

# Comparative Evaluation of Different Toothpastes to Determine antimicrobial Efficacy

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## Abstract:

*The objective of this in vitro study was to evaluate the antimicrobial effect of different marketed toothpastes containing natural and synthetic ingredients. The effectiveness of toothpastes was evaluated against microorganism such as E. coli using Agar cup plate method (single plate method). Sterile pyrogen free distilled water was used as a control. Four different types of toothpaste were taken and tested its antimicrobial activity. Agar plate was made. Well were made by using borer. The dentifrices were loaded into the wells. After incubation, the inhibition growth zone were measured. and statistical analyses ( $\alpha=0.05$ ) were performed. The results indicated that all formulations showed antimicrobial activity against E.coli. The toothpaste containing natural ingredient i.e.dantkanti able to inhibit the maximum growth followed by vicco toothpaste. The toothpastes containing synthetic component like colgate and close-up shows less antimicrobial activity against microorganism. In the present study, it has been demonstrated that triclosan containing toothpastes formulations are more effective in control of oral microflora compared to non-triclosan containing synthetic toothpastes.*

**Keywords:** toothpaste, antimicrobial efficacy, agar cup plate method.

## 1. Introduction

Dental care needs more attention because of bad care of measuring. All tooth may cause, tooth decay, bad breath, tooth sensitivity periodontal gum disease and dental caries arise from microbial activity of Buccal cavity<sup>[1,2]</sup>. Dental disease could be painful and the pain may be increased by cold heat, or food readings. drinks and sweet<sup>[3]</sup>. There are three types of dental problems that include formation of dental plaque, dental caries and periodontal disease<sup>[4]</sup>. Toothpaste is mainly used to promote oral cleanliness and also acts as an abrasive that helps to prevent the dental plaque, dental caries and periodontal disease.

### 1.1. Dental Caries

Dental Caries was first described in Miller's chemo parasitic theory in 1890. Earlier, it was defined as a localized, post-eruptive, pathological process of external surface involving softening of hard tooth tissue and progressing to the formation of a cavity' by the expert committee on dental health met in Geneva in November, 1961<sup>[10]</sup>. It was changed over a years with research. The more elaborated version of definition was found in Saunders Comprehensive Veterinary Dictionary, 2007. „The Dental caries is the demineralization and loss of substance of the hardtissues of the teeth, leading to continued destruction of enamel

and dentine, and cavitation's of the tooth<sup>[11]</sup>. In more sophisticated way, it is defined as "The dental caries is a localized, progressively destructive disease of the teeth that starts at the external surface (usually the enamel) with the apparent dissolution of the inorganic components by organic acids that are produced in immediate proximity to the tooth by the enzymatic action of masses of microorganisms (in the bacterial plaque) on carbohydrates; the initial demineralization is followed by an enzymatic destruction of the protein matrix with subsequent cavitation's and direct bacterial invasion; in the dentin, demineralization of the walls of the tubules is followed by bacterial invasion and destruction of the organic matrix. in medical dictionary for the dental professions<sup>[12]</sup>.

Dental caries is a localized, transmissible infectious process that ends up in the destruction of hard dental tissue. It results from accumulation of plaque on the surface of the teeth a biochemical activity of complex micro-communities. *Streptococcus mutans* is one of the main opportunistic pathogens of dental caries<sup>[5]</sup>. Which plays a central role in fermenting carbohydrates resulting in acid production, and leading to the demineralization of the tooth enamel.

Dental caries is the localized destruction of susceptible decisions. Car dental hard tissues by acidic by-products from bacterial that progress of fermentation of dietary carbohydrates<sup>[6,7]</sup>. Dental caries results from interactions over time between bacteria that produce acid, a substrate that the bacterial can metabolize, and many host factors that include teeth and saliva. Dental caries results from an ecological imbalance in the physiological equilibrium between tooth minerals and oral microbial biofilms<sup>[8,9]</sup>.

Bacteria. live on teeth in microcolonies that are encapsulated in an organic matrix of polysaccharides, proteins, and DNA secreted by the cells, which provides protection from desiccation. host defences and predators and provides enhanced resistance to antimicrobial agents<sup>[6,9]</sup>.

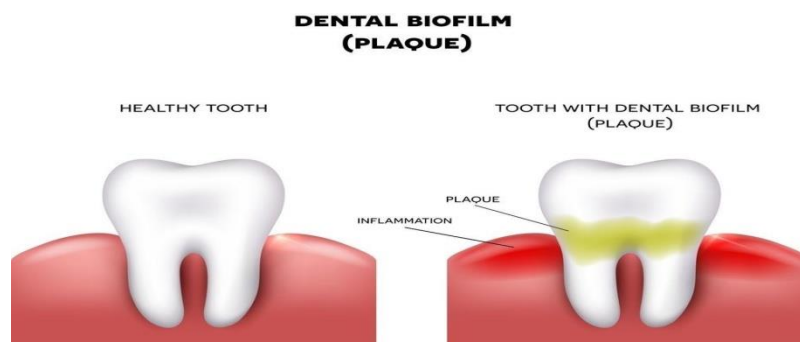
Teeth offer non-shedding surfaces for microbial colonisation and large numbers of bacteria and their by-products accumulate in a biofilm on tooth surfaces in health and disease<sup>[7,9]</sup>.

## **1.2.Dental Plaque:**

The oral cavity is haven for microbial flora where a very numerous and various microflora lives in together. The growth of such bacteria results in plaque formation which develops naturally on oral tissues, embedded in an adhesive matrix of salivary and bacterial polymers. This adherent deposit of bacteria and their products, forms on all tooth surfaces and is the cause of caries. This community works together, having a collective physiology, it means not a haphazard collection of bacteria but a community of micro-organisms attached to a surface. They are always metabolically active. Their ecology is constantly changing by external factors, such as daily food, dental care or possible antibiotic therapy<sup>[13]</sup>.

In addition, other microflora like *Escherichia coli* and *candida* are also associated with active

caries lesions. *C. albicans* is the most common yeast isolates from the oral cavity. It is by far the fungal species most commonly isolated from infected root canals, showing resistance to intercanal medication<sup>[14]</sup>. Poor oral hygiene is one of the reasons for accumulation of these microbes and their harmful activities.



### 1.3.Periodontal Disease :

Periodontal diseases are bacterial infections that affect the supporting structure of the teeth (gingival, cementum, periodontal membrane and alveolar bone) the endotoxins, hydrolytic enzymes and toxic bacterial metabolites are involved in this disease. Gingivitis, and inflammatory condition of gum, is the most common form of periodontal disease. Serious forms of periodontal disease that affect the periodontal membrane and alveolar bone may results in tooth loss. Streptococci, spirochetes and bacteroides are found to be the possible pathogens responsible for the disease.

An adult's mouth may contain 500 to 1000 different types of bacteria as part of the human oral flora about 100 to 200 species may live in it at any given time. Those who care for their teeth and have a relatively clean mouth have 1000 to 100,000 bacteria living on their tooth surfaces. Those that do not have a clean mouth have between 100 million bacteria on their tooth surfaces<sup>[15]</sup>.

Normal flora of the mouth include *Staphylococcus salivarius*, *Enterococcus faecalis*, *pyogenes*, *streptococcus pneumonia*, *streptococcus Neisseria meningitides*, *Escherichia coli*, *Heamoplus influenza*, *Actinoomycetes*, *Mycoplasmas*, *Pseudomonas aeruginosa*. Others are *Lactobacilli*, *Corynebacteria*, *Neisseria*, *Proteus*, *Clostridium* and other *Streptococci* and *Staphylococci*.

One of the problems of oral and dental health is the growth of oral microflora that causes dental plaque. The bacteria that play a dominant role in plaque formation and caries development are *Streptococcus mutans*. Plaque control is an effort to remove and prevent plaque build-up on tooth surfaces. These efforts can be done either mechanically or chemically. Mechanical discharges may include tooth brushing and dental floss use often do not produce maximum results due to lack of skills.



#### 1.4.Toothpaste

Toothpaste is a paste or gel dentifrice used along with a toothbrush as an accessory to clean and maintain the aesthetics and health of teeth. Toothpaste is used to promote the oral cavity by serving as an abrasive to remove food and dental plaque from the teeth<sup>[16]</sup>. Toothpastes are considered as one of the most used and common cosmetic and hygienic materials<sup>[17]</sup>. Toothpastes have been used since the ancient past and are one of main irreplaceable components of oral health care. Toothpaste is a dentifrice used to clean, maintain and improve the health of teeth<sup>[18]</sup>. To promote the attractiveness and to maintain the health of teeth the formulation is used called as toothpaste. For cleaning the teeth, toothpaste are widely used preparations<sup>[19]</sup>.

This primary function of cleaning is carried out with the help of a toothbrush. The objective behind the use of tooth paste is its ability to deliver form preventive and therapeutically active agents such as fluoride, metal salts and pyrophosphate. These agents may be useful for calcium inhibition also reduce the growth of plaque and to treat dentine hypersensitivity along with dental hygiene.

Nowadays, manufacturing companies formulated the toothpaste in various form for specific purpose. Non-toothpaste is an effective alternate for those who have some common sensitivity to fluoride. Some manufacturers now marketed low fluoride toothpastes for kids that contains less than 600 ppm fluoride. Sensitivity toothpastes are formulated for sensitive teeth can be effective in relieving the pain. Whitening toothpastes have fluoride and an enzyme system. Natural toothpaste has great demand today. They are made from herbal extracts and other natural ingredients. Toothpastes consists of abrasives, detergents, flavouring, coloring agents, and sweeteners, binding agents, humectants, preservatives, fluorides, anti-plaque agents, anti-calculus agents, herbal extracts and water. They are constituted from the following ingredients. The quality and quantity of ingredients varies by brand to brand.

To overcome the problem of bacterial infection it is recommended for the patient to use toothpaste with better antibacterial activity. Most of the toothpaste available in the market nowadays contains two types of ingredient which are the active and non-active toothpaste ingredient or the excipient. One of the active toothpaste ingredients is abrasive which helps in removing the plaque. It constitutes at least 50% from the total preparation of the toothpaste. It really helps in minimized periodontal disease.

The commonly used abrasives are sodium bicarbonate, calcium carbonate and aluminum hydroxide. Whitening agents helps to remove stain on the teeth but the effect is temporary. The commonly used whitening agents for toothpastes in the market are peroxide and bleach. Fluoride and its derivatives are used to strengthen the enamel and prevent cavities. The most common used fluorides are sodium fluoride, sodium mono fluorophosphate, olaflur and stannous fluoride. Between them, stannous fluoride shows effective controls of gingivitis.

The problem now is most people do not know the long-term consequence of using the commercial toothpastes. This is because the marketed toothpastes contain substance which consider as unhealthy and could harm the body in the future. Recently there is some issues arise on the harmful effect of fluoride when being used for longer period of time. The bleach and peroxide used as whitening agent in the toothpaste is consider as hazardous as they may cause irritation to the mouth and skin in small amount and chemical burn in large amount. The flavouring agents used are synthetically and chemically produced and commonly derived from petrochemical.



#### 1.4.1. Ideal properties of toothpaste: toothpaste should be

- Good abrasive effect
- Non irritant and non toxic
- Impart no stain in tooth
- Keep the mouth fresh and clean
- Prolonged effect
- Cheap and easily available

#### 1.5. Microorganisms

More than 700 bacterial species or phylotypes, of which over 50% have not been cultivated, have been detected in the oral cavity<sup>[20]</sup>. However, a recent report using pyrosequencing analysis estimated the number of phylotypes to be greater than 19,000<sup>[21]</sup>. Not surprisingly, the oral cavity also restrains the greatest biodiversity of any known human-associated biofilm. Though, more than 300 species of microorganisms have been identified in the mouth<sup>[22]</sup>.

1.5.1. **Anaerobes:** The human mouth provides a suitable habitat for numerous anaerobic bacteria. Anaerobic species constitute a significant part of the bacterial community of the mouth. Many researchers reported the presence of anaerobic genera or species of anaerobic members in the oral cavity. Such genera are Actinomyces, Bifidobacterium, Bacteroides, Leptotrichia, Eubacterium, Fusobacterium, Peptococcus, Peptostreptococcus, Veillonella, Propionibacterium, Lactobacillus, Selenomonas<sup>[23]</sup>, Abiotrophia, Rothia, Dialister, Olsenella<sup>[24]</sup>, Capnocytophaga, Leptotrichia, Campylobacter, and Treponema<sup>[25]</sup>.

These genera are causing typical dental plaque on human teeth as well as involvement in periodontal diseases, gingivitis and invasive human infections of the head and neck, chest, lung, liver and abdomen. Apart from bacilli, cocci and actinomycetes, a spiral shaped bacteria Treponema denticola also considered as the member of oral micro flora. It is a gram-negative bacterium from the Spirochetes family that is motile, slender, helically shaped and flexible. It is commonly found in the human oral cavity, specifically in sub gingival dental plaque, and it is often associated with periodontal disease<sup>[26]</sup>.

## **1.6.Diagnosis**

Caries diagnosis involves whether a lesion is detectable or not, studying the progression of caries, site of infection, deciding the type of caries etc. The proper diagnosis is depending on gathering the information like patient's history, nutritional analysis, clinical examination, salivary analysis and radiographic assessment and is useful in designing appropriate treatment planning. The classical and advance tools can assist the dentition to diagnose progression of caries. These include diagnostic techniques; caries risk assessment tests and indexing methods.

## **1.7.Prevention**

The global perspectives of preventive dentistry is based on the premise that every oral health activity implemented by the individual, the community or the dental professional is targeted towards the prevention of some aspects of the health-disease. Preventive dentistry encompasses all as acts of dentistry And those practices by dental professionals, individuals and communities that affect oral health. It has been conceptualized in a number of ways.

The action taken prior to the onset of disease, which removes the possibility that a disease will ever occur is called primary prevention. The concept of primary prevention is now being applied to the prevention of chronic diseases such as coronary heart disease, hypertension and dental caries, periodontal disease based on elimination or modification of "risk factors" of disease. A regular treatment is carries out to terminate a disease process and to restore tissues to as near normal as possible is called secondary prevention. The aim of tertiary prevention is to rehabilitate the patients to the normal as possible, after the failure of second prevention by replacing the lost tissues.

## **1.8.Good oral hygiene practices:**

Plaque control is an effective way of treating and preventing dental caries It is the removable of microbial plaque and prevention of its accumulation on the teeth. To date, the most dependable mode of controlling microbial plaque is still by mechanical cleansing with a tooth brush and other hygiene aids. Considerable progress has also been made with chemical inhibitors of plaque incorporated in mouthwashes or dentifrices (toothpastes). Bacterial plaque can be effectively removed by mechanical means. Mechanical means is safe and effective. It is brought by manual toothbrush, electronic tooth brushes, dental floss, tongue cleaner, toothpick with the aid of toothpastes, tooth powders and dantmanjan powder etc. Chemical control of dental plaque may involve prevention of plaque formation, removal on dispersion of existing plaque, inhibition of calcification of existing plaque, or altering the pathogenicity of plaque.

## 2. Aim and Objective:

The aim of the present study was to determine the prevalence of dental caries followed by isolation and identification of the causative microbial agents in dental caries. The study further extend to check the antimicrobial susceptibility of isolates against toothpastes According to above background the following objective have been set for the study

- a. To isolate and purify the microorganisms causing dental caries.
- b. To study the antimicrobial susceptibility of the isolates against different toothpastes.
- c. To determine the prevalence rate of dental caries.

## 3. Materials and Methods:

- Test tube
- Conical flask
- Test tube stand
- Measuring cylinder
- Micro tips box
- Petri plates
- Borer
- Spreader
- Micropipettes
- Inoculating needle

### 3.1. Media:

- ❖ EMB
- ❖ Nutrient agar
- ❖ Nutrient broth

### 3.2. Tooth paste formulations:

1. Dant kanti
2. Vico
3. Colgate
4. Close-up



**Table 1 - Toothpastes and their ingredients listed on their packages.**

Toothpastes	Ingredients as listed on packages
Dantkanti	Anacyclus pyrethrum, Azadirachta indica, Acacia arabica, Xanthoxylum alatum, Mentha spicata, Syzygium aromaticum, Pipersylvaticum, Barleriaprionitis, Mimusops elengi, Embelia ribes, Curcuma longa, Salvadoria persica, Quercus infectoria,
Vico	PASTE CONTAINS: Extracts of: Bahul (Acacia arabica, Willd-Bark- Powder) Jambul (Eugenia jambolana, Lam-Bark-Powder) Lavang (Caryophyllus aromaticus, Linn-Fruit-Powder) , Manjishtha (Rubia cordifolia, Linn-Stem-Powder) Dalchini (Cinnamomum zeylanicum, Blume-Bark-Powder) Bor (Zizyphus jujuba, Lam-Bark-Powder) Vajradanti (Barleria prionitis, Linn-Stem-Powder) , Acrod (Juglans regia, Lim-Bark- Powder) , Khair (Acacia catechu, Willd-Bark-Powder) , Patang (Caesalpiniasappan, Linn.-Stem-Powder) , Bakul (Mimusops elengi, Linn. Bark-Powder) , Jeshthamadh (Glycyrhiza glabra, Linn.-Root-Powder) Kavab-Chini (Chirfal) (Zanthoxylum rhetsa, DC-Fruit-Powder) . Anantmul (Hemidesmusindicus, R.Br-Root-Powder). Maifal (Quercus infectoria, Oliv.-Fruit- Powder), Trifala - (Amla - Emblica officinalis, Gaertn-Fruit-Powder, Hanta-Taminaia chebula Retz-Fruit-Powder, Behada - Terminalia belerica, Roxb-Fruit-Powder) . Ajwan (Carum copticum Benth & Hook-Fruit-Powder) Akkalkadha (Anacyclus pyrethrum, DC-Stem-Powder) and Excipients q.s.
Colgate	Calcium Carbonate, Sorbitol, Sodium Lauryl Sulphate, Arginine, Silica, Titanium Dioxide, Sodium Silicate, Flavor , Carrageenan, Sodium Monofluorophosphate, Sodium Bicarbonate, Potassium Nitrate, Benzyl Alcohol, Sodium Saccharin, Limonene, in aqueous base
Close up	Sorbitol, Water, Hydrated Silica, Sodium Lauryl Sulphate, PEG-32, Flavor, Cellulose Gum, Cocamidopropyl Betaine, Sodium Saccharin, Sodium Fluoride, Zinc Sulphate, Sodium Hydroxide, Synthetic Fluorophlogopite, Melaleuca Alternifolia (Tea Tree Extract), Eucalyptus Globulus (Eucalyptus Extract), Eugenol .

### 3.3. Sample collection:

1. A sample were collected from teeth and teeth of patients suffering dental caries problem from dental clinic in khapa.
2. The sterile cotton swab was vigoursly rubbed on the caries area and placed in sterile transport medium i.e. nutrient broth.
3. The broth culture was brought in laboratory and incubate at 37°C for 24 hours.
4. The broth cultures were streaked onto selective media such as EMB for E.coli. EMB agar plates were incubated at 37°C for 24 hours .
5. After the incubation period, the colonies were identified on the basis of colony morphology and the typical colonies from each sample plate were transferred to selective slant and incubated at 37°C for 24 hours for the gram staining.

### 3.4. Gram Staining:

The Gram Stain is differential staining technique most widely applied in all microbiology disciplines laboratories. It is one of the most important criteria in any identification scheme for all types of bacterial isolates. Different mechanisms have been proposed to explain the gram reaction. There are many physiological differences between gram positive and gram-negative cell walls. Ever since Christian Gram has discovered Gram Staining this process has been extensively investigated and redefined.

In practice, a thin smear of bacterial cells is stained with crystal violet, then treated with an iodine containing mordant to increase the binding of primary stain. A decolourizing solution of alcohol or acetone is used to remove the crystal violet from cells which bind it weakly and then counterstain (like safranin) is used to provide a colour contrast in those cells that are decolourized.

The gram-positive organisms or cells have more mucopeptide in their cell walls as compared to gram negative ones. Gram-negative bacteria have more content of polysaccharides and lipo-proteins in their cell walls. The polymers of glycerol or ribitol phosphate called as teichoic acids are also found in the cell walls of gram-positive organisms but are very less or almost not present in gram-negative organisms. In a properly stained smear by gram staining procedure, the gram positive bacteria appear blue to purple and gram negative cells appear pink to red.

### 3.5. Directions:

1. Prepare a thin smear on clear, dry glass slide.
2. Allow it to air dry and fix by gentle heat.
3. Flood with Gram's Crystal Violet for 1 minute.
4. Wash with tap water.
5. Flood the smear with Gram's Iodine. Allow it to remain for minute.
6. Decolourize with Gram's Decolourizer (S032) until the blue dye no longer flows from the smear.
7. Wash with tap water.

8. Counter stain with 0.5% w/v Safranin (S027). Allow it to remain for 1 minute.
9. Wash with tap water.
10. Allow the slide to air dry and examine under oil immersion objective.

### **3.6. Evaluation of Dentifrices:**

The study was aimed at knowing the brands of toothpastes that are mostly used. As a result, four toothpastes were selected for assessment of their in vitro antimicrobial activities.

They were purchased from local markets in Nagpur, Maharashtra, India.

The selected dentifrices solutions were made by mixing the calculated amount of toothpastes (2.0gm) in measured volume (2ml) of sterile pyrogen free distilled water.

### **3.7. Antimicrobial assay**

The Antimicrobial activity of different concentration of the dentifrices was determined by modified agar well diffusion method. In this method, nutrient agar plate were seeded with 0.5 ml of 24h broth culture of each isolate (brain heart infusion agar was used for streptococcus mutans strain). The plate were allowed to dry for an hour. A sterile 7 mm corkborer was used to cut one central and five well at equidistance in each plate. 0.2 g of the toothpaste was introduced into each of the five well. The plates were incubated at 37°C for 24 hr. the antimicrobial activity was evaluated by measuring the diameter of the zone of inhibition. All the plate were made five times and all the experiments were repeated 5 times.

### **3.8. Evaluation of toothpaste:**

#### **3.8.1. Organoleptics characters –**

##### **A. Colour:**

Colour of the prepared toothpaste was evaluated for its colour. The colour was checked visually.

##### **B. Odour :**

Odour was found by smelling the product.

##### **C. Taste:**

Taste was checked manually by tasting the product.

### 3.8.2. Physical characterization test:

#### A. Determination of pH :

Take 1 gm of the tooth paste in a 150 ml beaker and add 10 ml of freshly boiled and cooled water (at 27°C). Stir well to make a thorough suspension. Determined the pH of the suspension within 5 minutes, using digital pH meter.

We make 1% of sample solution for determine the ph or hydrogen ion concentration. Then we standardized the ph with buffer solution of pH 4 and pH 7. Then Immerse the Electrode in the solution under examination and measure the pH at the same temperature as for the standard solution. Measure the pH of the solution used to standardize the meter and the Electrode. All the samples were tested for 3 times and average of all three readings was used as final readings.

#### B. Foamability:

The foam ability of the product was evaluated by taking small amount of preparation with water in a measuring cylinder initial volume was noted and then shaken for 10 times. Final volume of foam was noted.

For the determination of Foaming Capacity (Index) we used Cylinder shake method. Firstly we prepare 1% of 50 ml dilute sample solution of toothpaste and kept in a 100 ml of Stoppard measuring cylinder and Shake well 10 times. The Total volume of Foam Content after 1 min of shaken was recorded the Height of the Foam generated was measured immediately.

### 3.8.3. Study of rheological properties:

#### A. Spreadability :

The Spreadability is term express to denote the extent of area to which the paste readily spreads on application area. One of the criteria for a paste to meet ideal quality is that it should posses good spreadability. About 0.5 gm of medicated dental paste was weighed and kept at the center of the glass plate and, another glass plate was placed over it carefully. 500 g weight was placed at the center of the plate (avoid sliding of the plate). The diameter of the paste in cms, after 5 min. was measured.

The Spreadability (S) can be calculated using the formula  $S = m.l/t$  Where, S-Spreadability. m-Weight tied to upper glass slide. l-Length moved glass slide. t-Time taken.

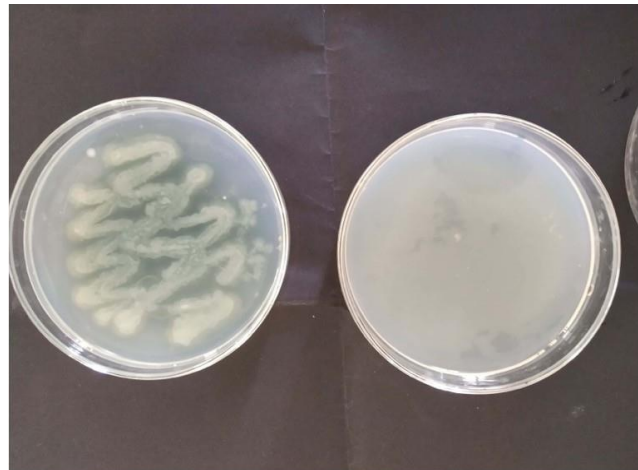
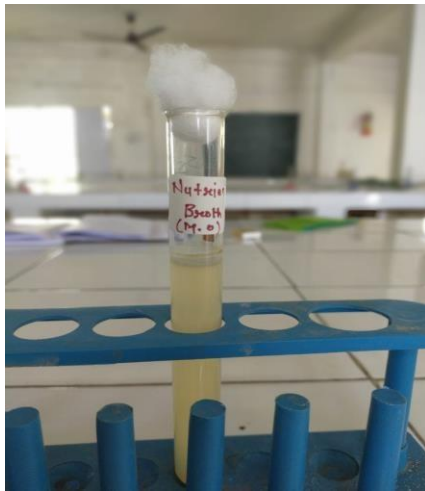
### 3.9. Microbiological studies:

Antibacterial activity of paste was tested by used against causative microorganism on agar plates. By taking microorganism such as E. coli.

## Testing of Antimicrobial Activity:

### 3.9.1. Preparation of Inoculum:

A loopful culture was inoculated from the stock slant culture in 5 ml of nutrient broth . Nutrient broth was incubated at 37°C in incubator for 24 hrs. After incubation a loopful of culture actively growing. This culture was used for the inoculum of agar plates.



(b)

### 3.9.2. Preparation of Nutrient agar medium :

Nutrient agar medium was prepared as per instructions of manufacturer. Suspend 6 gm of nutrient agar medium in 100 ml of distilled water.

Mix with continuous stirring to fully dissolve the agar powder. Now the prepared medium was transferred into Conical flask and was sterilized by using autoclave at 15lb for 20min.

## 4. Result and Discussion:

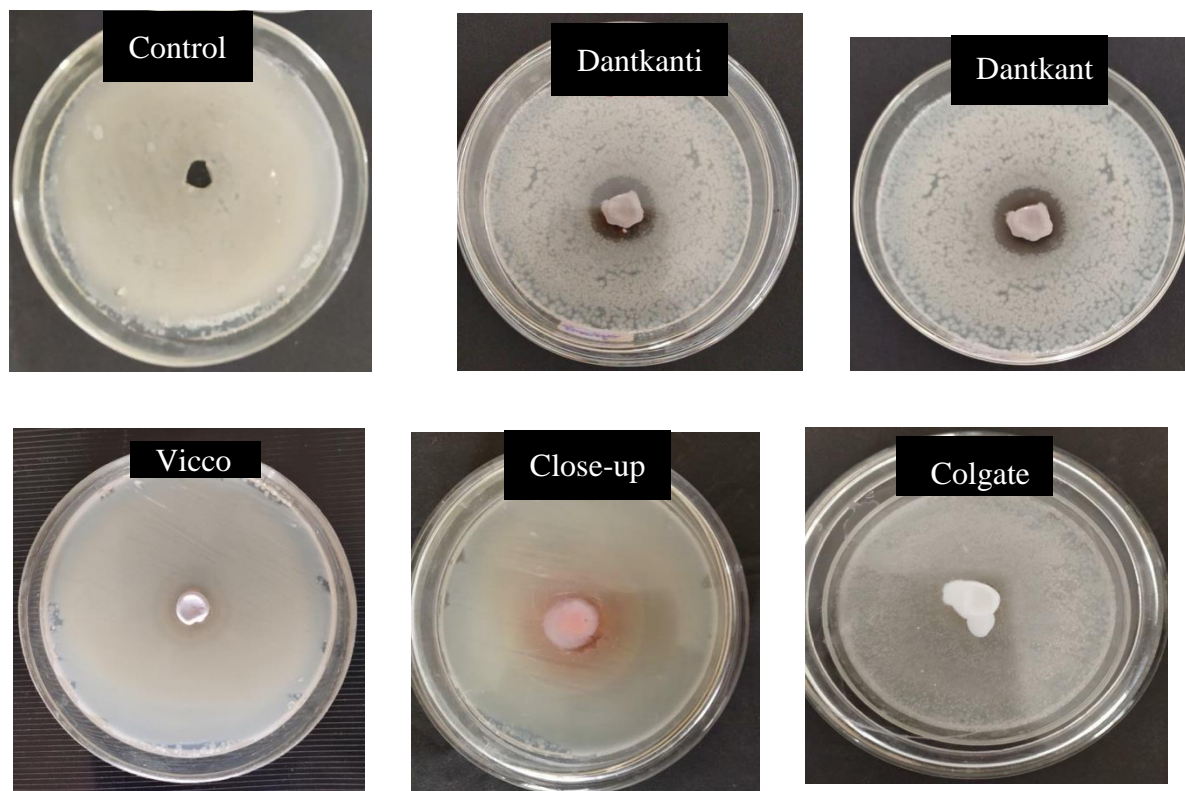
### 4.1. Antimicrobial activity

#### Agar cup plate method:

Cup plate method was performed throughout the experiment for different sample of toothpastes to the zone of inhibition of sample.

**Table 2: List of toothpaste used and their respective zone in diameter (in cm)**

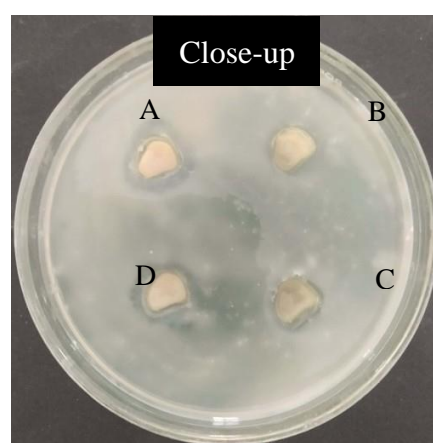
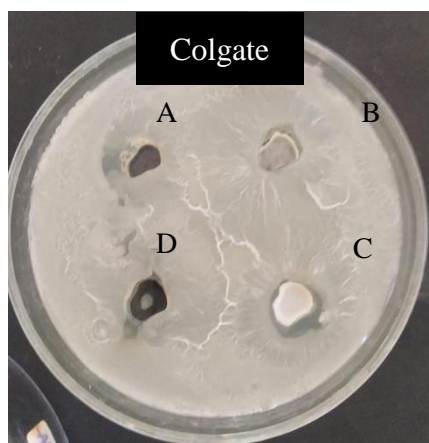
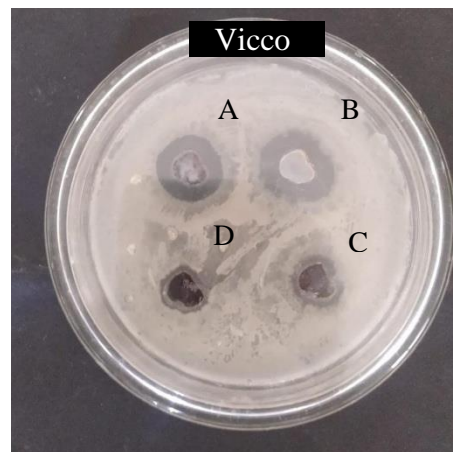
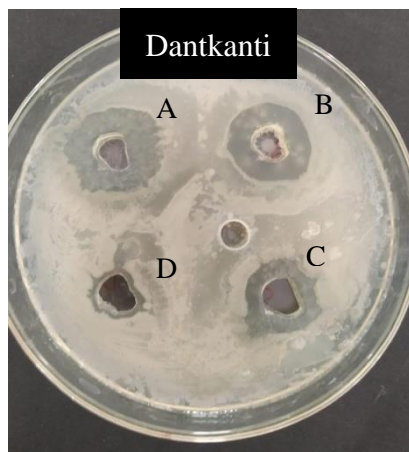
Sr no.	Name of Toothpaste	Zone of inhibition in diameter (in cm)
1.	Dantkanti	4
2.	Vico	2.5
3.	Close-up	3
4.	Colgate	2.5

**Plate 2 : Antimicrobial activity of different Toothpaste****4.2. Minimum Inhibitory Concentration (MIC):**

The MIC was performed to check at which percentage of different concentrations of various Toothpastes were able to inhibit the growth of Microorganisms.

**Table 3: Effect of different concentration of sample of toothpaste on micro-organism and the measurement of their zone site.**

	Zone of Inhibition diameter (cm)			
	Concentration ( $\mu\text{g/ml}$ )			
	A	B	C	D
Brands (toothpaste)	400 $\mu\text{g/ml}$	200 $\mu\text{g/ml}$	100 $\mu\text{g/ml}$	50 $\mu\text{g/ml}$
Dantkanti	1.0	0.9	0.7	0.6
Vicco	1.0	0.8	0.7	0.6
Close-up	0.5	0.4	0.2	0.0
Colgate	1.0	0.9	0.8	0.4



**Plate 3: Effect of different concentrations of different Toothpaste:**

### 4.3.Preliminary evaluation of toothpastes:

**Table 4 : Result of Appearance and Texture and odour**

Sr no.	Brands (toothpaste)	Apperance	Texture	Odour
1.	Dantkanti	Gray	Smooth	Strong
2.	Vicco	Brown	Smooth	Good
3.	Close-up	Red	Smooth	Good
4.	Colgate	White	Smooth	Good

**Table 5: Result of pH:**

Sr no.	Brands (toothpaste)	pH
1.	Dantkanti	7.42
2.	Vicco	7.17
3.	Close-up	7.49
4.	Colgate	7.21

**Table 6: Result of Sharp , Edge and Abrasive particles :**

Sr no.	Brands (toothpaste)	Sharp, Edge and Abrasiveparticles
1.	Dantkanti	Absent
2.	Vicco	Absent
3.	Close-up	Absent
4.	Colgate	Absent

**Table 7: Result of Foaming Power (in ml) :**

Sr no.	Brands (toothpaste)	Foaming Power (in ml)
1.	Dantkanti	61
2.	Vicco	54
3.	Close-up	57
4.	Colgate	52

**Table 8: Result of Spreadability of different brands of toothpastes :**

Sr no.	Brands (toothpaste)	Spreadability (in ml)
1.	Dantkanti	2.5
2.	Vicco	4.4
3.	Close-up	2.8
4.	Colgate	2.1



## 5. Discussion:

Maintenance of good oral hygiene is the main factor to prevent the dental diseases<sup>[4]</sup>. To maintain the oral health, it is necessary to brush everyday. The major problem of the dental problems is the formation of dental plaque. The dental plaque slowly causes the destruction of enamel<sup>[17]</sup> The microbes responsible for the formation of plaque are *S. mutans*, *E. coli* and *C. albicans*<sup>[27]</sup>

This study focuses the in vitro comparison of antimicrobial activity of different toothpastes. From the data collected, among all the toothpastes investigated the toothpaste Dantkanti appears to be most effective against the microorganisms. This is due to the ingredients present in the toothpaste formulation. The toothpaste vico was next to toothpaste Dantkanti, it showed good antimicrobial activity against the microorganisms. The effectiveness of the fluoridated toothpaste varies with its concentration<sup>[28]</sup>, In a previous study, Jenkins<sup>[29]</sup> had stated that triclosan toothpaste formulation have shown 30-70% reduce caries when compared to non triclosan therapy. The toothpaste formulations Colgate, Vico and close-up showed less antimicrobial activity against the Microorganisms.

The usage of natural substances for treatment of the diseases increased nowadays. This is because the contribution of the herbal products is comparatively more than modern products. The effective antimicrobial activity of herbs is due to the presence of secondary metabolites such as flavonoids, polyphenols, alkaloids and lectins<sup>[30]</sup> Nowadays Fluorides are used in the toothpastes to prevent dental caries<sup>[31]</sup>

## 6. Conclusion:

From this study we conclude that, the toothpaste Dantkanti is more effective against the microorganisms tested and helps in maintaining oral hygiene when compared to the other toothpaste formulations.

The effectiveness of toothpaste Dantkanti was due to the presence of the active ingredient. Hence, we conclude that the presence of this ingredient in toothpaste increases the effectiveness of that toothpaste formulation.

A product to maintain oral health should be viewed in all dimensions. Some ingredients are very helpful but its excess/ lower levels might lead to disease.

Example fluoride, triclosan. Because the formulation used in vivo is likely to be diluted by saliva, the level to which antimicrobial properties are buffered or lost in dilution in vitro of interest.

In our study 1:2 dilution shown effective result in triclosan containing toothpaste formulations and in Dantkanti as herbal formulation.

In the present study, it has been demonstrated that triclosan containing toothpastes formulations are more effective in control of oral microflora compared to non-triclosan containing synthetic toothpastes.

In herbal formulation Dantkanti toothpaste showed excellent antimicrobial activity against isolated oral pathogens.

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