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USE OF BIOSYNTHESISED SILVER NANOPARTICLES FROM *TRACHYSPERMUM AMMI* SEEDS(AJWAIN SEEDS) FOR PREPARATION OF TOOTHPASTE AND ASSESSMENT OF ITS EFFICACY AGAINST TARGET MICROORGANISM *STAPHYLOCOCCUS AUREUS*.

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Abstract : The use of nanotechnology in herbal medicine has been increasing significantly. Biosynthesised silver nanoparticles (AgNPs) from *Trachyspermum ammi* seeds (Ajwain seeds) were used for exploiting its antimicrobial property in the preparation of toothpaste. The biosynthesised silver nanoparticles were assessed as antimicrobial in which nanoparticle solution with 0.01M concentration of silver nitrate displayed the most potent activity *S.aureus* isolated from the oral cavity with the help of medical microbiology. The activity of the toothpaste was checked using the well diffusion assay. The procedure followed for synthesizing nanoparticles was cost effective, convenient and easy to perform.

keywords - Silver nanoparticles(AgNPs), *Trachyspermum ammi* seeds (Ajwain seeds), Antimicrobial property, toothpaste, oral cavity, medical microbiology, well diffusion assay.

INTRODUCTION

Nowadays most of the people complain about dental problems such as dental plaque, dental caries and so on. There is also a high prevalence of tooth decay and periodontal disease in India. Chronic inflammation, connective tissue loss, and alveolar bone destruction are the effects and symptoms of the chronic bacterial infection known as periodontitis. To tackle these problems, there are multiple toothpaste available in the market showing antimicrobial properties, but these contains various chemical constituents, including sodium fluoride (NaF), sodium lauryl sulphate, cocamidopropyl betaine, zinc lactate, and paraben, that have been linked to enamel discolouration, taste alterations, and mucosal irritation. These may also possess harmful effects on normal floral. To eliminate such deteriorating effects, synthesis of potent natural toothpaste is essential. Nanotechnology is being used in dental materials in recent years, with nanoparticles being integrated into the structure of dental composites and disinfection solutions due to their bacteriostatic as well as bactericidal effects. Preparation of home made toothpaste over commercial was chosen, with silver nanoparticles included in it based on the statistical data. Survey research is the practice of doing research through sending surveys to participants. The survey data is statistically evaluated after collection in order to obtain significant study conclusions. As one of the least expensive & most convenient ways to conduct surveys with extremely accurate results, online surveys. As a result, the study is built on data collected through surveying to select the research issue from a market viewpoint with a combination of scientific methodologies to take into account & satisfy consumer demands.. The prepared toothpaste can be used to avoid plaque formation and microbial deposit on teeth at home in

simple steps rather than visiting a dentist and cleaning and polishing teeth frequently, which would damage the enamel layer it found to be useful in previous research.

Hans Peter conducted studies that were found only in relation to oral infections such as periodontitis and apical periodontitis. Also, because of their unique physiology and energy metabolism, they are very likely more than just secondary colonisers of infected areas. Actively involved in the entire *polymicrobial* infection process. Swetha G, Rajesh Kumar S, and Lakshmi Thangavelu researchers highlighted the conclusion of their studies that the inhibition of oral pathogens and the growth of *S.mutans* and *Candida albicans was observed as an* antimicrobial effect *of* synthesised Silver nanoparticles. Silver nanoparticles generally interfere with and disrupt the integrity of bacterial membranes; silver ions bind to key biological components such as sulphur, oxygen, and nitrogen, preventing bacterial growth. It also prevents bacteria from producing proteins and duplicating DNA. The antimicrobial efficacy of the synthesised AgNps was dose-dependent, showing that *Pterocarpus santalinus* may be widely exploited in the synthesis of efficient antibacterial AgNPs for biomedical applications.

Silver nanoparticles have many different properties, including optical, chemical, electronic, catalytic, magnetic, antibacterial, and antimicrobial activities. They are harmful to bacteria but non toxic to animals, making them suitable for application in medical and healthcare products.Gram-positive and Gram-negative bacteria, including multidrug-resistant strains are affected because of a superior antimicrobial agent i.e. silver nanoparticles.(Bruna, Tamara *et al.*, 2021). When combined with other antibacterial agents like chemical compounds or antibiotics, silver nanoparticles have been found to have synergistic effects against harmful bacteria like *Escherichia coli* and *Staphylococcus aureus*.
The seminal paper reported by Sondi and Salopek-Sondi demonstrated the antimicrobial activity of AgNPs against *Escherichia coli*, in which *Escherichia coli* cells treated with AgNPs showed the accumulation of AgNPs in the cell wall and the formation of "pits" in the bacterial cell walls, eventually leading to cell death. Furthermore, the antibacterial activity of AgNPs is not only size—but also shape-dependent. Gram-negativeand Gram-positive bacteria, as well as methicillin-resistant Staphylococcus aureus, are highly susceptible to silver nanoparticles with an approximate size of 25 nm. Furthermore, a detailed study was carried out to investigate the efficiency of the antimicrobial effects of AgNPs against yeast, *Escherichia coli*, and *Staphylococcus aureus*. The results suggest that at low concentrations of AgNPs, the complete inhibition of growth was observed in yeast and *Escherichia coli*, whereas a mild effect was observed in *S. aureus*.

Nano-silver interacts with peptides and bacteria and serves as nanomedicine in various bacteria, fungi, and virus-mediated diseases. NPs have shown significant inhibitory effects against microbial pathogens, and are widely used as antimicrobial agents in a diverse range of products. Biological and irradiation methods have been included in Green synthesis approaches, which have advantages over conventional methods involving chemical agents associated with environmental toxicity. Numerous articles have been written about using different plant parts to make green silver nanoparticles (AgNPs). However, a method for synthesising AgNPs from fresh plant sources that is economical, environmentally responsible, and commercially viable is still needed (Chung et al., 2016).

From "Medical Microbiological Approach to Archaea in Oral Infectious Diseases." Japanese Dental Science, It was discovered that the microbial communities are more diversified than previously believed and contain a number of previously unidentified microorganisms. These unidentified or poorly defined organisms have been added to the list of research targets for microorganisms linked to oral disorders. The Archaea is one of these organisms. Based primarily on the frequency of detection or the increased numbers of these microorganisms in diseased areas in relation to the severity or symptoms of disease, a number of recent reports suggested that these microorganisms could be pathogens engaged in periodontitis and apical periodontitis. However, it cannot be concluded that Archaea are oral pathogens based on such circumstantial evidence.

In the experiment that served as the basis for this study, mouthwash was created through a series of stages involving chemical ingredients, to which red sandal-derived silver nanoparticles were added. The red sandalwood application, however, clearly showed the fundamental disadvantage of being an expensive & rare ingredient for any manufacturing that would benefit the users as a cost-effective/product with fair price when seen from the standpoint of market management. The more cost-effective natural ingredient Trachyspermum ammi (Ajwain), which, when combined with silver nanoparticles, has the same beneficial properties as the chemical ingredients found in any other commercial product, has replaced the red sandalwood-derived nanoparticles that were previously used in mouthwash. Various toothpaste chemical ingredients, including sodium fluoride (NaF), sodium lauryl sulphate, cocamidopropyl betaine, zinc lactate, and paraben, have been related to enamel discoloration, taste changes, and mucosal irritation owing to harm to the natural oral flora.

Using Obuli Ganesh Kishore S, et. al. 2022's study on the preparation of mouthwash using red sandal-mediated selenium nanoparticles as a reference, the further study related to production of homemade toothpaste was carried out. The majority of individuals in India suffer from periodontal disease and tooth decay. Periodontitis is a persistent bacterial

infection characterised by chronic inflammation, connective tissue loss, and alveolar bone destruction, which are all mediated by pro-inflammatory mediators. It is, however, highly dependent on the user's abilities. Mechanical treatments such as Brushing may also be difficult, challenging for persons who are disabled or traumatised. The use of supplemental treatments, such as mouthwashes, has been demonstrated to reduce plaque development. Mouthwashes containing chlorhexidine, for example, have been associated with enamel discoloration, taste changes, and mucosal irritation.

As a result, searching for an alternative antimicrobial agent with less side effects appears to be a possible choice. Because of their bacteriostatic and bactericidal properties, nanoparticles have been included into the structure of dental composites and disinfection treatments in recent years. From a market point of view, as an entrepreneur the cost effectiveness with more beneficiary products is the main goal considered in study with appropriate use of broad spectrum research studies. SYNTHESIS OF SILVER NANOPARTICLES FROM TRACHYSPERMUM AMMI SEEDS (AJWAIN SEEDS) AND ASSESSING ITS ANTIBACTERIAL ACTIVITY AGAINST TARGET MICROORGANISM STAPHYLOCOCCUS AUREUS the study from IJNRD science journal was referred for the study of application of AgNPs. With the green chemistry approach, the synthesis of AgNPs using *Trachyspermum ammi* seed extract was carried out. Biosynthesised silver nanoparticles showed antimicrobial activity against Gram-positive S. aureus. (Anagha *et. al.* 2023, Shloka *et. al.* 2023). For continuing the research further along with its applications, whether the efficacy of biosynthesised AgNPs against *S. aureus* even after its combination with homemade toothpaste is decreased or remained the same was checked.

METHOD AND MATERIALS:

Biosynthesis of Silver nanoparticles:

AgNP 1 and AgNP 2 were biosynthesized using 0.01M and 0.001 M silver nitrate respectively and ultrasonicated for 2 h.

Medical microbiological analysis

1. PREPARATION OF MEDIA

Enriched media:
· Nutrient agar

Selective media:
- Mitis salivarius agar
- Thioglycolate agar
- Cetrimide agar

Differential media:
- · Cysteine Lactose Electrolyte Deficient Agar
- · Mannitol salt Agar

Saliva sample was collected from the individuals having dental plaque by rolling sterile cotton swabs over the surface of both sides of the teeth and then placed into a sterile container containing saline. Using Pentagonal Streak technique, the sample was streaked onto the surface of sterile Nutrient agar, CLED agar, MSA agar, Cetrimide agar, Mitis salivarius agar, Thioglycolate agar and incubated for 24h. Subcultures of selected colonies were prepared on NA slants to use for further tests. The distinct colonies were examined using gram staining technique and observed on microscope under oil immersion lens at x100. After Gram staining to identify the species of the bacteria present in the samples biochemical test and special test were carried out as follows:

2. SPECIAL TEST:

I. Catalase test : This was carried out by putting a drop of Hydrogen peroxide on all 6 clean slides. With the edge of another slide, a colony of microorganism was picked and allowed to be in contact with the hydrogen peroxide.

II. Oxidase test : Moistened TMPPD disc was placed on the Colony taken on a glass slide.

3. BIOCHEMICAL TEST:

I. Sugar fermentation test : Sugar powders were dissolved in water and the addition of Andrade's indicator was carried out and kept for sterilisation. Loopful of culture was added in each tube and incubated for 24 hrs.

II. TSI test : TSI agar containing 1% lactose, 1% sucrose and 0.1% glucose was prepared for slants. The culture was streaked and stabbed on the TSI agar slant and the tubes were incubated at 37 degree C for 24hrs.

III. Indole test : One percent (1%) of tryptone broth was inoculated with a bacteria colony. Inoculated tubes were incubate at 37 degree C for 24 hours. After 24 hours of incubation, 1 ml of Kovac's reagent was added and the tubes were gently shake and allowed to stand for 20 minutes.

IV. Methyl red - Voges Proskauer test : MR-VP broth of pH 6.9 was prepared in a conical flask. 5 ml of broth was then poured in each of 7 test tubes and sterilised by autoclaving at 15 lb pressure for 15 min. The tubes were inoculated with test organism and incubated at 370C for 24hrs. After incubation each test tube were divided in 2 parts. To the 1st part, 5 drops of methyl red indicator was added. A red colour formation signifies a positive methyl red test and yellow colour signifies a negative methyl red test. To the 2nd part, 5 drops of 4% potassium hydroxide (KHO) was added followed by some 15 drops of 5% alpha naphthol in ethanol. The tubes were gently shaken for 1 min and allowed to reaction the complete for about 30-45 min.

V. Citrate test : The test organism was streaked onto Simmon's citrate agar slant (pH 6.9) and the tubes were incubated at 370C for 48 hrs.

VI. Urease test : The test organism was streaked onto Christensen urease agar (pH 6.9) and the tubes were incubated at 370C for 48 hrs.

4. CONFIRMATORY TEST- For *S. aureus*

Coagulase test : A loopful of plasma was added to the suspension then mixed gently and kept for 15 -30 min.

Preparation of toothpaste:

In a St. tube with lid, coconut oil and Baking soda was taken in equal proportion for mixing in between the burners maintaining sterility. constituents were gently mixed. Addition of some drops of rose essence was done and mixed to a thick consistency. The dilutions of toothpaste were prepared with a stock solution of 0.1mg of toothpaste and total volume of 1ml.

Table 1- Dilution table for testing efficacy of toothpaste

Concentration (ug/ml)	Toothpaste (mL)	Saline (mL)
0	0	1
200	0.2	0.8
400	0.4	0.6
600	0.6	0.4
800	0.8	0.2
1000	1	0

Testing the efficiency of toothpaste:

WELL DIFFUSION ASSAY METHOD :

A bacterial inoculum suspension of Gram-positive *Staphylococcus aureus,* a microorganism causing dental plaque obtained from Medical Microbiological analysis, was Bulk seeded in Nutrient Agar (NA) using the pour plate method. After the solidification of the agar, four wells were bored on each two plates using a well borer of internal diameter 8mm. Both the plates were incubated at 37 °C for 24 h, and the zone of inhibition was measured.

SWABBING OF DENTAL MODEL SAMPLE ON AGAR PLATE :

Swab from the dental model with layering of an earlier oral sample, without brushing teeth with toothpaste was collected and was swabbed onto NA, CLED, MSA agar plates. The plates were incubated at 37 °C for 24 hrs. Another sample was collected from the same dental model but after brushing with prepared toothpaste and was swabbed onto NA, CLED, SMA agar plates. These plates were incubated at 37 °C for 24 h.

OBSERVATION :

Survey

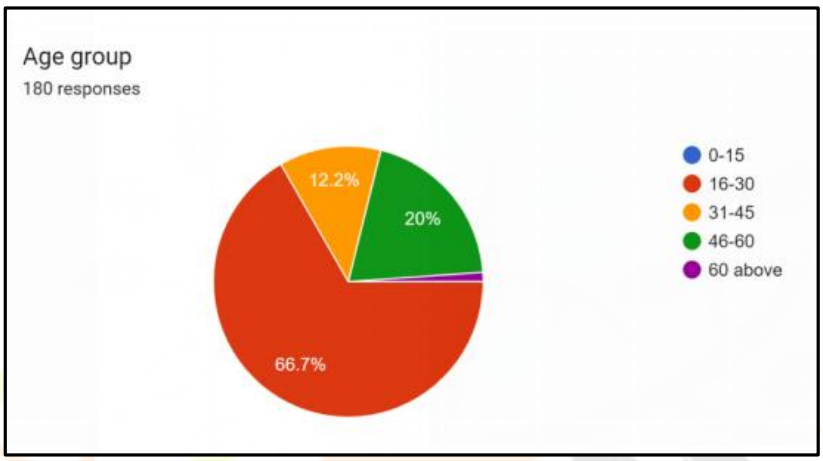

Figure 1- Pie chart of Question: 'Age group

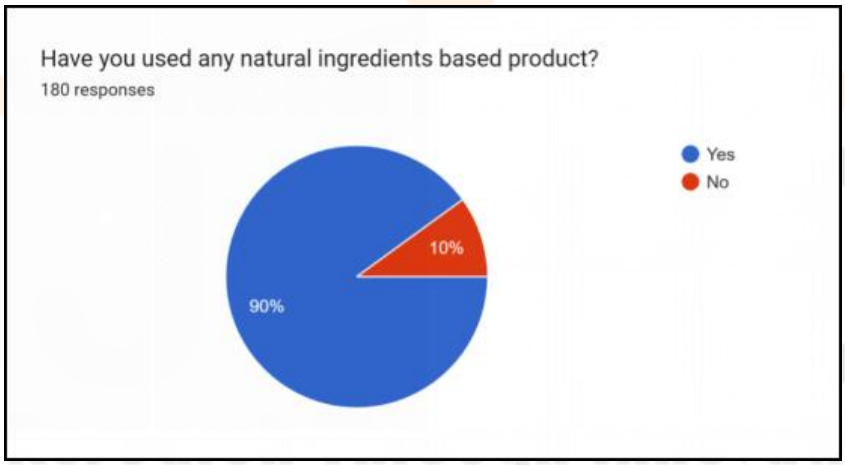

Figure 2- Pie chart of Question: 'Have you used any natural ingredients based products?

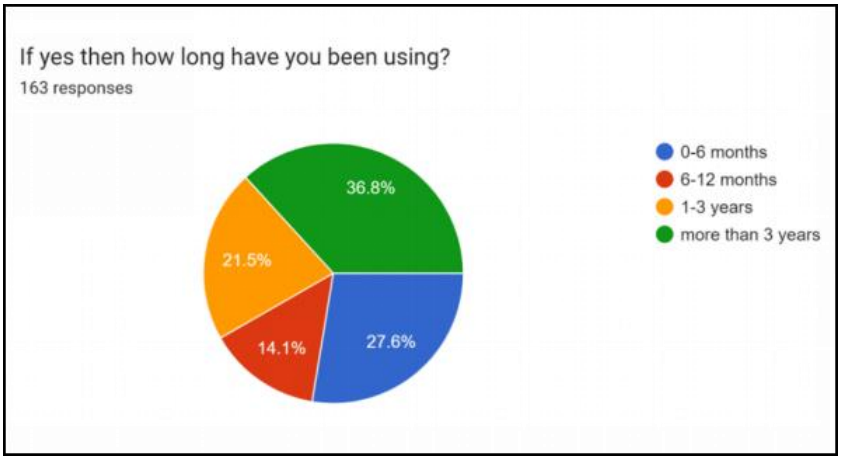

Figure 3- Pie chart of Question: 'If yes then how long have you been using?

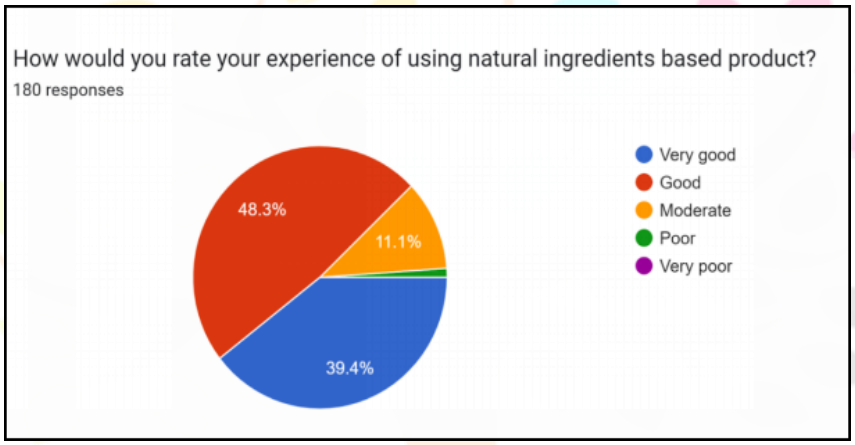

Figure 4: Pie chart of Question: 'How would you rate your experience of using natural ingredients based product'

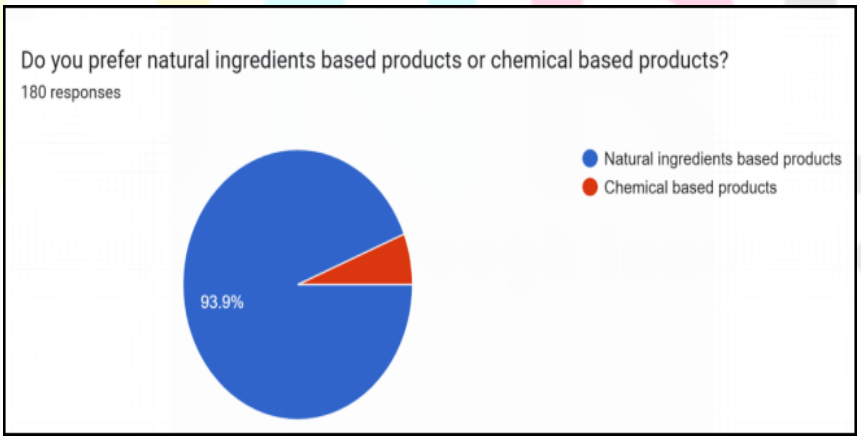

Figure 5: Pie chart of Question: 'Do you prefer natural ingredients based products or chemical based products'

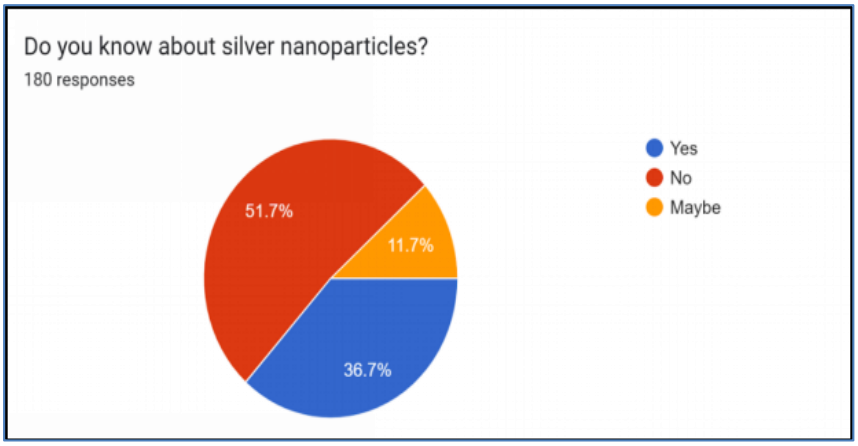

Figure 6- Pie chart of Question: 'Do you know about silver nanoparticles'

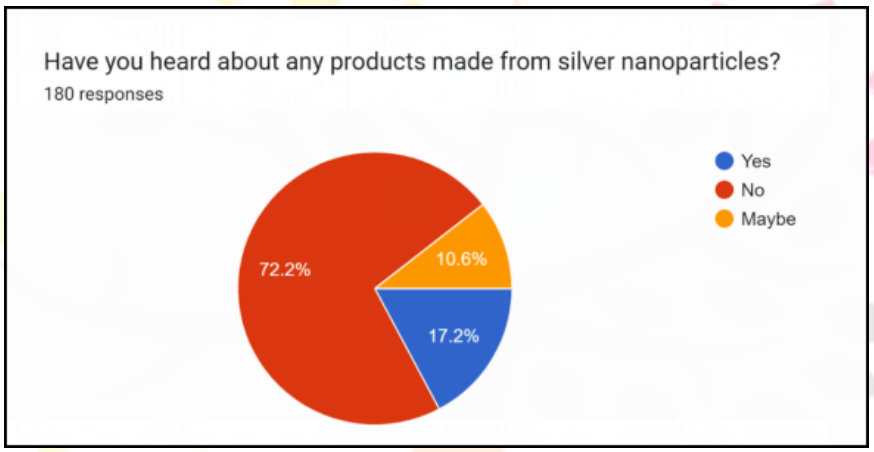

Figure 7- Pie chart of Question: 'Have you heard about any products made from silver nanoparticles'

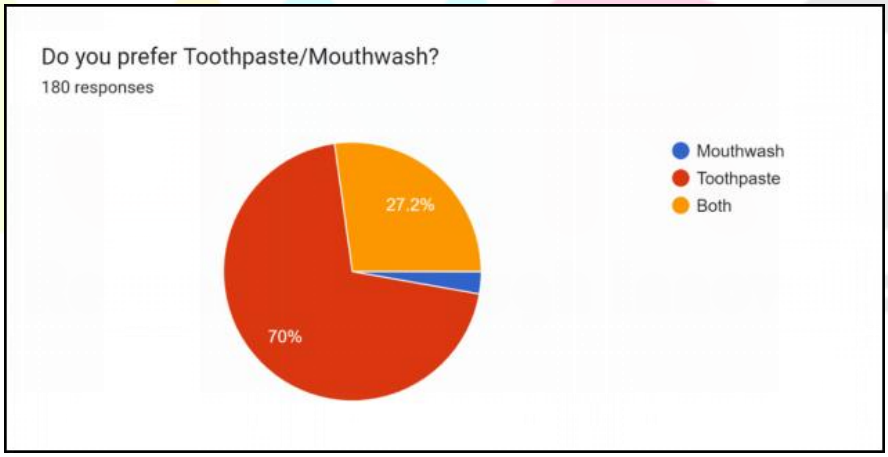

Figure 8- Pie chart of Question: 'Do you prefer Toothpaste/Mouthwash'

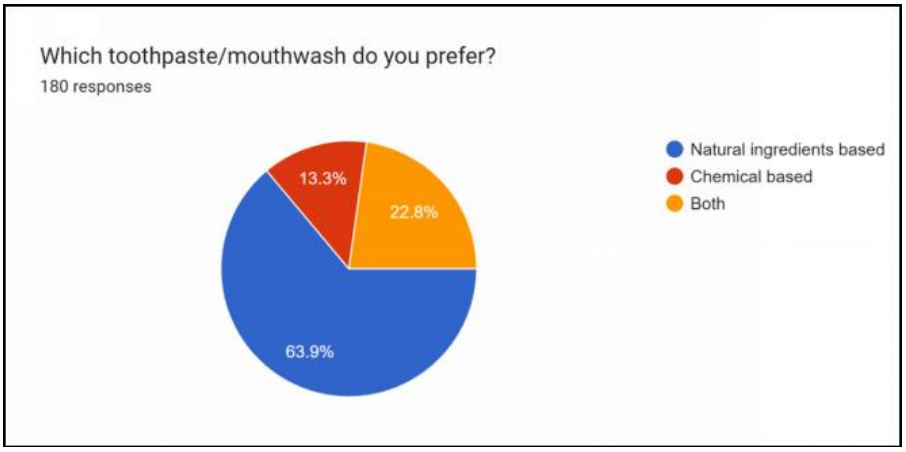

Figure 9- Pie chart of Question: 'Which toothpaste/mouthwash do you prefer?

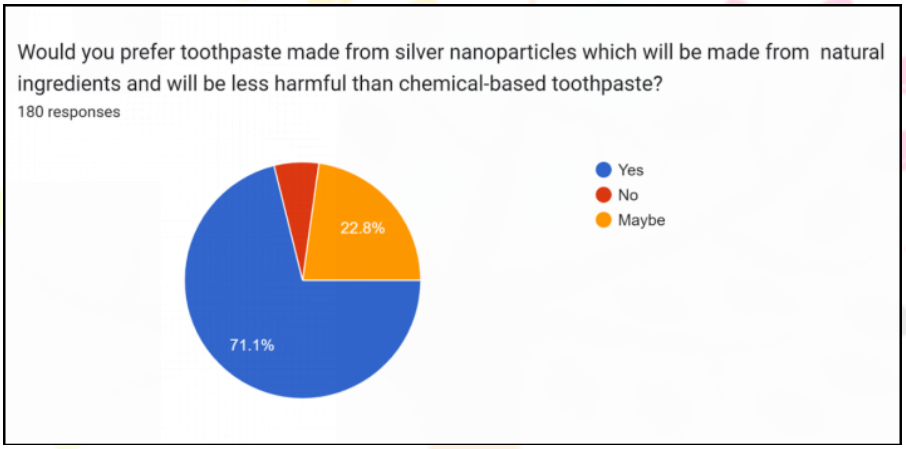

Figure 10- Pie chart of Question: 'Would you prefer toothpaste made from silver nanoparticles which will be made from natural ingredients and will be less harmful than chemical-based toothpaste'

Medical microbiological analysis

DAY 1 :

Figure 11- NUTRIENT AGAR

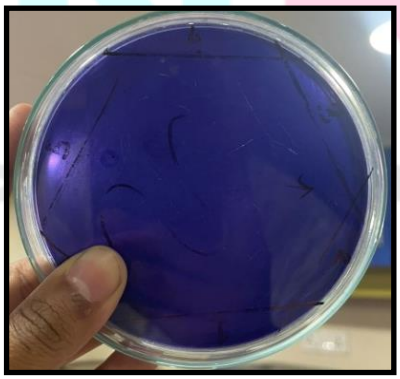

Figure 12- MITIS SALIVARIUS AGAR

Figure 13- THIOGLYCOLATE AGAR

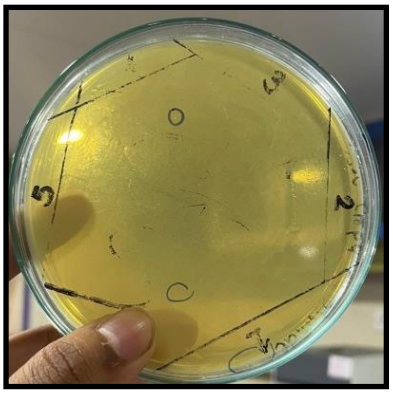

Figure 14- CETRIMIDE AGAR

Figure 15- CLED AGAR

Figure 16- SMA AGAR

DAY 2 : COLONY CHARACTERISTICS

Table 2 - Colony characteristics of colony 1 on Nutrient agar

Characteristics	Colony 1
Size	2mm
Shape	circular
Colour	yellow
Margin	entire
Opacity	opaque
Elevation	elevated
Texture	Smooth
Gram Staining	Gram positive cocci

Table 3 - Colony characteristics of colony 1 on thioglycolate agar

Characteristics	Colony 1
Size	Pinpoint
Shape	Spherical
Colour	white
Margin	Entire
Opacity	Opaque
Elevation	Elevated
Texture	Smooth
Gram Staining	Gram positive cocci

Table 4 - Colony characteristics of colony 1 on CLED agar

Characteristics	Colony 1
Size	2mm
Shape	Circular
Colour	White
Margin	Entire
Opacity	Opaque
Elevation	Elevated
Texture	Smooth
Gram Staining	Gram positive cocci

Table 5 - Colony characteristics of colony 1 on SMA agar

Characteristics	Colony 1
Size	4mm
Shape	Circular
Colour	Creamy yellow
Margin	Entire
Opacity	Opaque
Elevation	Elevated
Texture	Smooth
Gram Staining	Gram positive cocci

Table 6 - Colony characteristics of colony 1 and colony 2 on Mitis salivarius agar

Characteristics	Colony 1	Colony 2

Size	4mm	2mm
Shape	circular	circular
Colour	blue	colourless
Margin	irregular	entire
Opacity	opaque	translucent
Elevation	elevated	elevated
Texture	Smooth	Smooth
Gram Staining	Gram Positive cocci	Gram positive cocci

Table 7 - Colony characteristics of colony 1 and Ctrimide agar

Characteristics	Colony 1
Size	5mm
Shape	circular
Colour	white
Margin	entire
Opacity	opaque
Elevation	elevated
Texture	Smooth
Gram Staining	Gram negative cocci

GRAM STAINING :

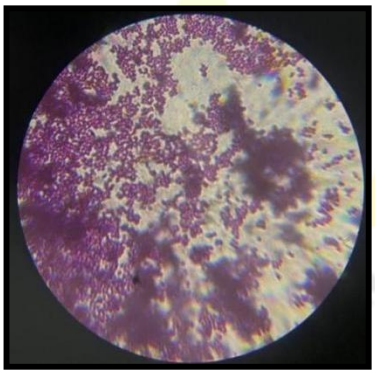

Figure 17 - NA colony 1

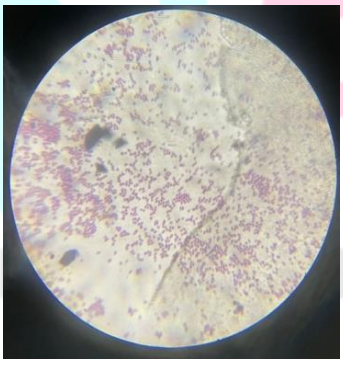

Figure 18 - Thioglycolate agar colony 1

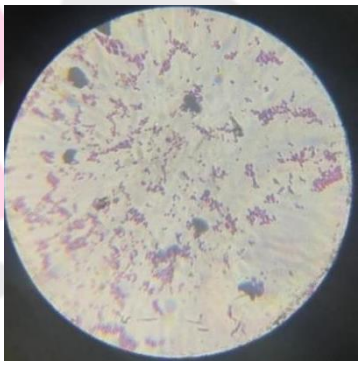

Figure 19 - CLED agar colony 1

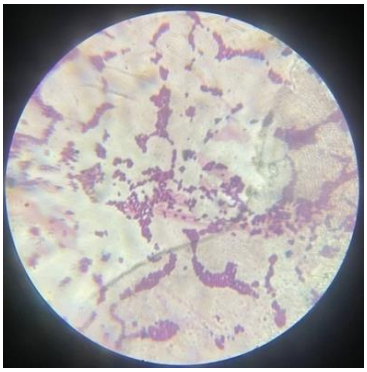

Figure 20 - SMA agar colony 1

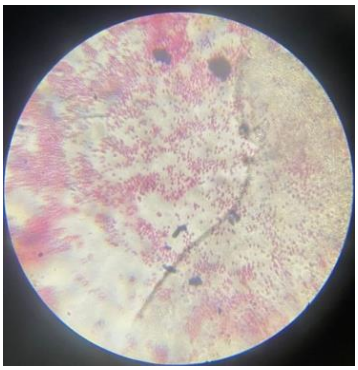

Figure 21 - Centrimide agar colony 1

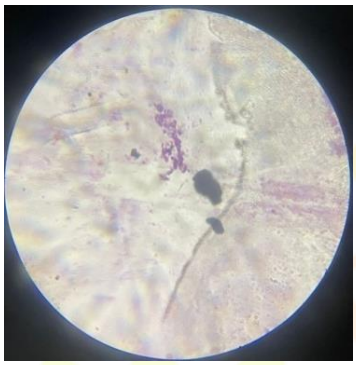

Figure 22- Mitis salivarius agar colony 1

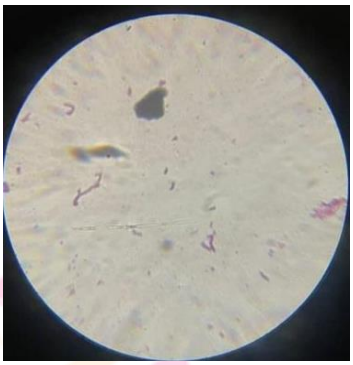

Figure 23 - Mitis salivarius agar colony 2

DAY 3 : BIOCHEMICAL TESTS

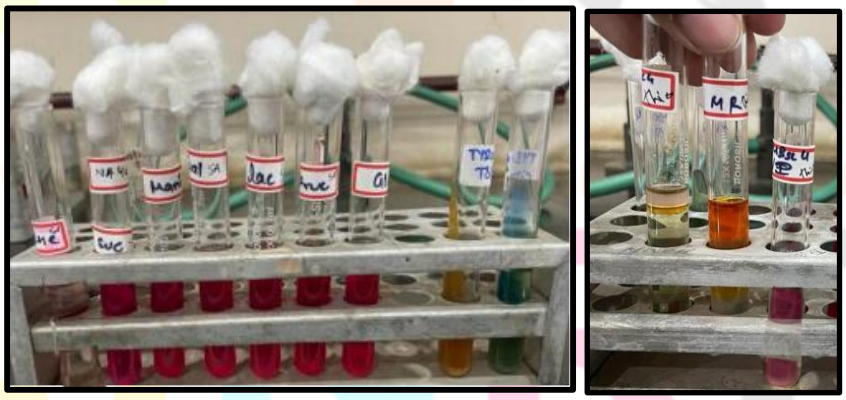

Figure 24 - NA colony 1

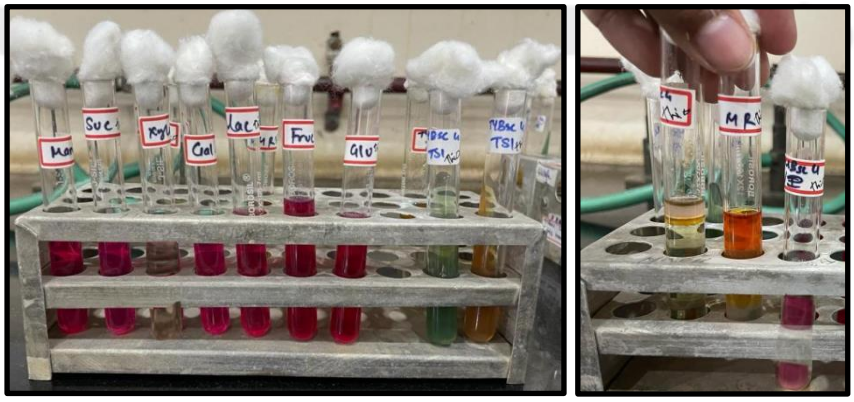

Figure 24 - Thioglycolate agar colony 1

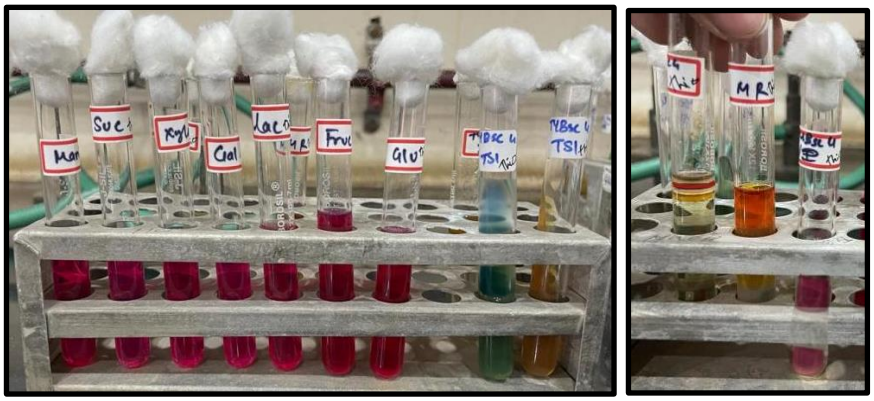

Figure 25 - CLED agar colony 1

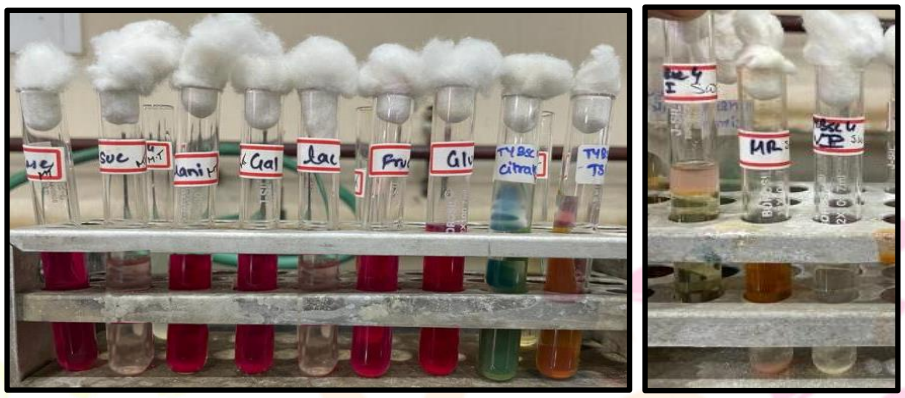

Figure 26 - SMA agar colony 1

Figure 27 - Centrimide agar colony 1

Figure 28- Mitis salivarius agar colony 1

Figure 29 - Mitis salivarius agar colony 2

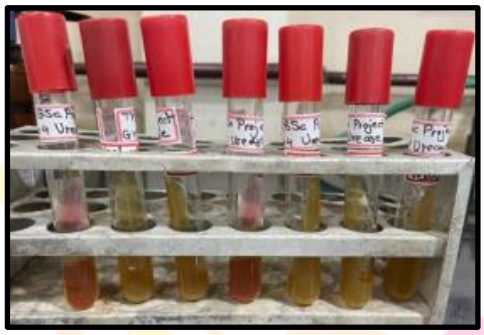

Figure 30 - Urease test result

Table 8 : Expected biochemical tests results:

	Streptococcus agalactiae	*Streptococcus mutans*	*Enterococcus faecalis*	*Staphylococcus xylosus*	*Providencia stuarti*	*Micrococcus luteus*	*Staphylococcus aureus*
Gram stain	Gram +ve Cocci	Gram+ve cocci	Gram +ve cocci	Gram +ve Cocci	Gram -ve Bacilli	Gram +ve Cocci	Gram +ve Cocci
Glucose	+	+	+	+	+	+	+
Fructose	+	nd	+	+	+	+	+
Lactose	variable	+	+	vari	-	-	+
Sucrose	+	+	+	+	vari	-	+
Mannitol	-	+	+	+	-	+	+
Galactose	+	+	+	+	+	+	+
Xylose	-	-	-	+	V	+	-
Indole	-	-	-	+	+	-	-
Methyl Red	+	+	nd	+	+	-	+
Voges proskauer	variable	+	+	+	-	-	+
Citrate	-	-	-	+	+	+	+
Urease	-	-	-	+	-	-	+
Triple sugar ion test	Both yellow	Both yellow	Both yellow	Both yellow	Both yellow	Both red	Both yellow
Catalase	-	-	-	+	+	+	+
Oxidase	-	-	-	-	-	+	+

Table 9 : Observed biochemical tests results:

	Mitis Salivarius Agar Colony 2	Mitis salivarius Agar colony 1	Thioglycolate Agar Colony 1	CLED agar Colony 1	Cetrimide agar Colony 1	MSA Colony 1	NA Colony 1
Gram stain	Gram +ve Cocci	Gram+ve cocci	Gram +ve cocci	Gram +ve Cocci	Gram -ve Bacilli	Gram +ve Cocci	Gram +ve Cocci
Glucose	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+
Lactose	+	+	+	+	-	-	+
Sucrose	+	+	+	+	+	-	+
Mannitol	-	+	+	+	-	+	+
Galactose	+	+	+	+	+	+	+
Xylose	-	-	-	+	+	+	-
Indole	-	-	-	+	+	-	-
Methyl Red	+	+	+	+	+	-	+
Voges Proskauer	-	+	+	+	-	-	+
Citrate	-	-	-	+	+	+	+
Urease	-	-	-	+	-	-	+
Triple sugar ion test	Both yellow	Both yellow	Both yellow	Both yellow	Both yellow	Both red	Both yellow
Catalase	-	-	-	+	+	+	+
Oxidase	-	-	-	-	-	+	+

Preparation of homemade toothpaste and its dilution :

Figure 31 - Prepared toothpaste

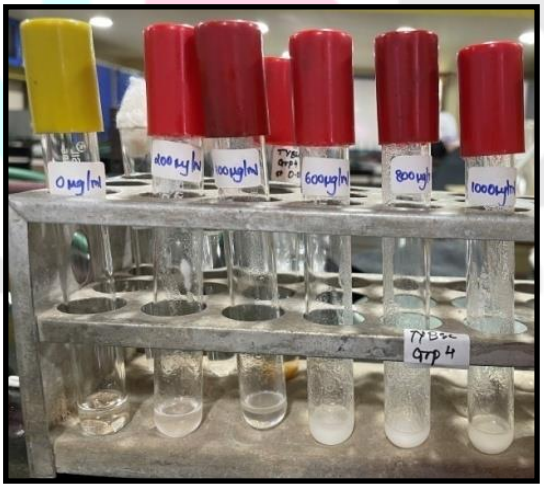

Figure 32 - Dilution of prepared toothpaste

Testing the efficiency of toothpaste:

WELL DIFFUSION ASSAY METHOD :

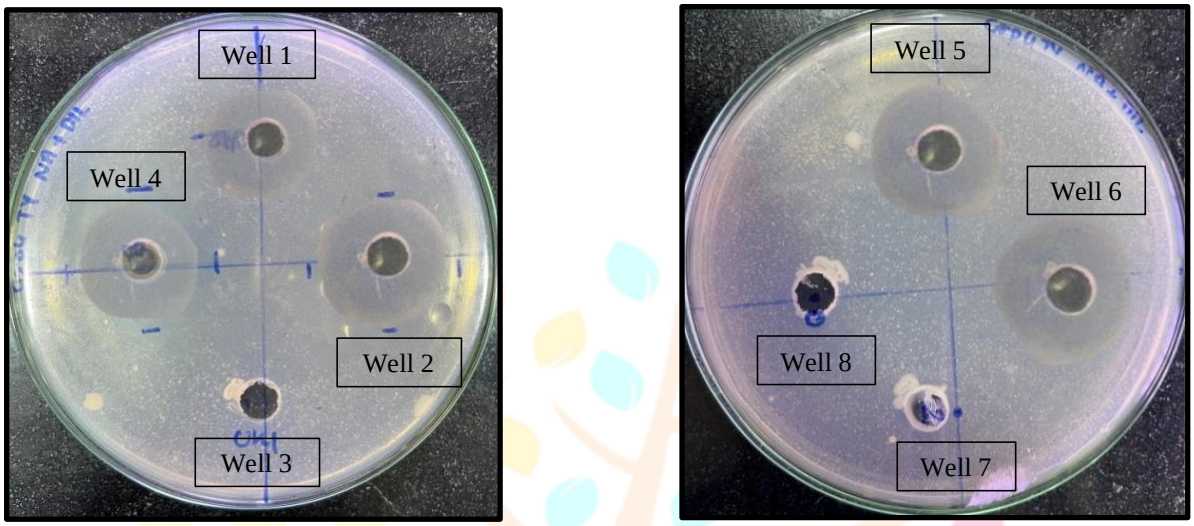

Figure 49- AST testing plates

Table 26- Result of AST for toothpaste

Concentration(µg / ml)	Toothpaste (ml)	Saline (ml)	ZOI 1 (mm)	ZOI 2 (mm)	ZOI (mm)
0 (well 8)	0	1	0	0	0
200 (well 7)	0.2	0.8	0	0	0
400 (well 1)	0.4	0.6	19	20	19.5
600 (well 5)	0.6	0.4	19	20	21
800 (well 4)	0.8	0.2	26	27	26.5
1000 (well 2)	1	0	29	28	28.5
Saline (well 3)	0	1	0	0	0
1mL ampicillin (well 6)	-	-	30	30	30

SWABBING OF DENTAL MODEL SAMPLE ON AGAR PLATE :

Before applying toothpaste

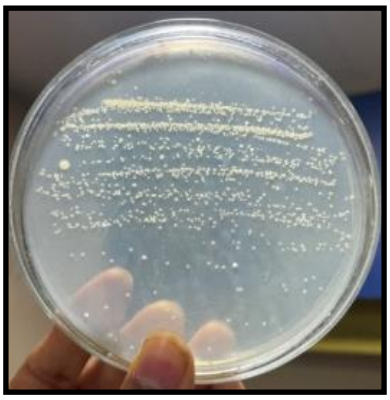

Figure 50- NA plate

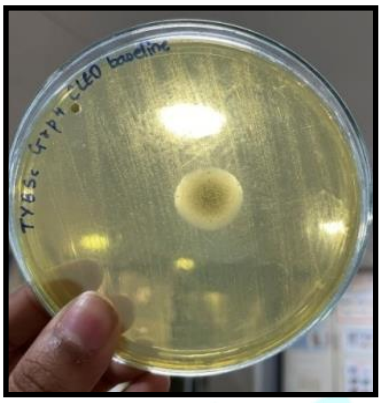

Figure 51 - CLED agar plate

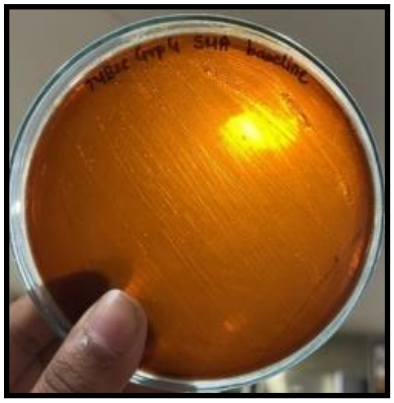

Figure 52- MSA plate

After applying toothpaste

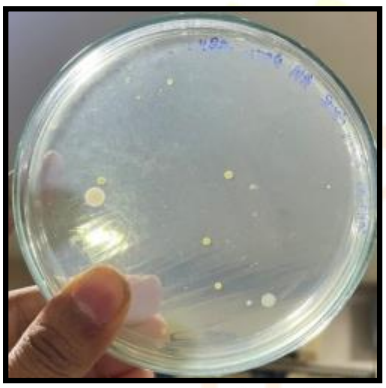

Figure 53- NA plate

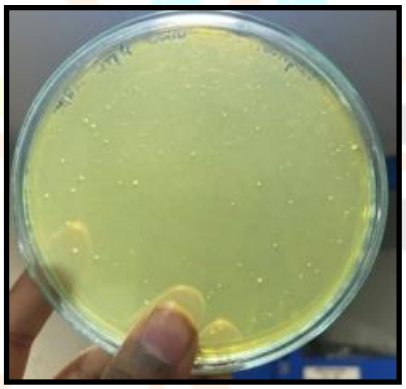

Figure 54 - CLED agar plate

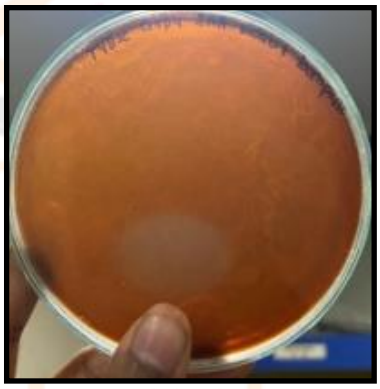

Figure 55 - MSA plate

RESULT

The results were obtained from an online survey conducted to check for people's preferences regarding natural ingredients and knowledge about silver nanoparticles. The most active age group who responded well to the survey was between 16-30 years old individuals. 93.9% of 180 individuals had been using and preferring natural ingredient based products over chemical based products for a long period of time. 36.7% of 180 individuals had the knowledge of silver nanoparticles and 7.2% of them have used products having silver nanoparticles. 70% of 180 individuals preferred toothpaste over mouthwash. 63.9% of these individuals preferred toothpaste prepared from natural ingredients. 71.1% of individuals preferred using toothpaste made by silver nanoparticles extracted from natural ingredients.

The study dealt with usage of biosynthesized silver nanoparticles from Ajwain seeds for preparation of homemade toothpaste. Through antibiotic sensitivity testing using well diffusion assay, AgNPs biosynthesised using 0.01M silver nitrate (AgNP 1) were found to be more effective against *S. aureus* as compared to AgNPs biosynthesised using 0.001 M silver nitrate (AgNP 2).(Anagha, Shloka *et.al.* 2023) Further, AgNP 1 solution was used for the preparation of toothpaste.

Isolation of bacterial species from oral cavity swab samples was carried out using the Medical Microbiology method as shown (Fig. 11 - 16).The colonies isolated from NA, CLED, Thioglycolate agar, Mitis Salivarius Agar and SMA were Gram positive cocci (Table- 9). Isolates of Gram negative bacilli were obtained from Cetrimide. After performing biochemical tests the results obtained were noted in table-. The results were found to be Mitis colony 1 was *S. mutans*,

Mitis colony 2 was *S. Agalactiae*, CLED was *S. xylosus*, SMA 1 was *Micrococcus luteus*, SMA 2 *S. aureus* and Thioglycolate was *E. faecalis*.

Toothpaste prepared from natural ingredients in sterile condition, was fused with biosynthesised AgNP 1. Serial dilution of nanoparticles was carried out for the fusion process from the results obtained; the concentration of 1000 µg / ml was found to be more effective for inhibiting growth of bulk seeded *S. aureus* after comparison of obtained ZOI (Table - 26) of each concentration. With the help of obtained results the swabbing of dental samples on NA, SMA, and CLED was carried out without application of modified toothpaste and after application of the same. Minimal growth of organism causing plaque was observed on NA, SMA, and CLED plates after application of prepared toothpaste compared to NA, SMA, CLED plates before application of prepared toothpaste as shown in (Fig. 50 - 55)

CONCLUSION

Antimicrobial toothpaste was prepared from natural ingredients and biosynthesized silver nanoparticles biosynthesised using 0.01 M silver nitrate. The efficacy of the toothpaste was checked using the target microorganism *Staphylococcus aureus* that selected from isolated oral flora . The undiluted form of tooth paste was found to be more potent against the test organism. Along with its antimicrobial property, the product synthesised required natural ingredients and was cost effective. However, further toxicity testing of the synthesised product needs to be carried out. Tests can also be conducted to check the antimicrobial activity of the product against various other microorganisms, including both normal flora and pathogens.

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