of zone of inhibition of different extracts of soap components against *Escherichia coli* and *Staphylococcus aureus*

Extracts	Diameter of zone of inhibition against <i>Escherichia coli</i> in cm	Diameter of zone of inhibition against <i>Staphylococcus aureus</i> in cm
Lime	1	1.7
Honey	2.1	3
Amla	1.5	3.2
Antibiotics	1.5	2.5

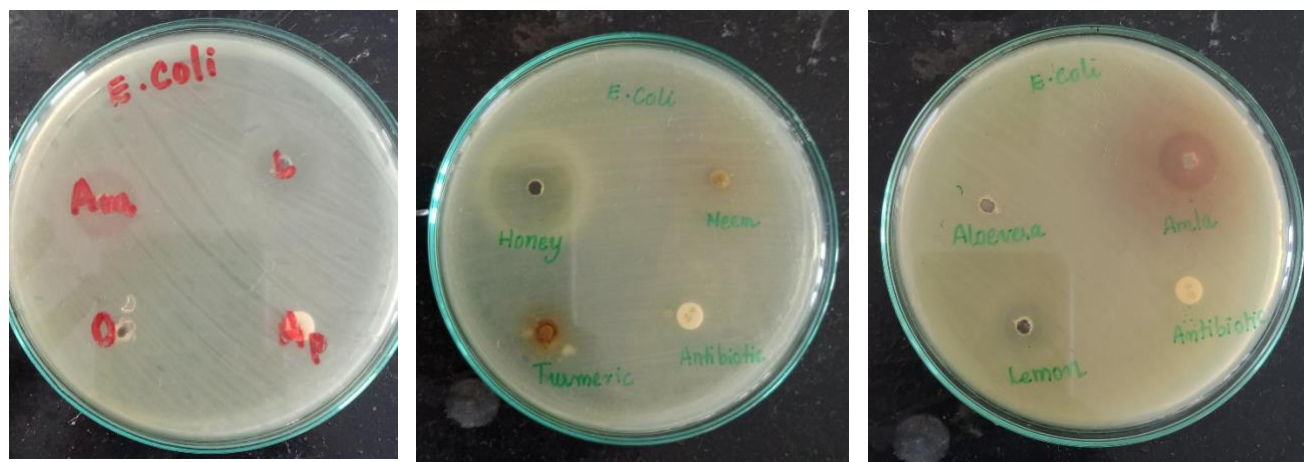


Figure 1: Photos showing the zone of inhibition of extracts of soap components against *Escherichia coli*

Figure 1 shows the antibacterial activity of extracts of soap components against *Escherichia coli*. Extracts of ingredients of soaps taken were Honey, Amla, Lime and antibiotic as a control. All of extracts had shown antibacterial activity against *Escherichia coli*. It was observed that the honey showed highest sensitivity, forming a zone of inhibition of 2.1 cm, followed by Amla and antibiotic

showing a zone of 1.5 cm each. It has been observed that the honey has more inhibition activity compared to the control antibiotics. The least sensitivity was observed in case of lime, with a zone of 1 cm. So, the honey has the most antibacterial activity among the compounds tested. The activity is in such a way:

Honey > Amla > Antibiotic > Lime

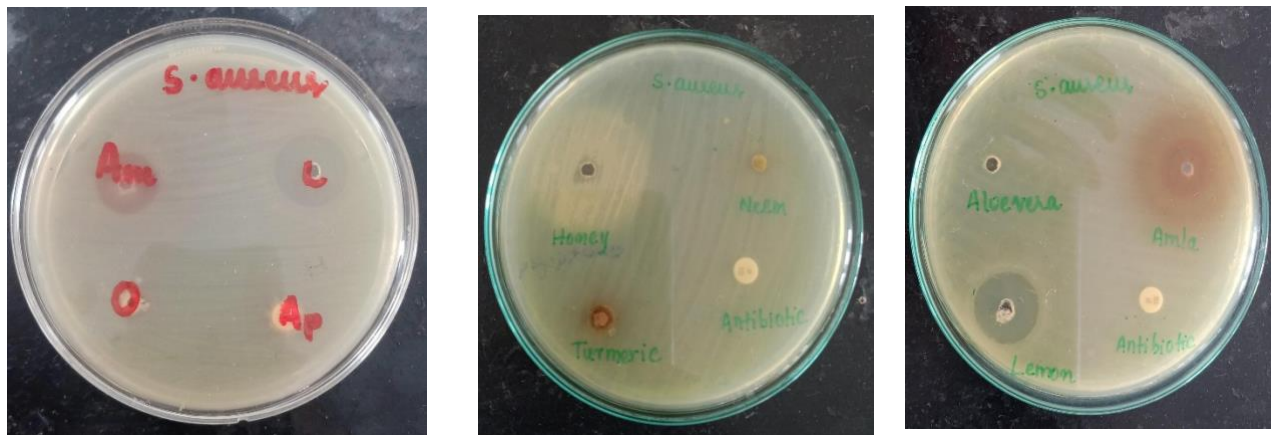


Figure 2: Photos showing the zone of inhibition of extracts of soap components against *Staphylococcus aureus*

In case of the antibacterial activity against *Staphylococcus aureus*, all of the extracts of soap components showed zone of inhibition. Amla showed highest sensitivity forming a zone of inhibition of 3.2 cm. Followed by Honey showing a zone of 3 cm. Then comes the antibiotics with a zone of inhibition of 2.5 cm. It is observed that the Amla and Honey has more antibacterial activity and higher sensitivity compared to the antibiotics taken as the control. The least sensitivity was observed in case of Lime extract, with a zone of 1.7 cm. The antibacterial activity against *Staphylococcus aureus* is as follows:

Amla > Honey > Antibiotics > Lime

In an overall scale the higher antibacterial activity was observed in case of Honey and the least was observed in case of Lime. In case of *Escherichia coli*, honey has the highest activity and Amla has the higher activity against the *Staphylococcus aureus*. Using soaps with a content of honey, Amla and lime are hence advised in order to avoid the above-mentioned bacteria, since it shows a considerable level of antibacterial activity.

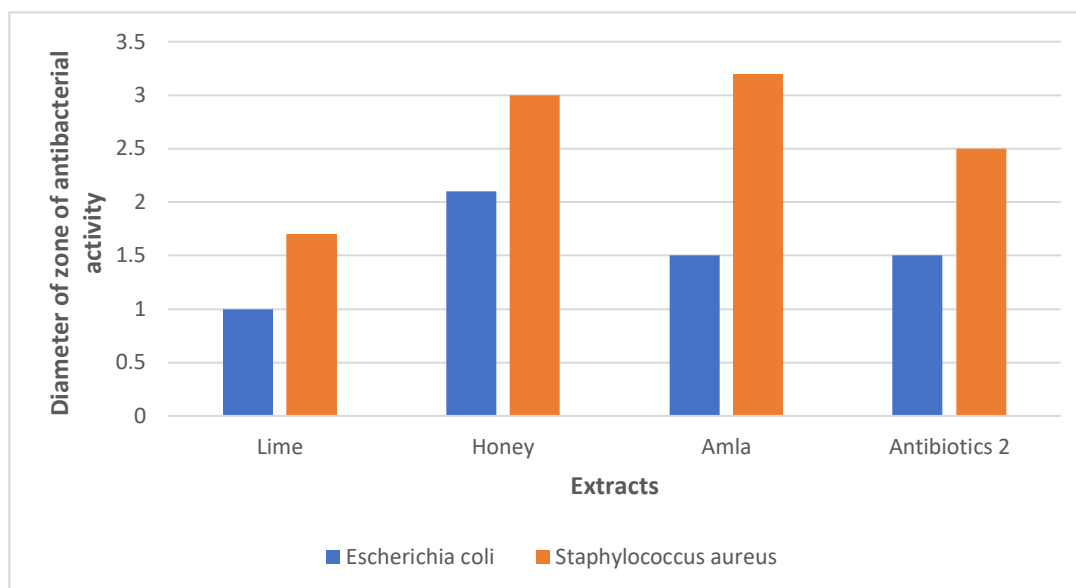


Figure 3: Bar graph showing the antibacterial activity of various extracts against *Escherichia coli* and *Staphylococcus aureus*

ANTIBACTERIAL EFFECT OF TOILET DISINFECTANTS

Table 2: Table showing antibacterial activity of various toilet disinfectants against *Escherichia coli* and *Staphylococcus aureus*

Compounds	Diameter of zone of inhibition against <i>Escherichia coli</i> in cm	Diameter of zone of inhibition against <i>Staphylococcus aureus</i> in cm
Bleaching powder	1	1
Domex	3	4
Florex	1	0.8
Phenoyl (C)	1	1.6
Homemade	0.6	0.6
Sanifresh	3.6	3.5
Texma	3	4
Antibiotics	1.9	3

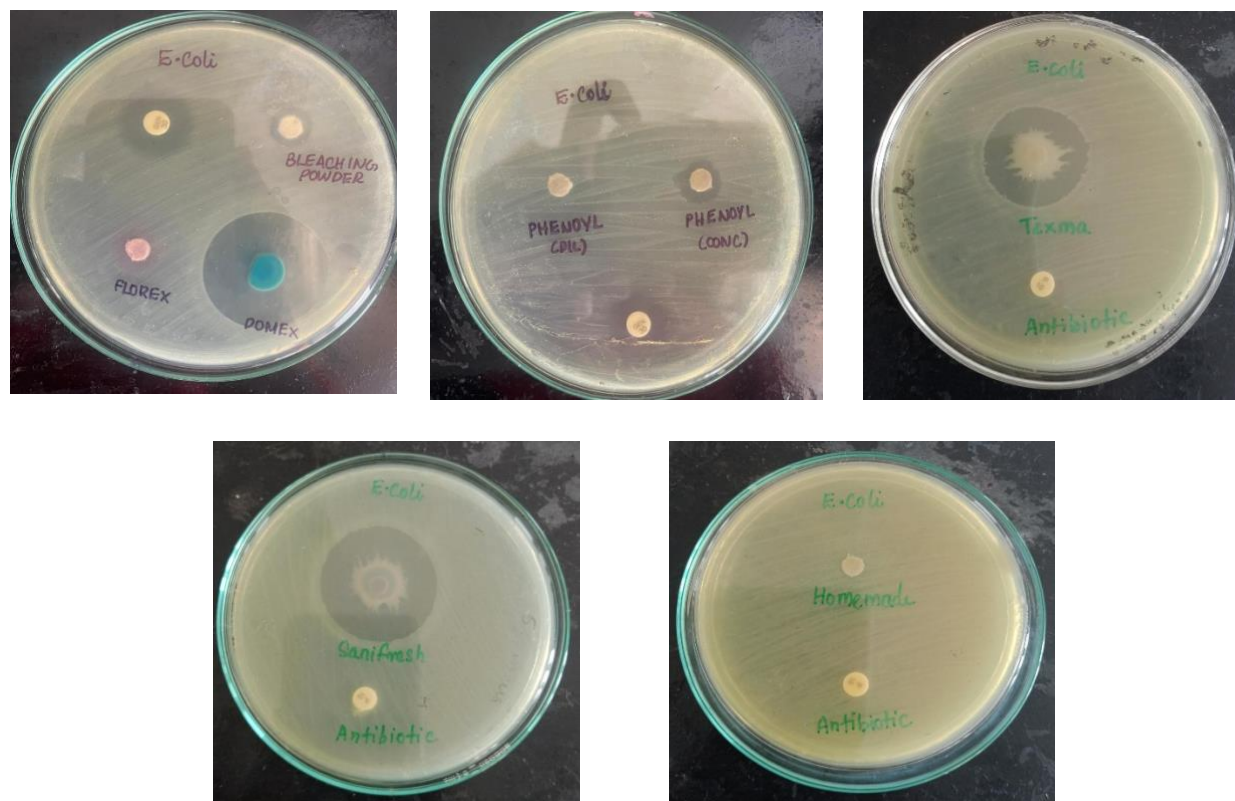


Figure 4: Photos showing the zone of inhibition of toilet disinfectants against *Escherichia coli*

The disinfectants taken were Bleaching powder, Domex, Florex, Phenoyl, Homemade disinfectant, Sanifresh and Texma. Antibiotic was also taken as a control. All disinfectants showed zone of inhibition against *Escherichia coli*. In case of *Escherichia coli*, the highest sensitivity was observed in Sanifresh with a zone of inhibition of 3.6 cm. Next to it Texma and Domex with a zone of inhibition of 3cm, followed by Bleaching powder, Florex, and phenoyl with a zone of inhibition of 1 cm and the least sensitivity was observed in case of Homemade disinfectant with a zone of 0.6 cm

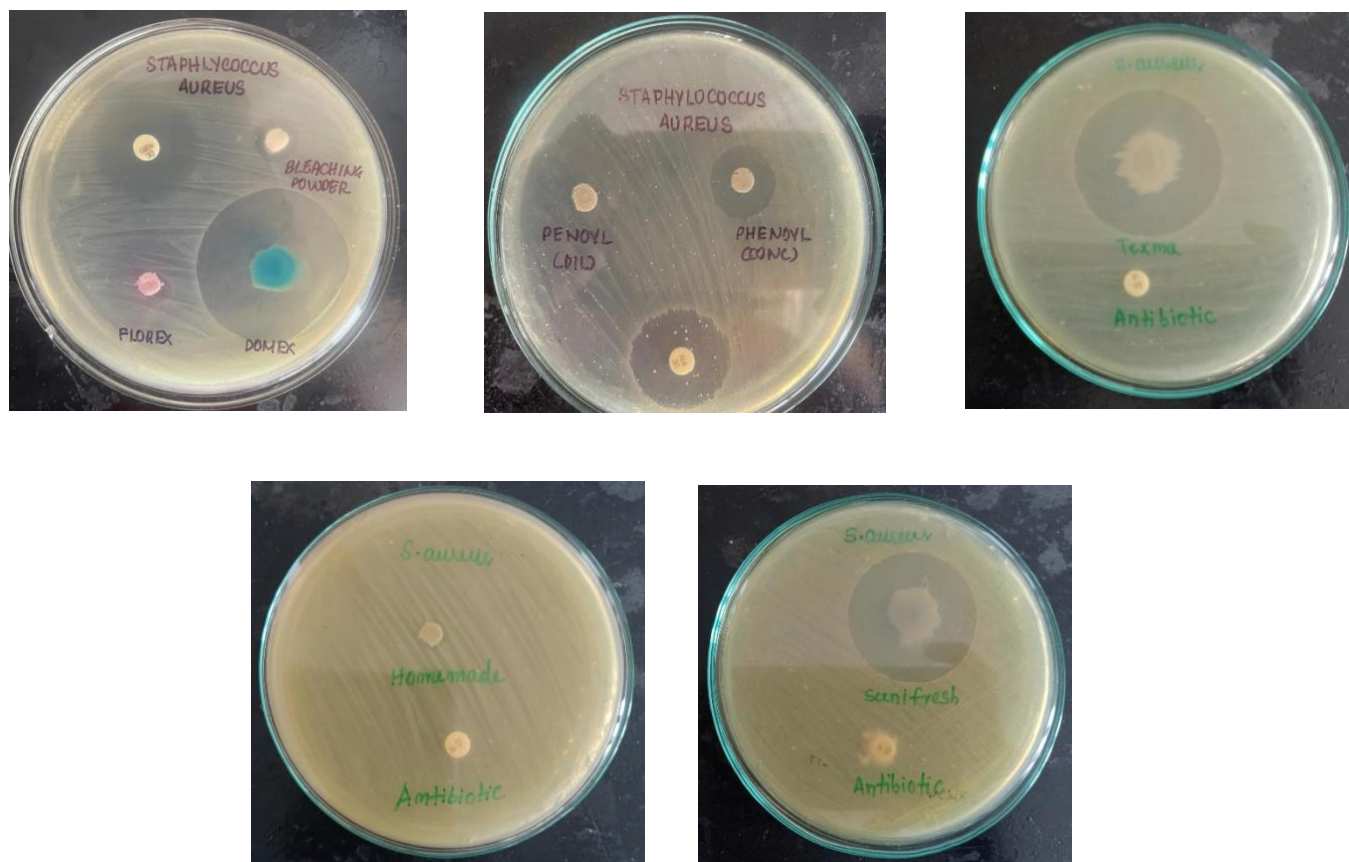


Figure 5: Photos showing the zone of inhibition of toilet disinfectants against *Staphylococcus aureus*

In case of *Staphylococcus aureus*, all the disinfectant showed zone of inhibition. The highest activity was shown by both Domex and Texma with an inhibitory zone of 4 cm, followed by Sanifresh with a zone of inhibition of 3.5 cm, followed by Phenoyl with a zone of inhibition of 1.6 cm. The least activity was observed in case of bleaching powder, Florex and homemade disinfectant with a zone of inhibition of 1cm, 0.8 cm and 0.6 cm respectively. Overall, the highest activity is observed in case of Domex, Texma and Sanifresh. The least activity was shown by Homemade disinfectant

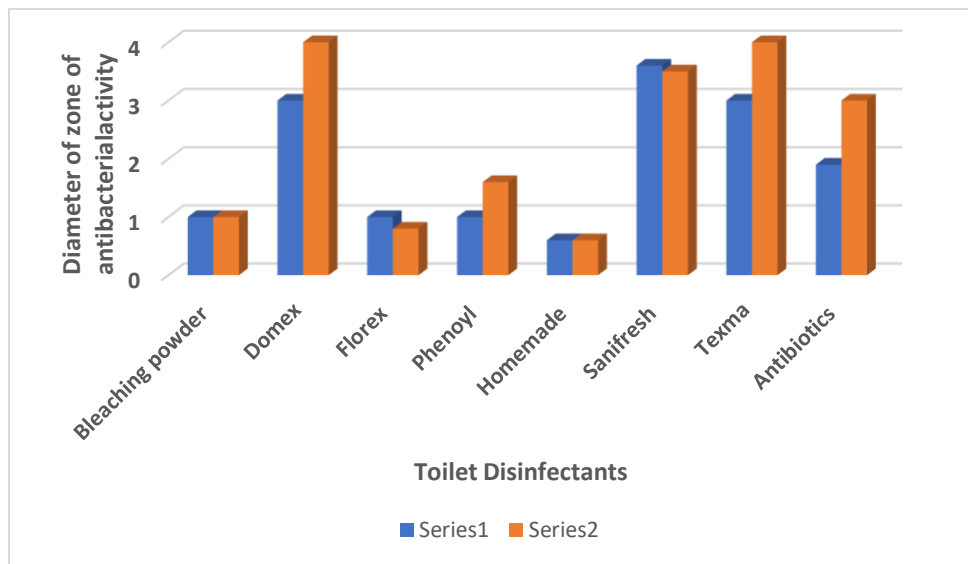


Figure 6: Bar graph showing the antibacterial activity of various toilet disinfectants against *Escherichia coli* and *Staphylococcus aureus*

DISCUSSION

The study was done for demonstrating antibacterial activity of various extracts of soap components and toilet disinfectants against *Escherichia coli* and *Staphylococcus aureus*. Disinfection describes a process that eliminates many or all pathogenic microorganisms, except bacterial spores, on inanimate objects and thus there is a need to find an effective disinfectant. Antibacterial soap can protect from commonly transmitted bacteria better than washing hands with non-antibacterial soap and water. Fewer bacteria on the hands may help prevent illnesses and result in fewer instances of cross-contamination of food and other household surfaces, so it is important to find the most perfect soaps in the market with the most efficient antibacterial component. All toilet cleaners are not antibacterial, research by other scientists has shown antimicrobial effects against *Staphylococcus aureus*, Influenza a flu virus, Rhinovirus, *Escherichia coli*, *Enterobacter aerogenes* and *Salmonella enterica*. Microorganisms are continuously acquiring resistance to new disinfectants, as a result, no single disinfectant will be appropriate for all pathogens (Tortora *et al.*, 1998). Therefore, it is necessary to evaluate the effectiveness of a disinfectant against a specific pathogen so that an appropriate agent can be easily selected.

Lime (*Citrus aurantifolia*) is a traditional plant that is widely used as antibacterial. It is used in many soaps as a key ingredient for fragrance well as for its antibacterial activity. In our study lime showed a zone of inhibition of 1 cm against *Escherichia coli* and 1.7 cm in case of *Staphylococcus aureus*. From previously conducted studies (Nerdy *et al.*, 2020), (Ibukun *et al.*, 2006), the lime had a zone of inhibition ranging 0.7-1.4 cm (*Escherichia coli*) and 0.7-2.2 cm (*Staphylococcus aureus*).

The antimicrobial activity in most honey is due to the enzymatic production of hydrogen peroxide. However, another kind of honey, called non-peroxide honey (viz., manuka honey), displays significant antibacterial effects even when the hydrogen peroxide activity is blocked. The zone of inhibition observed in our study was 2.1cm against *Escherichia coli* and 3 cm in case of *Staphylococcus aureus*. Previous studies (Steward *et al.*, 2023) showed a value of zone of inhibition ranging from 1.5-2.4 cm (*Escherichia coli*) and 1.3-2.3cm (*Staphylococcus aureus*) in 100% concentration. We also observed from other studies (Motior, 2010) that the zone of inhibition increases with increasing concentration and a concentration effective for one may not be effective for a different strain of bacteria. Honey of different botanical origin and geographical area showed wide range of variation in their antibacterial potency. The most potent honeys, such as Manuka, dark buckwheat, heather, or chestnut honeys have their MIC values ranging from 1% to 12.5% (Najla A. Albaridi, 2019).

The present study disclosed the importance of natural medicinal plant extract to control pathogenic bacteria which pose threat to human health and it also concludes that leaves of amla contain high antimicrobial properties. Antibacterial activity refers to chemicals that kill or limit the growth of bacteria on a local level while remaining non-toxic to surrounding tissue (Vikas *et al.*, 2023). In the previous studies (Lincy *et al.*, 2013). Amla showed an antibacterial sensitivity ranging 3-4 cm

against *Escherichia coli* and 1.5-3 cm against *Staphylococcus aureus*. The values we observed are similar to our study.

Public toilets can result in a buildup of pathogens within surfaces of the toilet and other areas. Surface contamination could occur within a short time of flushing. So, the disinfection of these surfaces using chemical and physical processes is essential. The efficiency of disinfectants varies greatly depending on various factors, some are specific for each disinfectant while others depend on the type of microorganisms. Disinfectants are chemical agents with an immediate and sustained activity which destroys microorganisms to such a level mandated for hygienic and surgical indication.

In this study, different types of toilet disinfectants had significant antibacterial activities. However, there were few which showed comparatively greater antibacterial activity and some showed lesser antimicrobial activity.

In the present study the highest activity was shown by Sanifresh. Its contents include Hydrochloric Acid and other ingredients like Water, Oleyl Amine Ethoxylate, Alkyl Trimethyl, Ammonium Chloride, Butylated Hydroxy Toluene, CL 61585, CL 45100. Hydrochloric acid is the main ingredient in most the disinfectant that prevents the growth of pathogenic bacteria.

The least activity was seen in case of homemade disinfectant against both *Escherichia coli* and *Staphylococcus aureus*. It may be due the natural consistency, less concentration and less amount of chemicals present in them.

In case of *Staphylococcus aureus*, the highest sensitivity was shown by Domex and Texma. It contains Sodium Hypochlorite, as recommended by the world's leading Health organizations for disinfecting surfaces. It removes tough stains and gives sparkling clean floors. Domex Disinfectant Floor Cleaner is proven to kill coronavirus instantly.

Sodium hypochlorite showed equal antimicrobial activity toward most types of bacteria and was the second effective antimicrobial agent. This is may be due to the fact that hypochlorite's preparations behave as wide spectrum with non-specific killing effects on bacteria even spores and virus (Yazd, 2008; McDonnell and Russell, 1999). Many findings reported activity of Sodium hypochlorite toward *E. coli* and *S. aureus* (Penna *et al.*, 2001; Saleh *et al.*, 2012). Although Hydrogen peroxide is oxidizing agent active against a wide range of microorganisms (Tasić *et al.*, 2009), it acted as the least effective against bacteria. That may be due to the production of catalase enzyme by aerobic organisms possessing cytochrome systems, which can degrade hydrogen peroxide to water and oxygen (Turner, 1983; Block, 2001).

CONCLUSION

The antibacterial activity of various extracts of soap components and toilet disinfectants was tested against the 2 strains of bacteria (*Escherichia coli* and *Staphylococcus aureus*) by using the well diffusion method and Kirby- Bauer disc diffusion method. Both of the bacteria taken showed sensitivity towards all the extracts as well as the disinfectant. *Escherichia coli* was observed to show zone of sensitivity ranging from 0.6 – 3.6 cm while *Staphylococcus aureus* showed zone of sensitivity ranging from 0.6 – 4 cm. The most active extract showing good inhibitory zone to both the bacterial strains is Honey. The effective disinfectants with highest sensitivity and great antibacterial activity are Domex, Texma and Sanifresh. The least active one was the Homemade disinfectant. From the study it is concluded that Domex, Texma, Sanifresh are the most effective disinfectants against the bacterial strains while the honey is the best antibacterial component present in the soap.

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