



## EFFICACY OF TOOTHPASTES AND ITS ANTIBACTERIAL ACTIVITY ON BACTERIA CAUSING DENTAL CARIES

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### ABSTRACT

India has one of the highest rates of oral cancers in the world. As dental diseases in India are a result of many factors, including poor oral hygiene, tobacco use and a sugary diet, the lack of awareness about the importance of dental health leads to the prevalence of dental health issues. The oral cavity is an ideal place for microbes as the oral fluids, nutrients help microbes to interact and produce unique ecosystems. Dental caries is biofilm-induced that result from action by microorganisms, which change the active formation of biofilms on tooth surfaces. Fifty percent of Indians having dental problems take advice from chemists other than a dentist. It is observed that 51% of Indians use toothbrush and toothpaste to brush their teeth. The main aim of the study was to observe the antimicrobial activity of six different toothpastes on *Streptococcus* sp. causing dental caries. Among the six different toothpastes, the herbal tooth paste showed the best antimicrobial activity. An extensive study was performed to check the antibacterial activity of antibiotics on *Streptococcus* sp. It was observed that Norfloxacin of 5mcg showed best antibacterial activity against *Streptococcus*. The use of natural products to inhibit the growth of pathogens has been the highlight of this swot. The study was designed to review the efficacy of natural products such as cloves, tulasi, cardamom, camphor and rock salt, of which cloves showed the best antistreptococcal activity, which is required in maintaining oral health in particular and overall health in general.

**KEYWORDS :** Dental caries, Toothpastes, *Streptococcus* sp., Norfloxacin, Natural Products.

### 1. INTRODUCTION:

Dental caries is one of the most common chronic infectious diseases in the world (1,2). *Streptococcus mutans* and *Streptococcus sanguinis* are two of the main bacteria involved in dental biofilm formation and responsible for dental hard tissue destruction (3). *Streptococcus mutans* is the bacteria mostly associated with tooth decay. When it comes into contact with sugar (through sugary diet) it releases acids that results in decay. There are many other factors that may increase the susceptible of an individual to dental caries or its sequelae (4). These include microbiological factors, behavioural and lifestyle-related factors like poor oral hygiene, inappropriate dietary habits like frequent intake of refined carbohydrates and sugars, frequent use of sugar-containing medications, and wrong feeding habits among infants (5, 6). It's also important to note that a diet high in processed foods reduces your intake of essential vitamins and minerals. Vitamins such as A, D and C are critical to healthy teeth and protecting against tooth decay. Therefore oral hygiene is linked to immunity and the overall health of an individual.

Caries of the permanent teeth was reportedly the most common oral condition as per the Global Burden of Disease Study of 2017 (7). The scenario in India is no different from other developed or developing countries (8). The available literature on the prevalence of dental caries in India shows a varied picture with prevalence of caries being very high in some areas and low in some areas (9, 10, 11, 12, 13). In 2005, the bulletin of the WHO stated, "Worldwide, the prevalence of dental caries among adults is high as the disease affects nearly 100% of the population in the majority of countries." (14). In a recent publication (15) that secondarily analysed the data provided in a publication in Lancet in 2017, (16) it was stated that as compared to South Asians, Indians had a higher incidence of dental caries and more females suffered from this problem as compared to males.

Toothpastes have antibacterial properties and decrease the occurrence of plaque-related conditions. However, they have additives with possible toxic effects on the oral mucosa (17,

18). Toothpastes contain three main ingredients of fluoride, abrasives, and detergents (19). Since these materials are in direct contact with the mucosa it can have an adverse effect on the person using it. The present study was aimed at isolating the organisms causing dental caries and to check the activity using various toothpastes on the organism. The organism was later exposed to various antibiotics and its antimicrobial susceptibility was noted. The later part of the study highlights on the use of aqueous extracts of the natural products and its sensitivity towards the organism.

### 2. MATERIALS AND METHODS:

#### 2.1 Materials Required:

Sterile loops, Petriplates, forceps, spreaders, incubator, cork-borer (0.4cm), sterile test bottles for storage, sterile tips.

#### 2.2 Medias:

Nutrient Broth: Peptone 0.5 gm, Beef extract 0.3 gm, sodium chloride 0.5 gm, Distilled water 100 ml, P<sup>H</sup> 7.

Nutrient Agar: Peptone 0.5 gm, Beef extract 0.3 gm, sodium chloride 0.5 gm, Agar 2.0 gm, Distilled water 100 ml, P<sup>H</sup> 7.

Blood agar: Peptone 0.5gm, beef extract/yeast extract 0.3gm, sodium chloride 0.5 gm, Agar 2.0 gm, 5% (vol/vol) sterile defibrinated blood, Distilled water 100 ml, P<sup>H</sup> 7.

#### 2.3 Commercial Tooth Pastes Used:

Six commercial toothpastes, of which toothpaste A, toothpaste B and toothpaste C are commercial tooth pastes with additives like amino acids, salt, charcoal respectively, while toothpaste D are herbal and toothpastes E, F have certain sensitive ingredients.

#### 2.4 Natural Products:

Aqueous extracts of Cloves (CL), Tulasi (T), Cardamom (Car), Camphor (CM), Rock salt (RS) were taken for the study. 2 g of Cardamom (Car), Camphor (CM), Rock salt (RS) powder of the natural products was added to 2 ml sterile distilled water and the aqueous extract was serially diluted i.e. 1:1, 1:2, 1:4, 1:8, 1:16. In case of cloves and tulasi, 2 g of powder was taken

and added to 4 ml of sterile distilled water and prepared 1:2, 1:4, 1:8, 1:16, 1:32 dilutions respectively.

**2.5 Microorganisms:**

Microorganisms on which it was investigated was Gram positive Streptococci isolated from dental caries.

**2.5.1 Isolation Of Microorganism:**

Conventional methods were successful in isolating Streptococci from dental caries and identified them based on cultural and biochemical tests.

**2.6 Antimicrobial Assay:**

The antimicrobial assay was performed by an agar well diffusion method. 1 ml of Streptococcus culture was inoculated onto the nutrient agar plate. In this method, a well was prepared in the plates with the help of a cork-borer (0.4 cm). 20µl of the sample (aqueous extract of toothpaste/ natural products) was introduced into the well. The plates were incubated overnight at 37 °C. Microbial growth was determined by measuring the diameter of the zone of inhibition. The result was obtained by measuring the zone diameter. The experiments were done in triplicates and the mean values are presented.

**3. RESULTS AND DISCUSSION:**

From a pool study conducted, microorganisms were isolated from the tooth cavity using a swab and spread on Nutrient agar, Blood agar plates respectively and incubated at 37°C for 24h. The colony morphology was identified on both the plates. On nutrient agar plates the organisms produced cream coloured pin point colonies and on blood agar medium after incubation for 24h the colonies are small circular with an area of clear haemolysis as shown in Figure 1, 2.

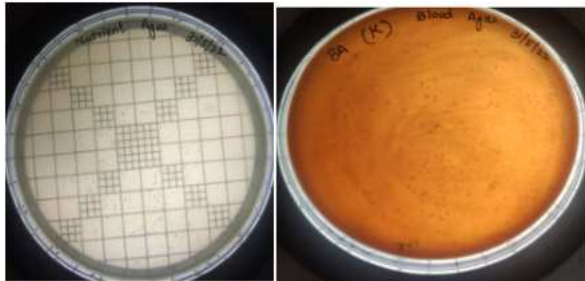


Figure 1: On Nutrient agar Figure 2: On Blood agar plates medium



Figure 3: Gram positive cocci in chains

The isolated organism was stained, observed microscopically and identified as Gram positive cocci in chains as shown in Figure 3. The biochemical tests of the organism was performed and the results confirmed the presence of Streptococci as per Table 1

**Table - 1 Biochemical Tests Of Streptococci**

Colony Morphology:	
Nutrient agar	Cream coloured pinpoints colonies
Selective medium:	Haemolysis
Blood agar	
Grams nature	Gram positive

Cellular morphology	cocci in chains
Indole	-ve
Methyl red	+ve
Voges Proskauer	-ve
Citrate	-ve
Catalase	-ve

Six different toothpastes A,B,C,D,E,F with the ingredients mentioned in Table 2 were taken and diluted serially with sterile distilled water and exposed to the isolated Streptococcus.

Among all the six toothpastes, toothpaste D which was a herbal toothpaste that showed the best antimicrobial property, a result which is in line with our work done (20, 21), followed by toothpaste B, which had salt as its ingredient, later toothpaste E and toothpaste F. Toothpaste A had charcoal, while toothpaste C contains amino acids which showed antimicrobial activity but not as much as the herbal toothpaste D.

**Table -2 Different Toothpastes With The Respective Ingredients.**

S.NO.	Toothpaste	Ingredients
1	A	Glycerin, Water, Hydrated Silica, Sodium Lauryl Sulfate, Arginine, Flavour, Zinc Oxide, Cellulose Gum, CI 77891, Poloxamer 407, Tetrasodium Pyrophosphate, Zinc Citrate, Benzyl Alcohol, Xanthan Gum, Cocamidopropyl Betaine, Sodium Saccharin, Phosphoric Acid, Sodium Fluoride, Mica, Charcoal Powder, Sucralose.
2	B	Calcium Carbonate, Silica, Sodium Silicate, Sodium Carboxymethyl Cellulose, Sodium Bicarbonate, Xanthan Gum, Pigment Blue (Ci 74160), Sorbitol, Sodium Lauryl Sulphate, Sodium Monofluorophosphate, Sodium Chloride, Sodium Saccharin, Benzyl Alcohol, Flavor, In Aqueous Base, Ci 77492.
3	C	Calcium Carbonate, Sorbitol, Sodium Lauryl Sulphate, Silica, Titanium Dioxide, Sodium Silicate, Flavor Carrageenan, Sodium Monofluorophosphate, Sodium Bicarbonate, Benzyl Alcohol, Sodium Saccharin, Triclosan, In Aqueous Base.
4	D	Lavanga Oil, Pippali, Miswak Extract, Babool Extract, Sunthi, Pudina Satva, Karpura, Tomar Beej, Garlic.
5	E	Sodium Fluoride (0.24 %), Sorbitol, Water, Hydrated Silica, PEG-8, Sodium Lauryl Sulfate, SD Alcohol 38-B, Flavor, Cellulose Gum, Sodium Saccharin, Titanium Dioxide.
6	F	Calcium Sodium Phosphosilicate 5%, Sodium Fluoride (1426ppm), Glycerin, PEG 8, Hydrated Silica, Cocamidopropyl Betaine, Sodium Methyl Cocoyl Taurate, Aroma, Titanium Dioxide, Carbomer, Sodium Saccharin.

The following statistical results were calculated as seen in Table 3.

**Table -3 Antimicrobial Activity Of Six Different Tooth Pastes.**

	1 : 1	1 : 2	1 : 4	1 : 8	1:16
Tooth paste A	1	0.7	0.6	0	0
	1	0.6	0.9	0	0
	1	0.7	0.8	0	0
Mean	1	0.67	0.77	0	0
S.D	0	0.06	0.15	0	0
M + SD	1.00	0.72	0.92	0	0
M - SD	1.00	0.61	0.61	0	0
Tooth paste B	1.4	1.2	1	0	0
	1.3	0.7	0.6	0	0
	1.4	1	0.8	0	0
Mean	1.37	0.97	0.8	0	0
S.D	0.06	0.25	0.2	0	0
M + SD	1.42	1.22	1.00	0	0
M - SD	1.31	0.72	0.60	0	0
Tooth paste C	1	0.8	0.7	0	0
	1	0.6	0.5	0	0
	1	0.7	0.6	0	0
Mean	1	0.7	0.6	0	0
S.D	0	0.1	0.1	0	0
M + SD	1	0.8	0.7	0	0
M - SD	1	0.6	0.5	0	0
Tooth paste D	1.5	1.1	1	0	0
	1.4	1.1	0.9	0.6	0
	1.5	1.1	1	0.6	0
Mean	1.47	1.1	0.97	0.4	0
S.D	0.06	0	0.06	0.35	0
M + SD	1.52	1.10	1.02	0.75	0
M - SD	1.41	1.10	0.91	0.05	0
Tooth paste E	1.2	1.1	0.8	0.7	0.6
	1.2	0.8	0.6	0.6	0.6
	1.2	1	0.7	0.7	0.6
Mean	1.2	0.97	0.7	0.67	0.6
S.D	0	0.15	0.1	0.06	0
M + SD	1.2	1.12	0.80	0.72	0.6
M - SD	1.2	0.81	0.60	0.61	0.6
Tooth paste F	1.3	0.8	0.7	0	0
	1.1	0	0	0	0
	1.2	0.5	0.3	0	0
Mean	1.2	0.43	0.33	0	0
S.D	0.1	0.40	0.35	0	0
M + SD	1.3	0.84	0.68	0	0
M - SD	1.1	0.03	-0.02	0	0

Toothpastes are compounds containing more than one ingredient. The study was extended to check the antibacterial activity of various antibiotics on Streptococcus sp. as shown in Table-4. When their effectiveness is tested with different antibiotics of various dosages, the results differed depending on the concentration. It was observed that Norfloxacin of 5mcg showed best antibacterial activity against Streptococcus.

**Table-4 Antibacterial Activity With Various Antibiotics.**

S.NO.	Antibiotics	Dosage (mcg)	Zone of inhibition (cm)
1	Erythromycin (E)	10	3
2	Vancomycin (VA)	10	1.8
3	Streptomycin ( S)	25	2.5
4	Gentamycin (GEN)	50	3
5	Norfloxacin (NX)	5	2.6
6	Clindamycin (CD)	10	3.3

The use of natural products has served as powerful therapeutics against various pathogenic bacteria. Natural products have become a hot research topic in current years due to their promising antibacterial effects. These products are an important source to control bacterial pathogens. A poly-herbal tooth powder was redesigned to toothpaste form, it was standardized as per the guidelines (22, 23). The present study concentrates on the antimicrobial activity of natural

products like cloves, tulasi, cardamom, camphor, rock salt on Streptococcus. Cardamom, camphor, rock salt did not show any antibacterial effect on the organism. It was observed that natural products like cloves of 1:2 and 1:4 dilutions and tulasi of dilution 1:2 showed a positive effect in inhibiting Streptococcus effectively as shown in Table 5.

**Table- 5 Antimicrobial Activity Of Five Natural Products**

	Dilutions taken				
	1:2	1:4	1:8	1:16	1:32
Cloves (CL)	1.8	1.2	0	0	0
	1.7	1.2	0	0	0
	1.8	1.2	0	0	0
Mean	1.8		0	0	0
SD	0.05	0	0	0	0
M+SD	1.86	1.2	0	0	0
M-SD	1.74	1.2	0	0	0
Tulasi (T)	0	0	0	0	0
	0.5	0	0	0	0
	0.5	0	0	0	0
Mean	0.33	0	0	0	0
SD	0.29	0	0	0	0
M+SD	0.62	0	0	0	0
M-SD	0.04	0	0	0	0
	Dilutions taken				
	1:1	1: 2	1:4	1:8	1:16
Cardamom (Car)	0	0	0	0	0
	0	0	0	0	0
	0	0	0	0	0
Camphor (CM)	0	0	0	0	0
	0	0	0	0	0
	0	0	0	0	0
Rock salt (RS)	0	0	0	0	0
	0	0	0	0	0
	0	0	0	0	0

Among the two natural products which showed antibacterial properties, it was observed that cloves had shown the best activity against Streptococcus and the results are in correlation with the work done by Rahim ZH et al. (2006) (24).The antimicrobial activity of tulasi on Streptococcus strain in the present work is in relevance to the previous study on the organism responsible for tooth decay, which suggests that it can be used as a herbal mouth wash for treating bad breath, gum disease and mouth ulcers.(25,26). It is therefore necessary to bring back into usage of natural products, as they are very effective and with minimal side effects. Creating awareness on traditionally available herbal medicines will help to minimize oral and other universal problems and thereby treating them effectively.

**4. CONCLUSION:**

Toothpaste is used to maintain our personal oral and dental hygiene. The chemicals in the toothpaste can have an adverse effect on the normal flora which is beneficial to our body. Natural ingredients in toothpastes are increasingly being used to prevent caries formation and improve oral health. The study evaluated the effectiveness of toothpastes and extended it effects with natural ingredients on pathogenic microorganism. The work showed a positive impact of aqueous extracts of natural products that inhibited growth of microorganisms like Streptococcus which causes dental and oral health deterioration. It is encouraging to see the results obtained from the present study with natural products. Further studies of safety and efficacy of these agents will be important to establish whether they offer therapeutic benefits either alone or in combination with conservative therapies, that can help to address the issues of oral diseases worldwide.

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