ORGANIC TOILET CLEANER

An eco-friendly alternative to chemical toilet cleaner

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ABSTRACT

A clean and hygienic society is very necessary for today's world. This lacks in many regions of the world. This does not leave any exception of facilities like toilets. So, to ensure this hygiene and deplete the harmful effects of chemical toilet cleaners we decided to make an effective organic alternative for it.

To work on this problem, we did literature survey and gathered information about each activity involved in the process. We also enlisted variables and represented its interdependence by drawing mind map. This helped us to predict our hypothesis. It stated that the solution would act anti-microbial and anti-stain on the toilet microflora and surface respectively.

Designing the experiments and performing them was our next step. The experiments were performed in three stages. Anti-microbial activity was tested in the school laboratory using well diffusion method. In the second stage, we tested the concentrations of the prepared solution on plates as well as on toilet. The fourth stage involved testing of swabs before and after cleaning the toilet with solution in the colorimeter.

After experiments, observations were noted. Data was analysed and graphs were interpreted. Conclusion was drawn that the solution acts anti-microbial and anti-stain on the toilet. It can also be preserved. The hypothesis was proved right and objectives were achieved.

Future plans were made considering the application of the Organic Solution in the society and MIC (minimum inhibitory concentration) would be taken of the solution. We see that the solution made would be helpful in the development of the human resource.

INTRODUCTION

The research started by brainstorming in which various topics were enlisted (around 250 topics were listed down). Our literature surveys helped us to find about the topics which we had chosen to work on. After referring many books and sites we finally succeeded in creating the base of our project. One of the researches conducted by 'The Department of microbiology and The Department of Biochemistry' of the Federal University of Technology, Owerri; said that the tobacco extract showed bacteriostatic activity on *Staphylococcus aureus* and *Escherichia coli.* This gave us an idea to use tobacco in our research project. It was a challenge for us to work under the field of microbiology as all the methods and concepts were new for us. This also led us to take more information and do more literature survey in order to know the field. After conducting experiments, ourselves regarding the bacteriostatic effectivity of tobacco on Escherichia Coli, we concluded its positive results. Then our focus shifted on application of this bacteriostatic property of Tobacco on Escherichia Coli.

On searching more about presence of E. coli we came to know that they are found in toilets. This gave us an idea to use tobacco in toilet cleaner. Since, hygiene is a major factor contributing in the development of the society it is vital to work on it. For securing the hygiene of the society it is necessary to consider the fields in which hygienic conditions are lacking. One of the most common places where hygiene lacks the most are toilets. Unhygienic toilets are responsible for various harmful diseases. And the spread of the microbes is not limited to a specific area but instead provokes epidemics. Usually, we see people using chemical toilet cleaners to clean their toilets. But these cleaners have more adverse effects than its uses. The chemical toilet cleaners are hazardous as well as human beings. The chemicals used in the cleaner pollute the air, water and soil resources.

Statement of necessity

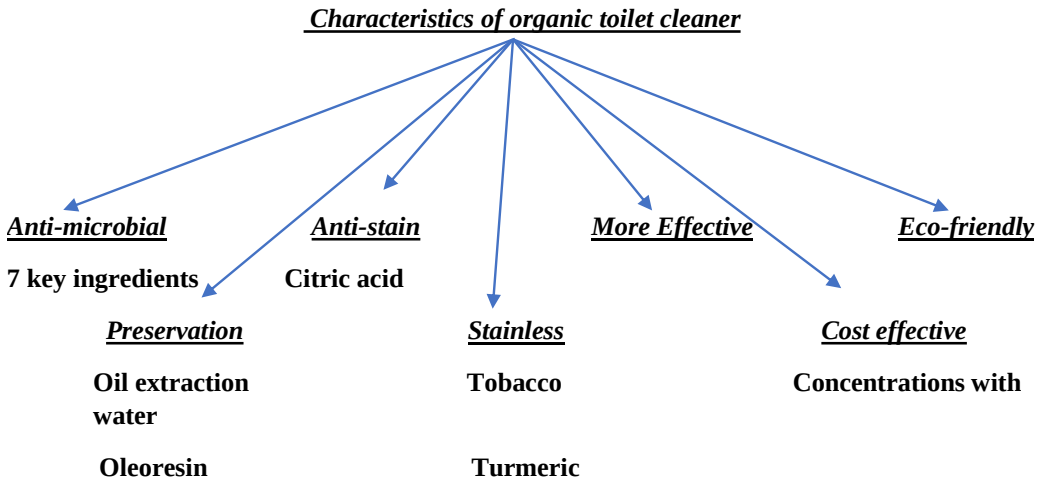
A. The unhygienic toilets cause diseases. Thus, a solution to disinfect them is necessary.
B. The chemicals in the chemical toilet cleaner are hazardous to the environment as well as human beings. So, an Organic, eco-friendly alternative is vital for society.

Problem statement: To make an effective Organic Toilet cleaner.

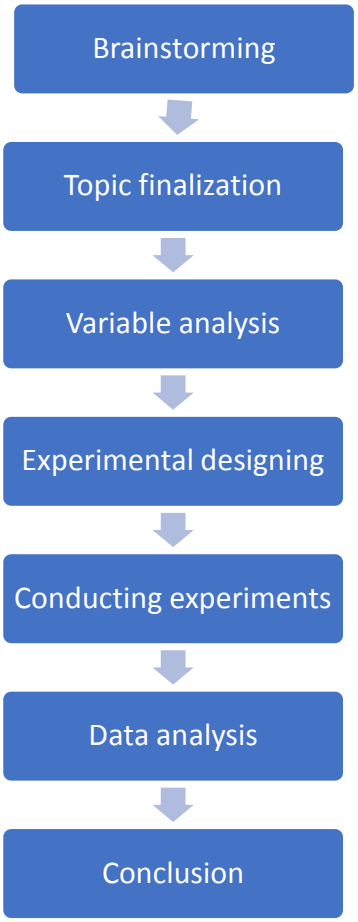
Objective:

- To make an effective organic natural toilet cleaner with the help of natural ingredients.
- The toilet cleaner should be eco-friendly for environment.
- Essential oils used in the cleaner would show antimicrobial effect on the sample collected.
- The solution would show ant stain properties.
- The solution should last long without changing the chemical composition of contents used in the solution.
- The solution should be effective than other chemical toilet cleaners in market.

- It was a challenge for us to work under the field of microbiology as all the methods and concepts were new for us. This also led us to take more information and do more literature survey in order to know the field.



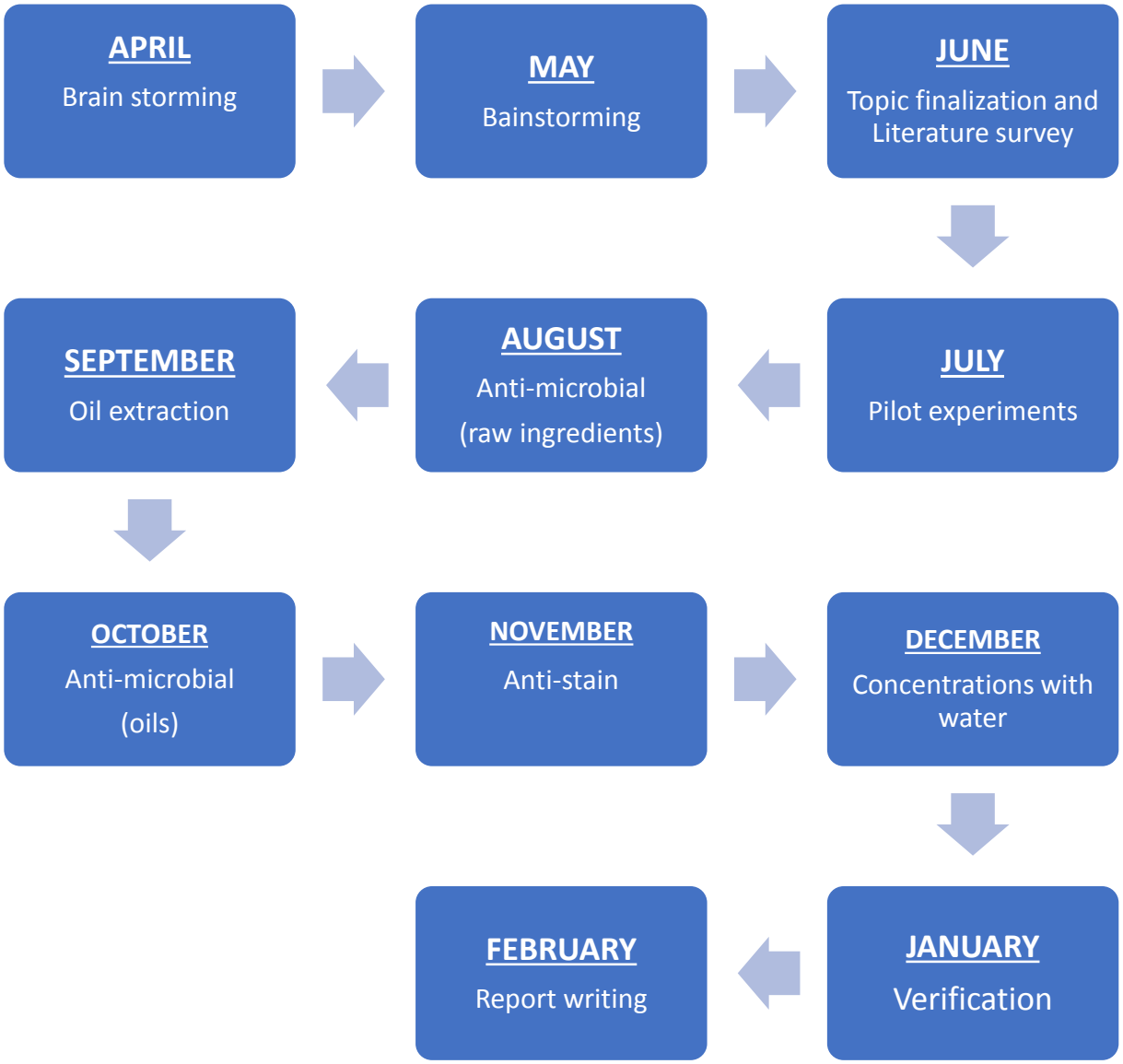
STUDY DESIGN AND EXPERIMENTAL SETUP

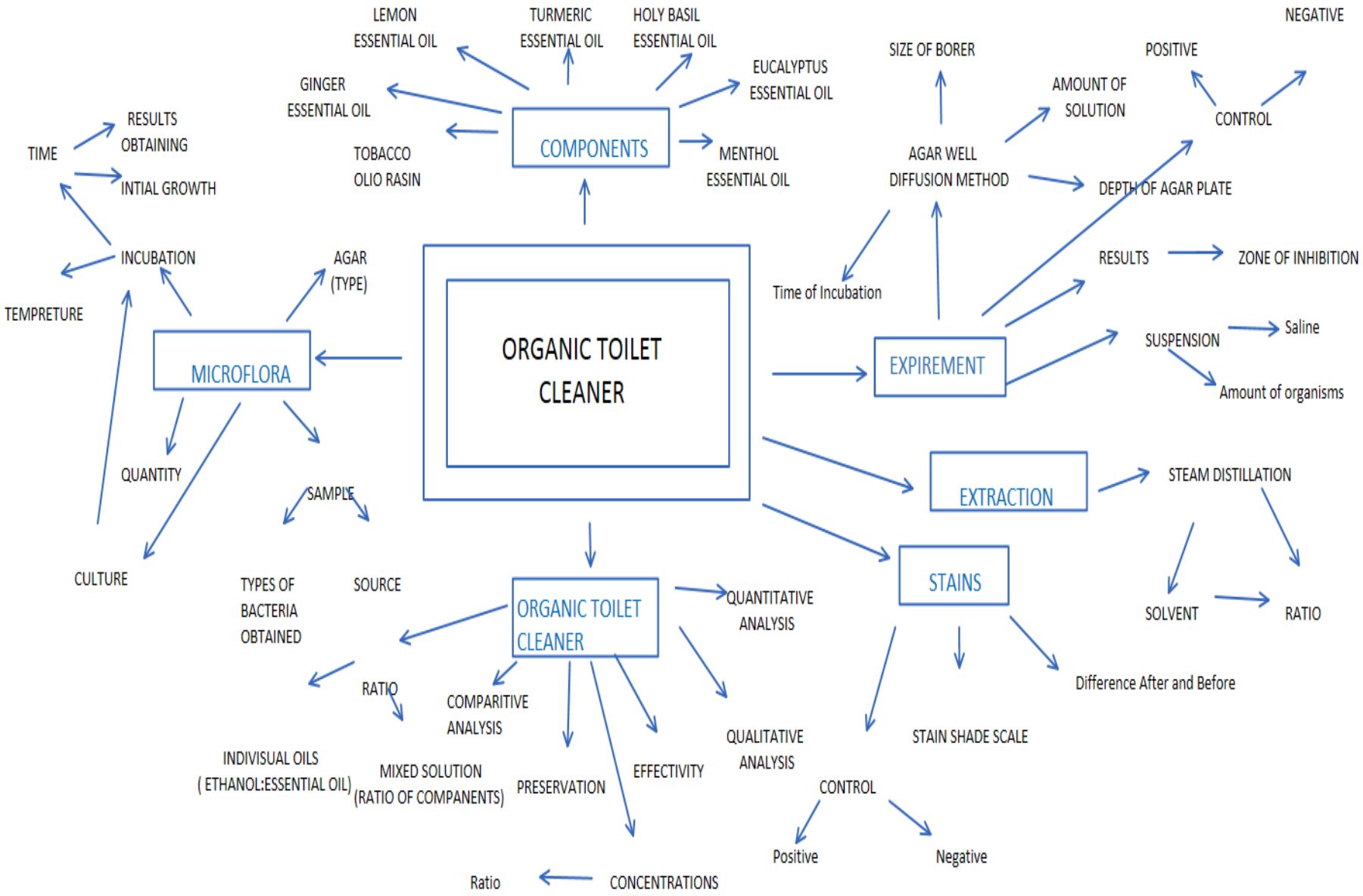


For the project, many topics were enlisted. Brainstorming was done on them to finalize one. Deciding the work plan was our next action as time was limited. Along with it, literature survey was done to get clear idea of the field in which we are working. Variable analysis was done by drawing a mind map of possible variables affecting the experiments. Following is a mind map of variables and their interdependence.

Experimental design was our next step. First, we tested the anti-microbial effects of the selected solutions by using well diffusion method and second, we tested the anti-stain properties.

Work plan:


APRIL
Brain storming
MAY
Bainstorming
JUNE
Topic finalization and Literature survey
JULY
Pilot experiments
AUGUST
Anti-microbial (raw ingredients)
SEPTEMBER
Oil extraction
OCTOBER
Anti-microbial (oils)
NOVEMBER
Anti-stain
DECEMBER
Concentrations with water
JANUARY
Verification
FEBRUARY
Report writing


ORGANIC TOILET CLEANER
COMPONENTS
LEMON ESSENTIAL OIL
TURMERIC ESSENTIAL OIL
HOLY BASIL ESSENTIAL OIL
EUCALYPTUS ESSENTIAL OIL
MENTHOL ESSENTIAL OIL
TOBACCO OLIO RASIN
GINGER ESSENTIAL OIL
MICROFLORA
AGAR (TYPE)
INCUBATION
TIME
RESULTS OBTAINING
INTIAL GROWTH
TEMPRETURE
QUANTITY
SAMPLE
CULTURE
TYPES OF BACTERIA OBTAINED
SOURCE
EXPIREMENT
AGAR WELL DIFFUSION METHOD
SIZE OF BORER
AMOUNT OF SOLUTION
CONTROL
POSITIVE
NEGATIVE
DEPTH OF AGAR PLATE
Time of Incubation
RESULTS
ZONE OF INHIBITION
SUSPENSION
Saline
Amount of organisms
EXTRACTION
STEAM DISTILLATION
SOLVENT
RATIO
STAINS
Difference After and Before
STAIN SHADE SCALE
ORGANIC TOILET CLEANER
QUANTITATIVE ANALYSIS
QUALITATIVE ANALYSIS
RATIO
COMPARITIVE ANALYSIS
INDIVISUAL OILS (ETHANOL:ESSENTIAL OIL)
MIXED SOLUTION (RATIO OF COMPANENTS)
PRESERVATION
EFFECTIVITY
CONCENTRATIONS
Ratio
CONTROL
Positive
Negative

Hypothesis:

1) We predict that the 7 key ingredients (Tobacco, Eucalyptus, Holy Basil, Ginger, Lemon, Turmeric and Menthol) would show anti-microbial effect on swab taken from toilet bowl.

Following are the reasons for choosing the given ingredients:

1) Lemon essential oil: Lemons have powerful antibacterial properties; experiments have proven that the extract of lemons destroy the bacteria of malaria, cholera, diphtheria, typhoid and other deadly diseases. Citric acid binds well with minerals and helps to remove them from the toilet surface. Citrus acid is a benign cleaning agent. It also has a pleasant scent that makes it preferable for household use instead of harsh chemicals.
2) Eucalyptus essential oil: The results obtained showed that essential oil of the leaves of *E. globulus* has antimicrobial activity against gram negative bacteria (*E. coli*) as well as gram positive bacteria (*S. aureus*).
3) Mint essential oil: It is proven that the distilled concentrations of essential oil inhibits the growth of microorganisms.
4) Turmeric essential oil: Turmeric is also anti-bacterial. Curcumin, the compound that gives turmeric spice its characteristic bright yellow hue, has well-known antimicrobial properties.
5) Ginger essential oil: Ginger (Zingiber+6 officinale Roscoe) has been used widely as a food spice and an herbal medicine. In particular, its gingerol-related components have been reported to possess anti-microbial and antifungal properties, as well as several pharmaceutical properties.
6) Tobacco: Researches reveal that tobacco leaves extracts have anti-bacterial effects on bacterial growth.
7) Holy Basil oil: Tulsi oil completely inhibited the growth of *S. aureus*, including *E. coli*. The results showed that Tulsi essential oil had only bacteriostatic activity against the examined bacterial strains.

   Thus, these ingredients were chosen.

2) We predict that the citric acid would show anti-stain effects on the toilet surface.

Reason: The stains that build up in showers and bathrooms are often caused by soap and hard water. Both soap and hard water are alkaline, which are broken up by acids. Citric acid is gentle enough that it can be used in the home and the bathroom. In general, cleaning your bathroom on a regular basis will help ensure that less stains and calcium build up on your shower and in your bathtub.

3) We predict that by extracting essential oils of all raw ingredients, the ingredients would preserve for a longer period of time.

Reason: By extracting essential oils, the water content as well as degradable material is taken away from the ingredients, thus, preserving the solution.

4) We predict that by extracting essential oils of all raw ingredients, the effectivity of ingredients would increase.

   Reason: In essential oils, only the main acting agents are extracted, thus making the ingredient more effective.

5) We predict that by extracting essential oils of all raw ingredients, the natural stains of ingredients (tobacco and turmeric) would vanish.

   Reason: By extracting essential oils, the material causing natural stain is removed.

6) We predict that the effectivity of solution (mixture of all oils and citric acid in ratio 1:3) would be almost same as concentrated solution when diluted with water.

   Reason: since, the components of the solution are very concentrated, we thought that on adding water the effectivity would be considerably stable.

Anti-microbial activity

After searching about toilet cleaners, we came to know that the toilet cleaners available in the market are harmful for environment as well as for living beings. These toilet cleaners after being used, emit fumes which are hazardous for humans. So, we felt a need to make an Organic Toilet Cleaner. The organic ingredients we chose were as follows:

Tobacco (*NicotinaTabaccum*)

Eucalyptus (*Eucalyptus globulus)*

Holy basil (*Ocimumtenuiflorum*)

Turmeric (*Curcumin***)**

Ginger (*Zingiberofficinale*)

Menthol *(Mentha balsamea)*

Lemon (*Citrus Limonum)*

Our work started by extracting nicotine from tobacco. At first, we boiled tobacco with water for 20 minutes and then filtered it. The effect of filtrate was then checked by using Disc Diffusion Method. But, after repetitions and literature survey we realised that nicotine is alcohol soluble and not water soluble. So, we then soaked tobacco in ethanol for 24 hours and extracted nicotine from it (procedure explained on page no.). This extract showed positive effect on sample of E. coli.

Along with it we used juices of other ingredients to test its activity on water sample collected from toilet. But these juices used were not preserved for longer period of time. So, as a solution to this, problem we extracted essential oils of these ingredients. Not only the ingredients were preserved but also its effectivity increased three times the previous one. By extracting essential oils, the natural stains of turmeric and tobacco vanished too. But, as the agar was water based and oils do not dissolve in water, we used ethanol (an alcohol) in to help oils diffuse easily in agar. (oils: ethanol is 1:5).

The effectivity of these oils was measured by using Disc Diffusion Method. In this technique, small discs of Whatman's paper are soaked in the solution for a particular time. Then, the disc is placed in the centre of the nutrient agar plate. But, after many trials, we observed that the quantity of solution absorbed by the disc is very less thus leading to false results.

An alternative to this technique we used Well Diffusion Method. In this technique, a well is bored in the centre of nutrient agar plate. A fixed amount of solution is pore in it.

Before pouring the solution into the well, we used to spread the water sample from toilet on the plate. But it was the seen that not all bacteria in the water are live. This led to uneven spreading of bacterial culture on plate and no proper zone of inhibition could be measure. So, to make improvement in the procedure, a suspension of bacteria was made. In it, fully grown bacterial colonies were mixed with saline (water + 0.85%NaCl) and then spread on the plate. This led to even growth of culture and accurate measurement of zone of inhibition.

By combining these two techniques i.e. by making suspension and by using well diffusion method we obtained the results of seven key ingredients and harpist (positive control). According to their effectivity, we decided the proportion of these ingredients in the anti-microbial solution.

The ratios were as follows:

Eucalyptus oil: 5

Holy basil oil: 5

Lemon oil: 3

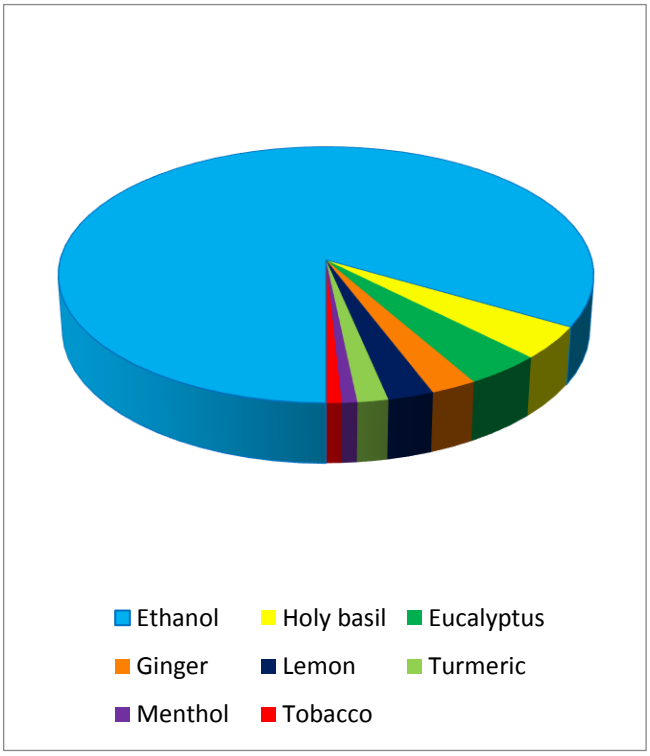
Ginger oil: 3

Turmeric oil: 2

Menthol oil: 1

Tobacco extract: 1

After mixing the ingredients in above mentioned ratio, we found that the total effectiveness was greater than each ingredient. This result stated that the ratio was appropriate with respect to our objective.



Finally, the anti-microbial solution was ready.

Anti-stain activity

A toilet cleaner should not only have anti-microbial properties but should also have anti-stain properties to be a good toilet cleaner.

Thus, adding anti-stain agent was necessary.

Sub objectives of anti-stain activity:

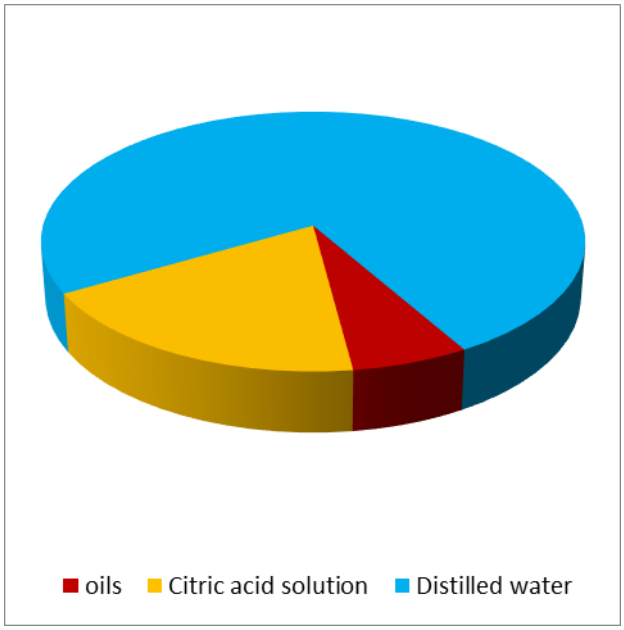
1) To make a stain shade scale to compare shade of stains before and after cleaning toilet bowl.

2) To compare the effectively of chemical toilet cleaner with organic solution made by us.

3) To check whether the anti-microbial solution itself is anti-stain.

4) To check anti-stain activity of other ingredients like lemon and citric acid.

5) To check the effectively of solutions on western as well as Indian toilets.



After searching for organic anti-stain agents, we decided to try citric acid and lemon on the toilet bowl. we decided to try both lemon and citric acid because there may be some other chemicals present in lemon including citric acid which would affect the results. Thus, two separate sets of experiments were performed.

Simultaneously, the previously made anti-microbial solution was also tested to check whether it itself is anti-stain or not.

After analysing the observations, it was noted that citric acid showed comparatively good results than other agents which were tested.

Now, the next step was to try concentration of citric acid and previously prepared anti-microbial solution. Three ratios of anti-microbial solution are to citric acid were tested i.e. 1:3, 1:1 and 3:1. After performing experiments, is was proved that 1:3 ratio is best among all. But it was necessary to check whether this ratio which included citric acid did not affect anti-microbial activity of the solution. So, this ratio was tried on nutrient agar plates too. It was founded that 1:3 ratio was best at anti-microbial as well as anti-stain activities.

Thus, in this way a solution containing anti-microbial and anti-stain properties was made.

Concentrations

As, a part of curiosity, we decided to take concentrations of above-mentioned solution with water to check whether it retains its effectiveness even after dilution. On doing so, we obtained results which showed that even after diluting the solution the effectiveness on agar plates and on toilet surface were almost same compared to concentrated solution which contained anti-microbial and anti-stain properties.

Along with it, the solution turned cost effective as more quantity was made with less core ingredients. Thus, our final product was made.

Procedures

Nutrient agar plates making procedure:

a) Take 3gm of nutrient agar for 3 plates.

b) Measure 50ml distilled water for 3 plates.

c) Mixed the agar in distilled water in a conical flask. Plug it with cotton and cover it with newspaper using rubber band.

d) Wrap the plates in newspaper.

e) Preheat the autoclave with container.

f) With the apparatus preheat autoclave and close the nob.

g) Set the dial at 45 minutes (15 lbs for 20mins)

Tobacco extraction procedure:

a) Take 2gm of tobacco mixture.

b) Crush it into small pieces.

c) Measure 14ml ethanol and 6ml distilled water to make a solution.

d) Soak the crushed tobacco in the solution in a beaker and cover it with butter paper and watch glass.

e) Keep it at room temperature for 24 hours.

f) Filtered the solution using Whatman's paper.

g) Transfer it in an open container and blow hot air to vaporize the ethanol.

Procedure 1:

Equipment used:

- Micro-pipette
- Spreader
- Petri dishes
- Borer
- Autoclave Machine
- Weighing scale

Chemicals used:

- Nutrient Agar
- Sodium Chloride
- Organic Ingredients
- Ethanol

a) Nutrient agar plates were made in the laboratory using autoclave.

b) Swab was taken from the toilet bowl and spread on the plate. (The plating was done between two burners to reduce the risk of contamination).

c) After bacterial growth was observed on the plate, suspension was made using saline (0.85% NaCl in water) and bacterial colonies.

d) Suspension was then poured and spread using pipette and spreader respectively.

e) Well was bored in the plate by a borer (15mm in diameter) and the solution was poured into it. Before pouring the solution, they were mixed with Ethanol so that the diffusion of the oils in the agar would be properly done.

f) The plate was incubated for 24 hours at 37 degree Celsius.

g) Results we obtained by measuring the 'zone of inhibition' given by the solutions.

h) Each experiment was repeated thrice.

i) The ratio of all the ingredients were decided according to the results obtained.

j) The solution was then tested on the toilet bowl.

k) Swabs were collected 'before' and 'after' washing the surface with solution.

l) These were incubated for 24 hours and then tested in the colorimeter.

Procedure 2:

Equipment used:

- Brush
- 5cm x 5cm marking stick
- Syringe
- Gloves
- Masks
- Stain shade scale

Chemicals used:

- Charcoal powder
- Citric acid
- Lemon juice
- Organic solution
- Chemical cleaner

a) The charcoal powder was mixed with water to make a thick paste. 5cm x 5cm marking stick was then dipped in the paste and mark was obtained on the toilet surface.

b) By using the stain shade scale, the colour of the stain before washing was noted down.

c) 5ml solution was taken in the syringe and was spread over the marked area.

d) The solution was kept stand still for 20 minutes and then washed with water.

e) The 'After washing' results were obtained by comparing the reduced stain with the scale made.

f) Each experiment was conducted thrice.

RESULTS

Observations:

Once we conducted all the experiments, we did a detailed measurement and calculation of the effects. We did a detail analysis of the results obtained and compared both of the main cases of the methods of toilet cleaning, to find out which one was better. After comparison, we found out that our natural methods of cleaning were better than the chemical methods used in the daily households. In fact, the natural toilet cleaners proved to be three times better than the chemical toilet cleaners in antimicrobial activity.

The results were found out using the zone of inhibition method. This method includes calculating a clearance zone, or a zone where there is no bacterial growth. The area considered surrounds the well into which the material to be tested is inserted. It was measured in millimetres using a scale. The diameter of the area is taken into consideration.

Following are the results obtained for **antimicrobial activity of the organic natural solution**.

Photo plate: Images showing zone of inhibition for different treatments

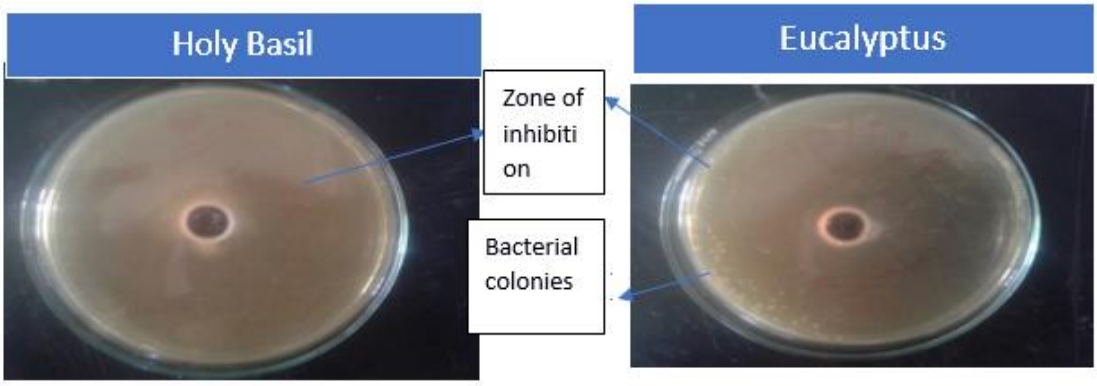


Figure 1: Photo plate showing zone of inhibition of Holy Basil

Figure 2: Photo plate showing zone of inhibition of

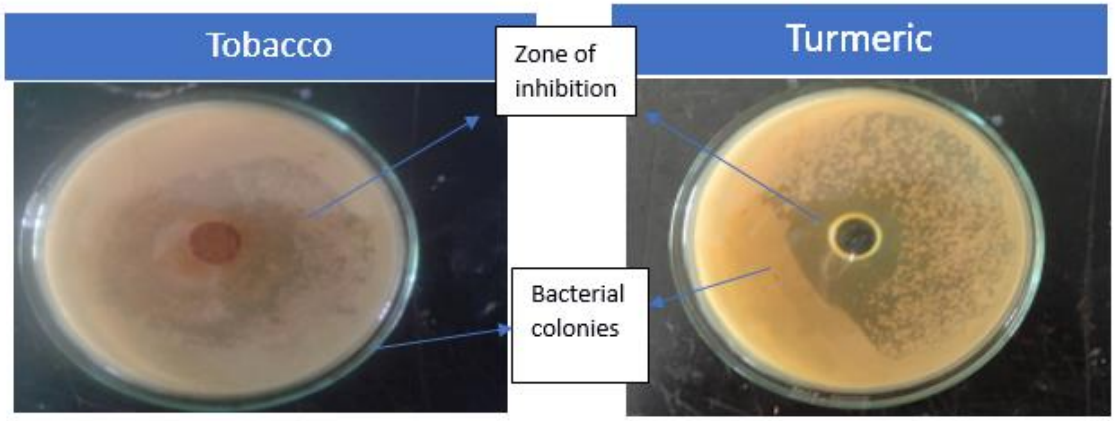


Figure 3: Photo plate showing zone of inhibition of tobacco

Figure 4: Photo plate showing zone of inhibition of Turmeric

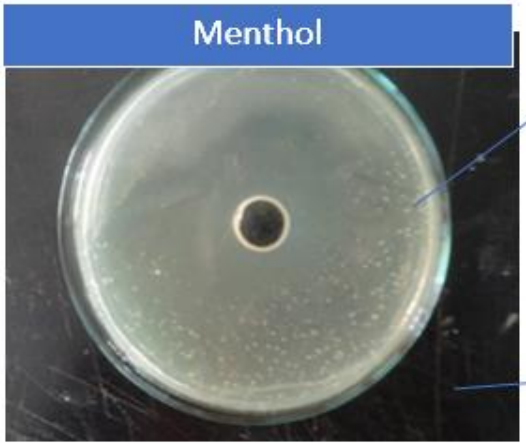
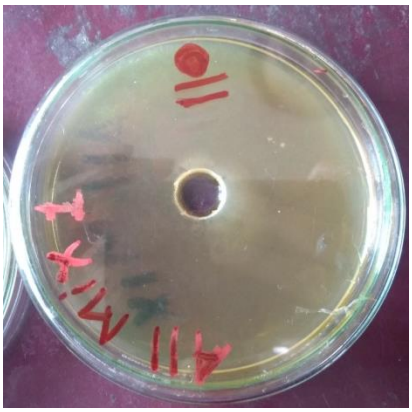


Figure 5: Photo plate showing zone of inhibition of Menthol

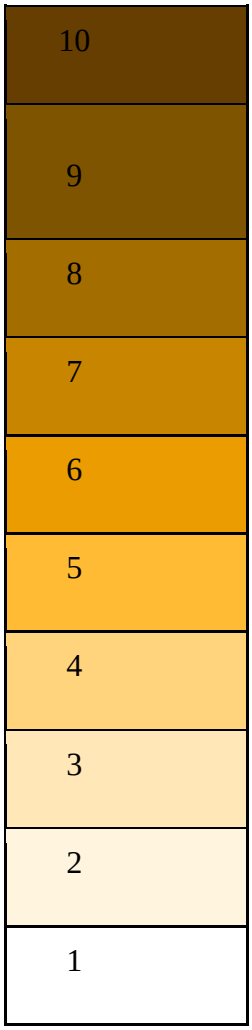
Figure 6: Photo plate showing zone of inhibition of Ginger

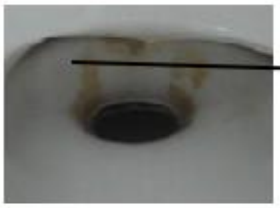


What we found out was that the natural toilet cleaners are better than the chemical toilet cleaners. The zones of inhibition recorded on the plates where we used essential oils were much larger than the ones on the plates where we used chemical products.

After measuring the zone of inhibitions of various solutions, we conducted experiments regarding the stain removing properties of the organic solution. We compared the stain (before cleaning with solution and after cleaning with solution) with our own standardise Stain Shade Scale. Following are the images of cleaning the toilets (before and after) with various solutions and the scale.

Stain Shade Scale



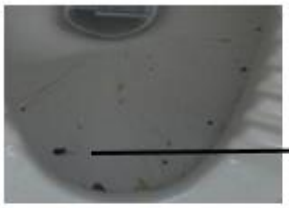


Before using the Organic Natural Solution

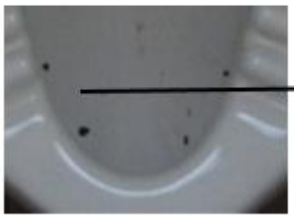
After using the Organic Natural Solution

Before using phenyl

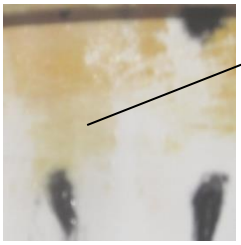
After using phenyl



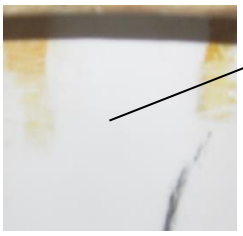
Before using citric acid



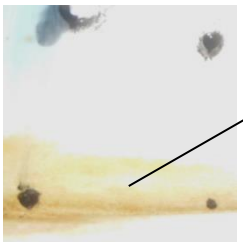
After using citric acid



Before using the solution (only oils: citric acid concentrations (1:3))

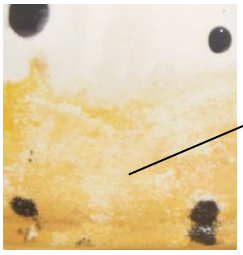


After using the solution (only oils: citric acid concentrations (1:3))



Before using the Organic toilet cleaner

After using the Organic toilet cleaner



Before using the Harpic

After using Harpic

Table 1: Data showing comparison between zone of inhibition diameters (in mm) within and among different treatments of organic ingredients.

Ingredients	Repetition 1 (In Mm)	Repetition 2 (In Mm)	Repetition 3 (In Mm)	Average (In Mm)	Standard Deviation
Holy Basil	88	87	89	88	0.816497
Turmeric	53	47	51	50.3	2.494438
Tobacco	43	55	53	50.3	5.249339
Eucalyptus	87	91	90	89.3	1.699673
Menthol	55	49	51	51.6	2.494438
Lemon	82	78	81	80.3	1.699673
Ginger	74	68	71	71	2.44949
Only oils	94	93	93	93.3	0.471405

Table 2: Comparison among and within the stages of making an organic toilet cleaner.

Name of the ingredient	Repetition 1 (in mm)	Repetition 2 (in mm)	Repetition 3 (in mm)	Average	Standard Deviation
Raw ingredients	34	26	30	30	1.41421356
Only oils	92	93	93	92.67	0.47140747
Only oils +citric acid	94	94	94	94	0
Only oils + citric acid+ water	92	94	93	93	0.81649658
Harpic	88	90	87	88.34	1.24722358

Table 3: Comparison among and within the treatments of anti-stain agents.

Sr. No.	Lemon		Citric Acid		Organic toilet cleaner		Harpic		Phenyl	
	Before	After	Before	After	Before	After	Before	After	Before	After
R1	4	2	7	2	8	6	5	2	6	3
R2	5	4	6	2	5	2	4	2	4	2
R3	6	3	5	3	6	2	6	3	5	4
Average	5	3	6	2.3	6.3	3.3	5	2.3	5	3
Difference	2		3.7		3		2.7		2	

Table 5: Comparison within the ratios of solution and water

(Only oils +Citric Acid): water					
01:01		01:03		03:01	
Before	After	Before	After	Before	After
5	1	5	1	6	2
Difference: 4		Difference: 4		Difference: 4	

Table 6: Comparison among and within the optical density of swabs collected before and after washing the toilet surface.

Name of The Solution	Repetitions	Before or After	Frequency (630 µ)
Organic Toilet Cleaning Solution	Repetition 1	Before Testing the Solution	0.13
		After Testing the Solution	0.06
	Repetition 2	Before Testing the Solution	0.21
		After Testing the Solution	0.03
Harpic	Repetition 1	Before Testing the Solution	0.15
		After Testing the Solution	0.12
	Repetition 2	Before Testing the Solution	0.05
		After Testing the Solution	0.03
Control (Water)	Repetition 1	0.18	0.03

Table 7: Comparison among and within the final solution and harpic.

Sr.no.	Organic toilet cleaner		Harpic		Negative control(water)	
	Before	After	Before	After	Before	After
Repetition 1	6	1	6	4	6	3
Repetition 2	6	2	6	2	6	3
Average	6	1.5	6	3	6	3
Difference	4.5		3		3	

Graphs:

Chart 1: Comparing zone of inhibition among and within different treatments

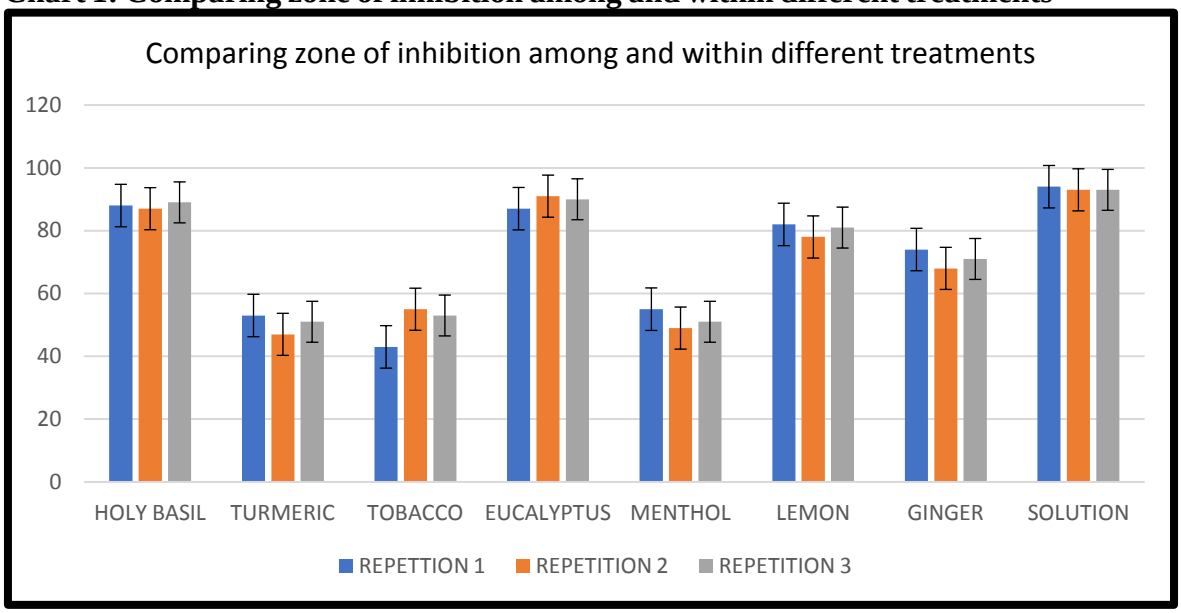


Chart 2: Comparing mean zone of inhibition (in mm) among different treatments

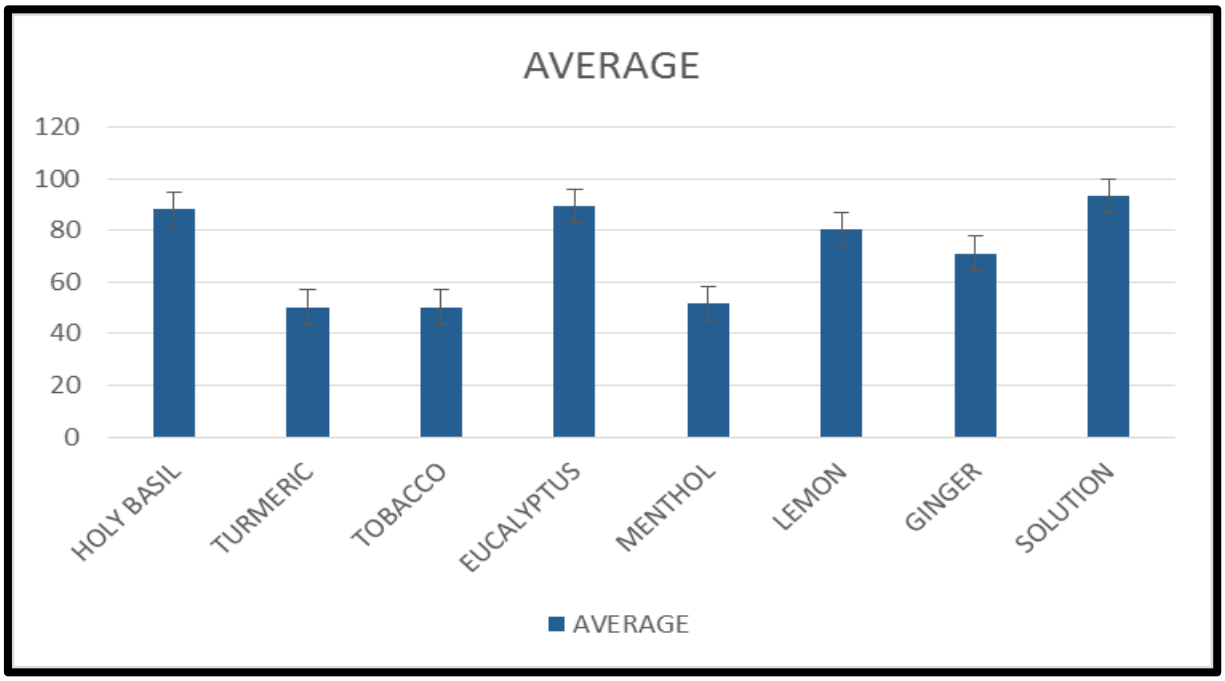


Chart 3: Comparing among and within anti-stain properties of various solutions

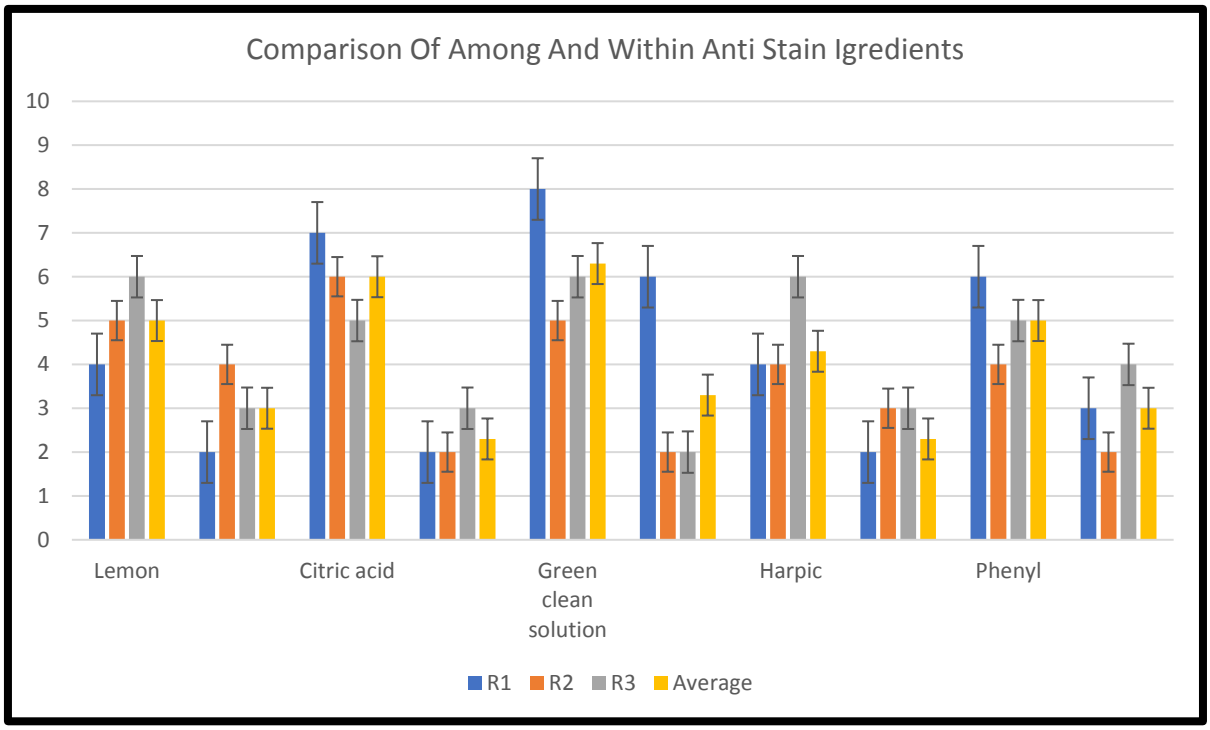


Chart 4: Comparison Of among and within Concentrations of Solution – Anti Microbial activity (organic Toilet Cleaner: water)

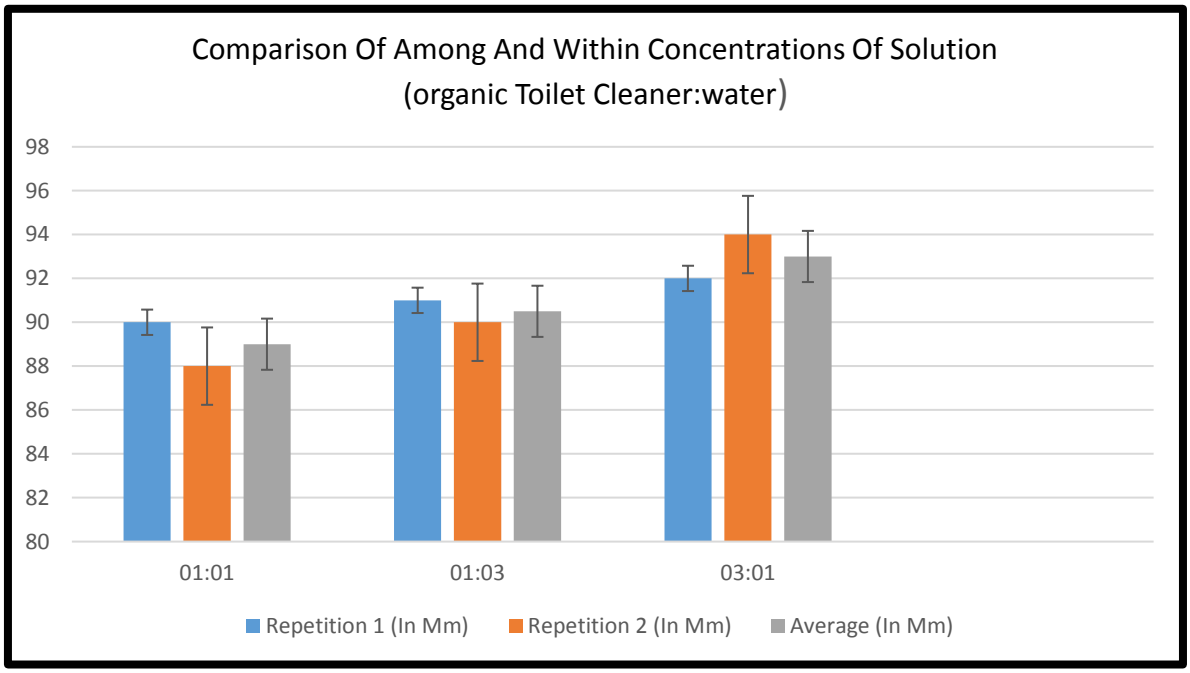


Chart 5: Comparison Among and Within Concentrations of Solutions -Anti Stain Affectivity (Organic Toilet Cleaning Solution +Citric Acid: Water)

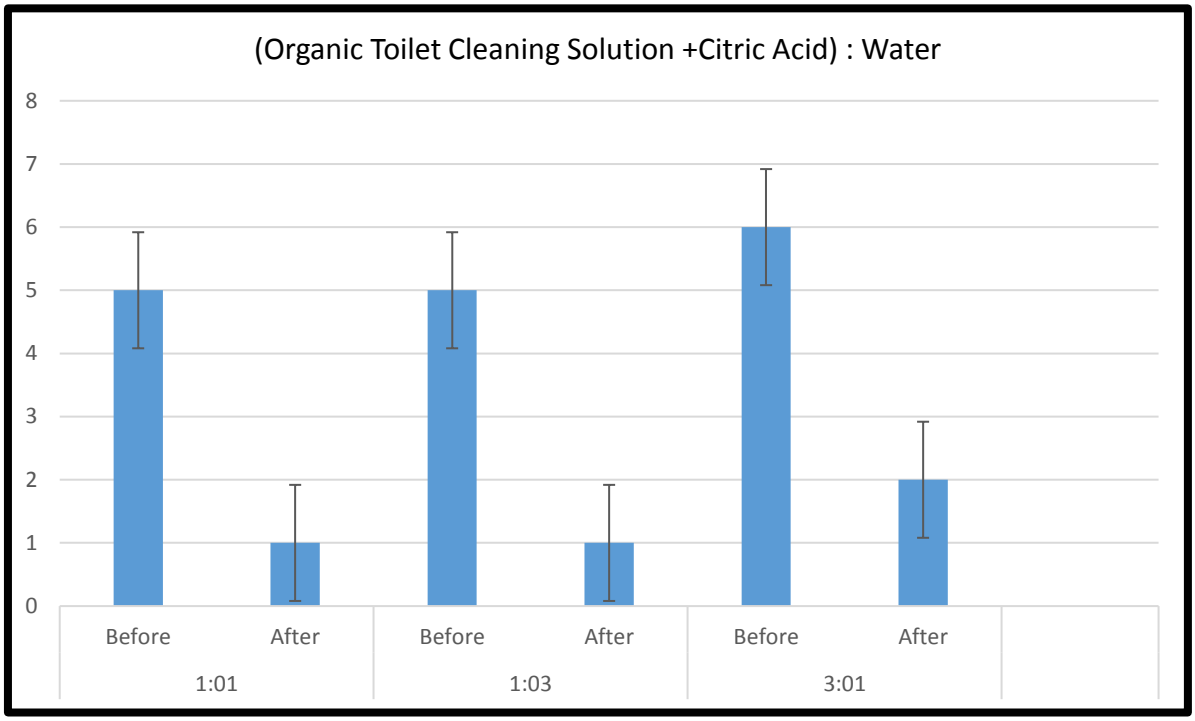


Chart 6: Comparison among and within the optical density of swabs collected before and after washing the toilet surface.

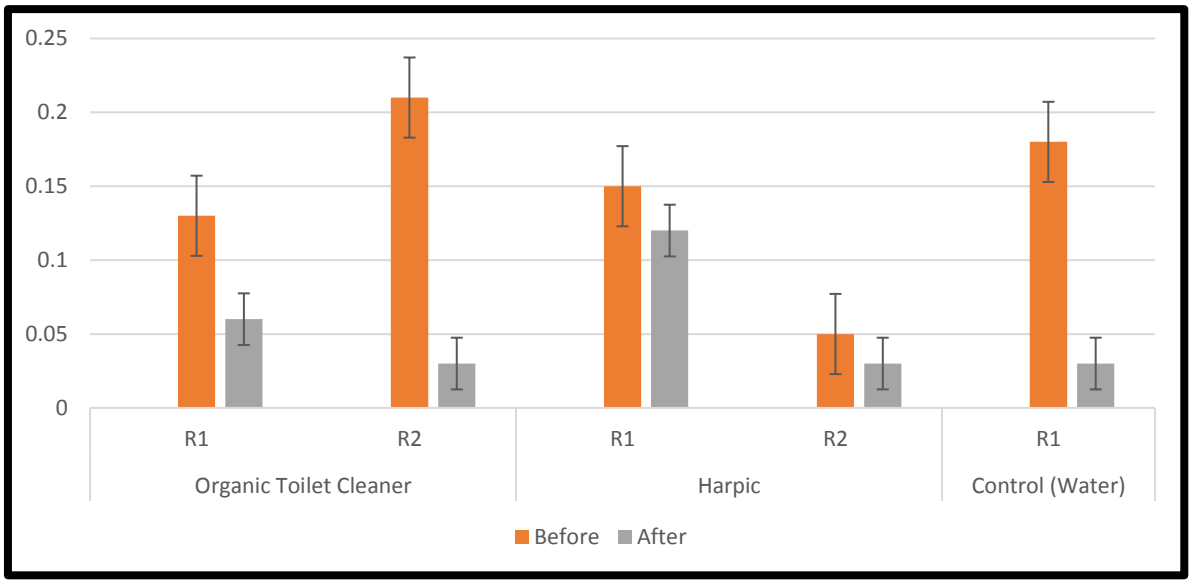
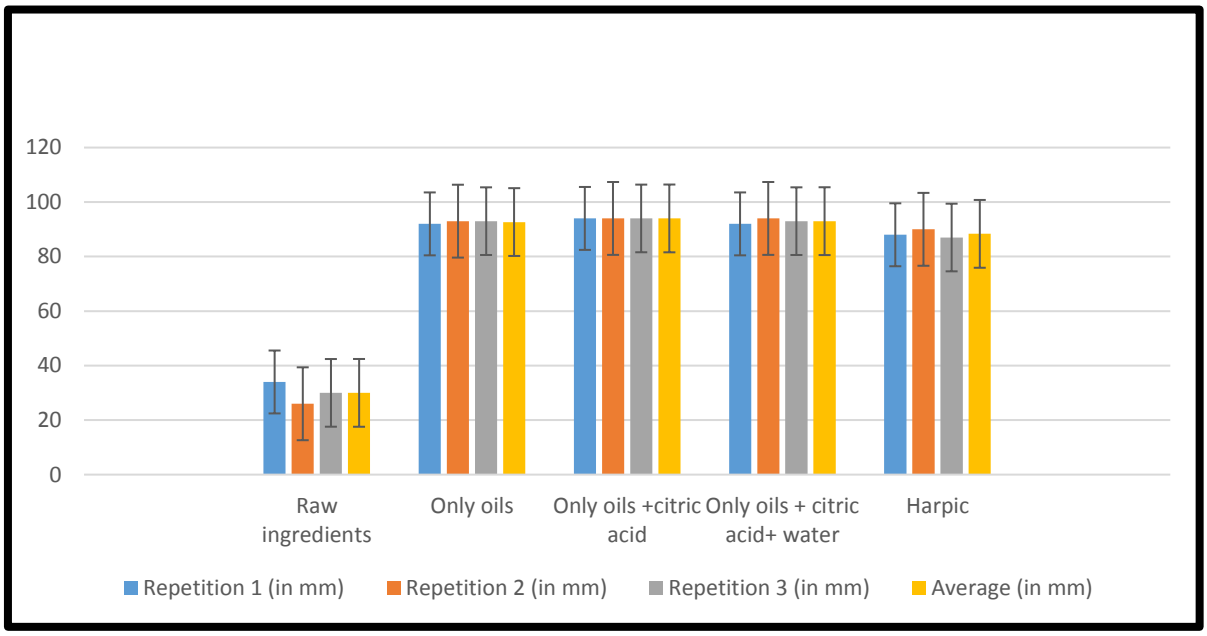


Chart 7: Comparison Among and Within Different Stages of Organic Toilet Cleaner and Harpic



We also conclude that the stain removing agents (i.e. Citric acid) performed very well in the test conducted. Organic toilet cleaner acted as antimicrobial as well as anti-stain in the experiments. The solution also performed better than Harpic (a well-known chemical toilet cleaner) in killing bacteria as well as showing anti stain properties.

We also collected the toilet swabs before and after using the Organic Toilet Cleaner. We checked the results using colorimeter. In scientific terms the word colorimeter generally refers to the device that measures the absorbance of particular wavelengths of light by a specific solution. We used this instrument to measure the count of the bacteria present before and after cleaning the toilet. This proves that the organic toilet solution is antibacterial and antimicrobial too.

We also conclude that the stain removing agents (i.e. Citric acid) performed very well in the test conducted. Organic natural solution acted as antimicrobial as well as anti-stain in the experiments. The solution also performed better than phenyl in killing bacteria as well as showing anti stain properties.

CONCLUSION

The basic aim of our project was to avoid the use of chemical toilet cleaners which in turn give harmful and toxic effects to humans as well as the environment. We obtained results which supported our aims and goals. We focused on antimicrobial property, so we designed experiments accordingly and to scientifically prove them we decided to measure the zone of inhibition. We found satisfactory results on the antimicrobial activity of organic toilet cleaner. The next challenge in our project was to make it a good cleanser or add anti-stain properties. We created our own Stain shade scale to measure the range of shade. The next problem we faced was its preservation, so, we decided to get essential oils of the chosen ingredients. This also increased effectivity of our solution three times than before. But we also faced certain limitations while completing this project such as: we could not keep the stains or organisms same as we had to cover all the aspects for a dirty toilet, we could do more experiments and researches if it was not time-bound. After we found a solution to one problem more questions came in our mind then we started working on them too. We thought that when we have made additions to the solutions its effectivity with respect to all factors should not decrease. So, we tested the solutions in the colorimeter. We observed positive results. The methods we used to verify our results can be said scientific as they have a proper reason, measure and they are specific. The Cost of the solution made is rupees 103/ litre.Thus, we think that this is a solution which satisfies all the properties of a good toilet cleaner. We think that this is a need of utmost necessity in a country like India where the population can be utilised for the benefits of the country. and this can be ensured only when the people are healthy.

FUTURE PLANS:

1)Eco-friendliness: Though the ingredients used in the cleaner are theoretically organic and eco-friendly, it is necessary to prove it by conducting experiments.

2)Testing on various species: We are interested in testing our organic solution individually on various bacterial species.

3) Launching of product: After considering all characteristics of our Organic toilet cleaner, we feel that it is capable to be launched as a new product!

Health & Safety

The entire experiments were conducted in the school laboratory. Our laboratory instructor guided us about the use of equipment and safety measures that should be taken while working in the laboratory. Following is a set of instructions we followed while working in the laboratory:

(a) Suitable clothes should be wore while working near fire sources.

(b) While operating the autoclave machine, proper handling of the instrument should be done to avoid burns and injuries.

(c) Using the burners under the supervision of the guide is mandatory.

(d) While disposing the contaminated plates, care should be taken about that the plates do not get directly in contact with anything else. This might spread diseases.

(e) Dealing with the bacterial growth is risky. Therefore, masks and gloves are mandatory during experiments.

(f) Even during working in the toilets, masks and gloves were worn to avoid infections.

Details about the laboratory:

The laboratory of Jnana Prabodhini Prashala School, Pune, Maharashtra, India was used to perform experiments. We worked under our supervision of guide, Mrs. Maya Bhanu.

Dr. Aditya Ponkshe, Mrs. Shantala Kulkarni also helped in the project.

Acknowledgements

We would like to acknowledge our guides Mrs. Maya Bhanu, Mr. Aditya Ponkshe and Mrs. Shantala Kulkarni. We would like to thank Mr. Yogesh Kulkarni for providing us essential oils time to time. We would also like to thank Mrs. Lata Bhoir and Mr. Dhanajay Bhide for helping us in completing the report. We would also like to thank our school for providing us a variety of instruments and well-equipped labs. We would like to acknowledge our parents, classmates, teammates and everyone who helped in making our project succeed towards progress.

Equipment's list:

- Colorimeter
- Incubator
- Micro-pipette
- Spreader
- Petri dishes
- Borer
- Autoclave Machine
- Weighing scale
- Nutrient Agar
- Sodium Chloride
- Organic Ingredients
- Ethanol
- Brush
- 5cm x 5cm marking stick
- Syringe
- Gloves
- Masks
- Stain shade scale
- Charcoal powder
- Citric acid
- Lemon juice
- Organic solution
- Chemical cleaner
- Standardise Stain shade scale

Limitations

- After searching for universally approved or defined stain colour scale to measure the stains of the toilet and ultimately arrived to the point to make our own stain shade scale. So, we defined this scale numerically and so may not match other colour scale for stain of toilet.

- The microbes are grown initially on nutrient agar plates. Some fixed quantity of toilet water sample (swab) is transferred to the saline to make a suspension which is then spread over other nutrient agar plates for experiments. The amount of microorganism transferred was measured by wire loop which cannot be completely considered as a unit for measurement. Since such highly an accurate equipment which could measure such small quantity as microbial culture was not available in our school laboratory this stood as a limitation in our research. But still, we greatly managed to keep this quantity same throughout all experiments.

To incubate the nutrient agar plates consisting of solutions to check the anti-microbial activity, we used the school laboratory incubator which is often used by other students as well. This may, we think some temperature differences in the experimental researches.

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