

STUDY OF EFFECTIVENESS AND RESISTANCE OF VARIOUS TOOTHPASTE ON MOUTH MICROBIAL FLORA

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ABSTRACT

There are various toothpastes, which are used for brushing the teeth in different parts of the world. Several studies have been reported on the resistance and effectiveness of toothpastes on oral bacteria. This study was conducted to evaluate the resistance and effectiveness of various class of toothpastes on oral microflora. The present study was conducted to assess resistance and effectiveness of various concentration of toothpastes on oral microflora. The well method was used to test the resistance on oral bacteria. Wells were prepared on blood agar and Mueller Hinton agar plates with the help of punch having 6mm diameter. The plates were left for 1h at room temperature and then incubated at 37°C for 24hr and examined for zone of

inhibition. Red toothpaste shown no zone of inhibition with *Enterococcus* and *Streptococcus* in any concentration and with *staphylococcus*, observed zone of inhibition in 100% and with *Lactobacillus* observed zone of inhibition in 100% and 5% concentration. Chemical toothpaste shows no zone of inhibition with *Staphylococcus* and *Lactobacillus* in 10% concentration and observed higher zone of inhibition with *Streptococcus* in 100% concentration. Herbal toothpaste shows significantly highest zone of inhibition with *Enterococcus* in 100% concentration. Both herbal and chemical toothpastes had sensitivity against *Streptococcus*, *Enterococcus*, *Lactobacillus* and *Staphylococcus* bacteria, and powder toothpaste have resistance against *Lactobacillus* and *Staphylococcus* bacteria in only with

100% and 5% concentration. Resistance was significantly higher in herbal toothpaste than chemical toothpaste.

KEYWORDS: *Streptococcus*, *Enterococcus*, *Lactobacillus*, *Staphylococcus*, Microflora, Resistance, Blood Agar, Mueller Hinton Agar, Zone of inhibition. Toothpaste.

INTRODUCTION

The evolution of the modern tooth- brush has its origin in chewing sticks that were used by the Babylonians as early as 3500 BC(Wu CD *et al* 2001). The toothpaste containing *Neem* as well as fluoridated toothpaste were equally efficacious against caries-producing bacteria. Acetone extract from the bark of *Neem* is bactericidal against *S. sobrinus* hence indicates its anti-cariogenic activity (Bhuiyan *et al* 1997). Plaque is a complex biofilm found on the tooth surface that is a major cause of the development of dental caries (Benson *et al.*, 2004). Bacteria form an important group of microorganisms found in both healthy and diseased mouths (Robert, 2005). There have been more than 300 types of bacteria found in the mouth (Robert, 2005). More over, a microorganism accumulation on oral surfaces could be a major think about the event of most of the common dental unwellnesss like decay and penitential disease (Williams and Cummins, 2003). Toothpaste is a dentifrice which improves the aesthetic appearance and the health of the teeth it is commonly found and sold in flexible tubes or sachets for the promotion of oral hygiene, removal of dental plaque and food debris from the mouth as well as the elimination of halitosis from the mouth (Nwakanma *et al* 2014). Toothpaste is classified as drugs not cosmetics because drugs should contain an ingredient to achieve the effect the consumer desires (Regos and Hitz, 1974). Many mechanical aids are used worldwide to remove or control plaque, including toothbrushes, dental floss, and mouth rinses, and dentifrices (Barnes VM *et al* 2010). Brushing with *Neem* toothpaste after every meal and using a mouthwash with *Neem* extract is recommended treatment for preventing gingivitis (Bhambal *et al* 2011). Various parts of the *Neem* tree possess astringent and antiseptic activity (Laxmi *et al* 2015).

MATERIALS AND METHODS

Sample Collection

The present invitro study was conducted to assess sensitivity and effectiveness of 10%, 30%, 50% and 100% concentration of chemical, herbal and powder toothpaste on oral microflora which has been isolated from 20 different age group person between 20 to 25 in the department of life sciences, CV Raman University, Bilaspur Chhattisgarh. Materials used in

the study included three different class of toothpastes like chemical, herbal and powder, Micro-organisms (*Streptococcus*, *Enterococcus*, *Stephylococcus* and *Lactobacillus*– which was isolated from samples), Muller Hinton Agar plates, Blood agar plates, MacConkey agar plates, Petri-dish, Vernier calipers, Punch, Distilled water, Weighing machine, Sterile test tubes.

Preparation of different concentrations of toothpastes

One gram three grams five grams and pure form of each toothpastes were used in the experiment. The toothpastes were kept in laminar air flow for 10 mints for sterilization before experiment. The weighted toothpastes i.e. 1gm, 3gm and 5gm and pure was kept separately in test tubes. Distilled water was added to each test tube of toothpastes and made volume.

PREPARATION OF AGAR PLATES

Blood agar media

Suspended 28g of nutrient agar powder in 1 litre of distilled water. Heated this mixture and stir it to fully dissolve all components. Autoclaved the dissolved mixture at 121 degrees Celsius for 15 minutes for sterilization. After the nutrient agar has been autoclaved, allowed it to cool but not solidify. When the agar has cooled to 45-50°C, Added 5% (vol/vol) sterile defibrinated blood that has been warmed to room temperature and mixed gently but well. Poured it into sterile plates while liquid. Stored the plates at 2-8°C, sealed plastic bags to prevent loss of moisture. (HI Midia).

Mueller Hinton Agar

Beef extract 2 gram acid hydrolysate of casin 17.50grams starch 1.5gram agar 17.00grams these 38 gm of the medium Suspend in one liter of distilled water. Heat with frequent agitation and boil for one minute to completely dissolve the medium. Autoclaved it at 121°C for 15minutes. Cooled it at to room temperature but not solidify. Poured cooled Mueller Hinton Agar into sterile petri plates. Allowed to cool at the to room temperature. Checked for the final pH 7.3 ± 0.1 at 25°C. Stored the plates at 2-8°C. (HI Midia).

MacConkey Agar

Peptone 17 gram Protease peptone 3gram Lactose monohydrate 10gram bile salt 1.5 gram Sodium chloride 5 gram Neutral red 0.03gram Crystal Violet 0.001gram Agar 13.5g these 49.53 grams of dehydrated medium Suspend in 1000ml purified/distilled water. Heated to boiling to dissolve the medium completely. Sterilized by autoclaving at 15 lbs pressure

(121°C) for 15 minutes. Cooled to 45-50°C. Mixed well and poured into sterile Petri plates. (HI Midia).

Well Method For Sensitivity Test

The ditch plate method was used to test the microbial sensitivity. Wells were prepared on agar plates with the help of punch having 6 mm diameter. Sterile distilled water was used as control. The plates were left for 1h at room temperature and then incubated at 37°C for 24h and examined for zone of inhibition. The zones was recorded in millimeters.

BIOCHEMICAL ANALYSIS

Gram's Staining Gram staining was performed to differentiate in Gram negative and positive bacteria.

Bacitracin Test selected a well isolated colony with a sterile inoculating loop from an 18-24 hours old culture. Spreadad the colonies onto a blood agar plate. Using heated forceps, placed a bacitracin disk in the center of the plate. Pressed the disk with forceps to ensured adequate adherence with the agar surface. Incubated the plate in ambient air at 35°C-37°C for 18-24 hours. After incubation, observed the zone of inhibition around the bacitracin disk.

Bile Esculin Test

Using sterile loop, picked one colony from an 18-24 hours culture. Inoculated onto the surface of slant of bile esculin medium with an S-shaped motion. Incubated the inoculated tube at 35-37°C for 24 hours. Observed the result.

Catalase Test

Transferred a small amount of bacterial colony to a surface of clean, dry glass slide using a loop or sterile wooden stick Placed a drop of 3% H₂O₂ on to the slide and mixed. A positive result is the rapid evolution of oxygen (within 5-10sec.) as evidenced by bubbling. A negative result is no bubbles or only a few scattered bubbles.

Coagulase Test

Dipped a flamed and cooled straight inoculating wire into the undiluted plasma at room temperature, withdraw, and stir the adhering traces of plasma (not a loopful) into the staphylococcal suspension on the slide. Flamed the wire and repeated for the control suspensions. Read as positive a coarse clumping of cocci visible to the naked eye within 10

seconds. Read as negative the absence of clumping or any reaction taking more than 10 seconds to develop.

Carbohydrate Fermentation Test

Carbohydrate Phenol red broth base medium was used as a medium for this test. Different sugar substrates namely, arabinose, sucrose, maltose, lactose, sorbitol and glucose were used. 0.1g (0.1% w/v) of each sugar substrate was added to 100 ml of the medium. 5ml of each mixture was transferred into each tube. For gas detection, Durham tube was inserted into the test tube containing glucose. All the tubes were sterilized for 15 min at 121°C. The tubes were inoculated with a single colony of the bacteria under study. The positive reaction of the bacteria was indicated by the changes in the colour of the medium (Thoesen et al 1994).

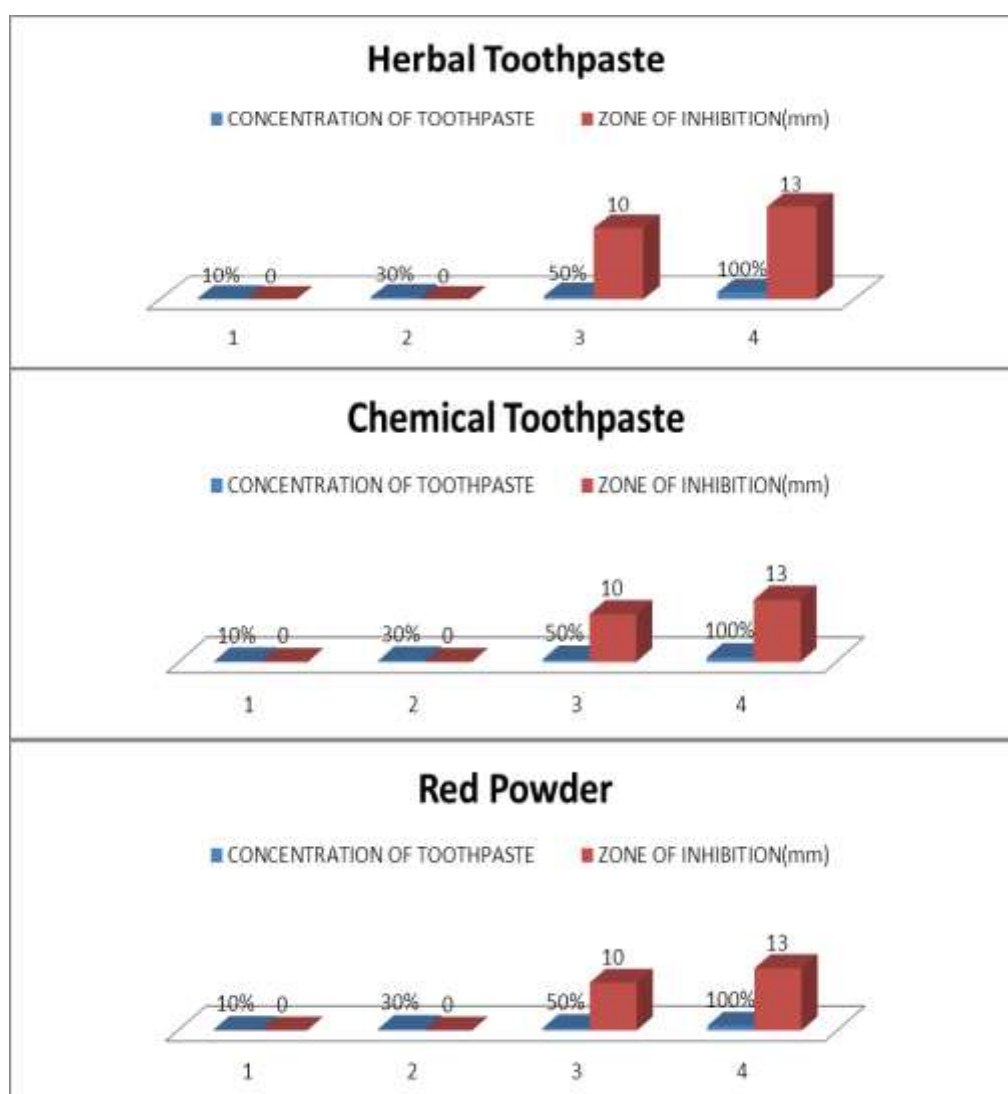
RESULTS AND DISCUSSION

The present invitro study was conducted to assess resistance and effectiveness of different concentration of toothpastes on oral microflora. Zone of inhibitions were measured from the edge of the punched hole (well) to outer border of bacterial inhibition at selected perpendicular places. These zones of inhibitions were measured after 24h. [Table-1] shows effect of various concentrations of chemical herbal and powder toothpastes on *Enterococcus*. There was no zone of inhibition observed with 10%, 30%, 50% and 100% concentration of powder toothpaste. Zone of inhibitions of 9, 14, 18, 24mm were observed with herbal toothpaste in 10%, 30%, 50% and 100% concentration. Zone of inhibitions 9, 10, 13, and 16mm were observed in 10%, 30%, 50% and 100% concentration of chemical toothpaste. [Table-2] shows effect of various concentrations of chemical herbal and powder toothpastes on *Streptococcus*. There was no zone of inhibition observed with 10%, 30%, and 50% concentration of powder toothpaste but in 100% concentration it shows 11mm zone of inhibition. Zone of inhibitions 12, 19, 22, 30mm were observed with herbal toothpaste in 10%, 30%, 50% and 100% concentration. Zone of inhibitions 8, 11, 12, and 14mm were observed in 10%, 30%, 50% and 100% concentration of chemical toothpaste. [Table -3] shows effect of various concentrations of chemical herbal and powder toothpastes on *Stephylococcus*. There was no zone of inhibition observed with 10%, 30%, and 50% concentration of powder toothpaste but in 100% concentration it shows 11mm zone of inhibition. Zone of inhibitions of 11, 14, 16 and 19mm were observed with herbal toothpaste in 10%, 30%, 50% and 100% concentration. Zone of inhibitions 10, 13 and 16mm were observed in 30%, 50% and 100% concentration of chemical toothpaste but it shows no zone

of inhibition in 10% concentration. [Table-4] shows effect of various concentrations of chemical herbal and powder toothpastes on *Lactobacillus*. There was no zone of inhibition observed with 10%, and 30%, concentration of powder toothpaste but in 50% and 100% concentration it shows 10 and 13mm zone of inhibition. Zone of inhibitions of 12, 13, 14 and 17mm were observed with herbal toothpaste in 10%, 30%, 50% and 100% concentration. Zone of inhibitions 11, 14 and 15mm were observed in 30%, 50% and 100% concentration of chemical toothpaste but it shows no zone of inhibition in 10% concentration.

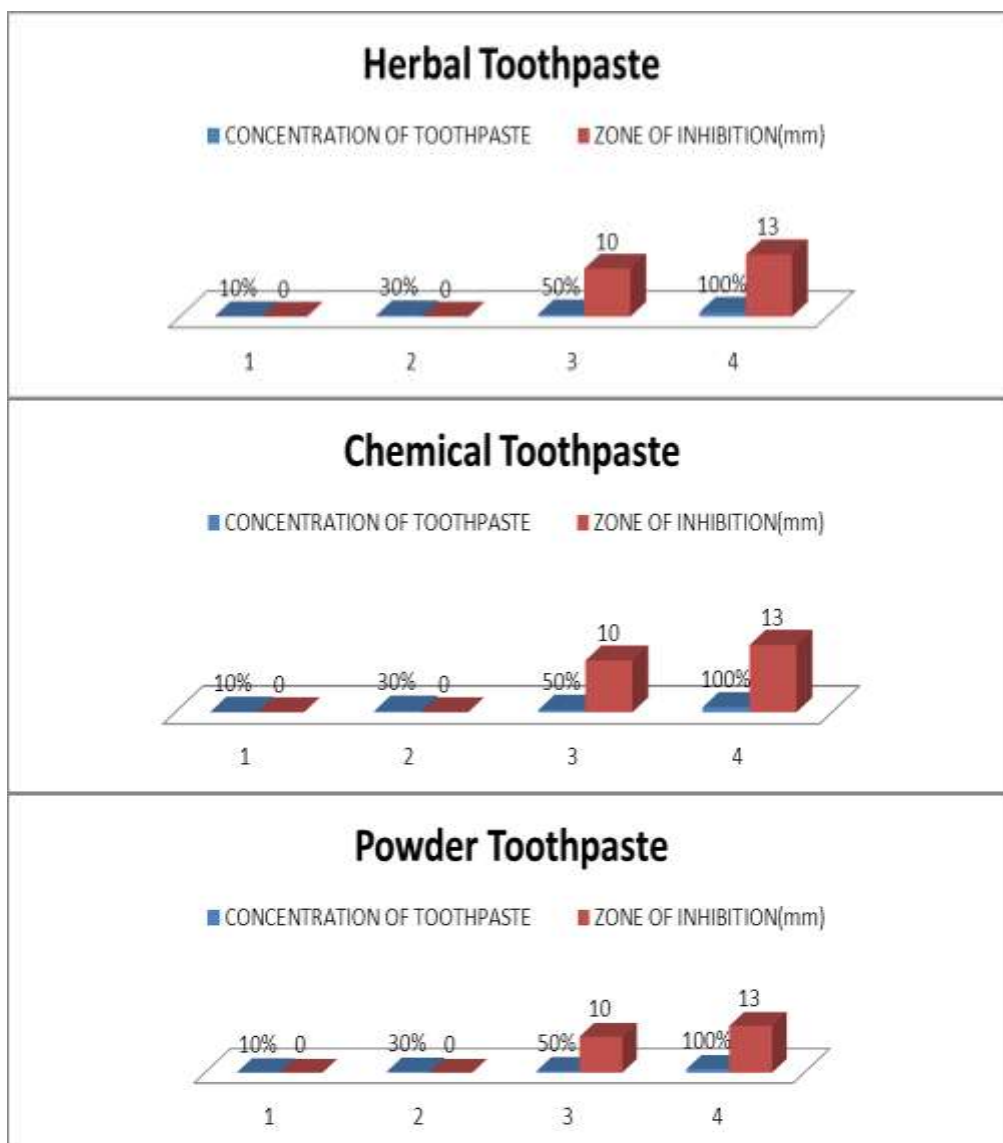
[Table/Fig-1]: Effect of various concentrations of toothpastes on *Enterococcus*

CONCENTRATION OF TOOTHPASTES %	ZONE OF INHIBITION(mm)		
	HERBAL	CHEMICAL	RED POWDER
10	9	9	00
30	14	10	00
50	18	13	00
100	24	16	00

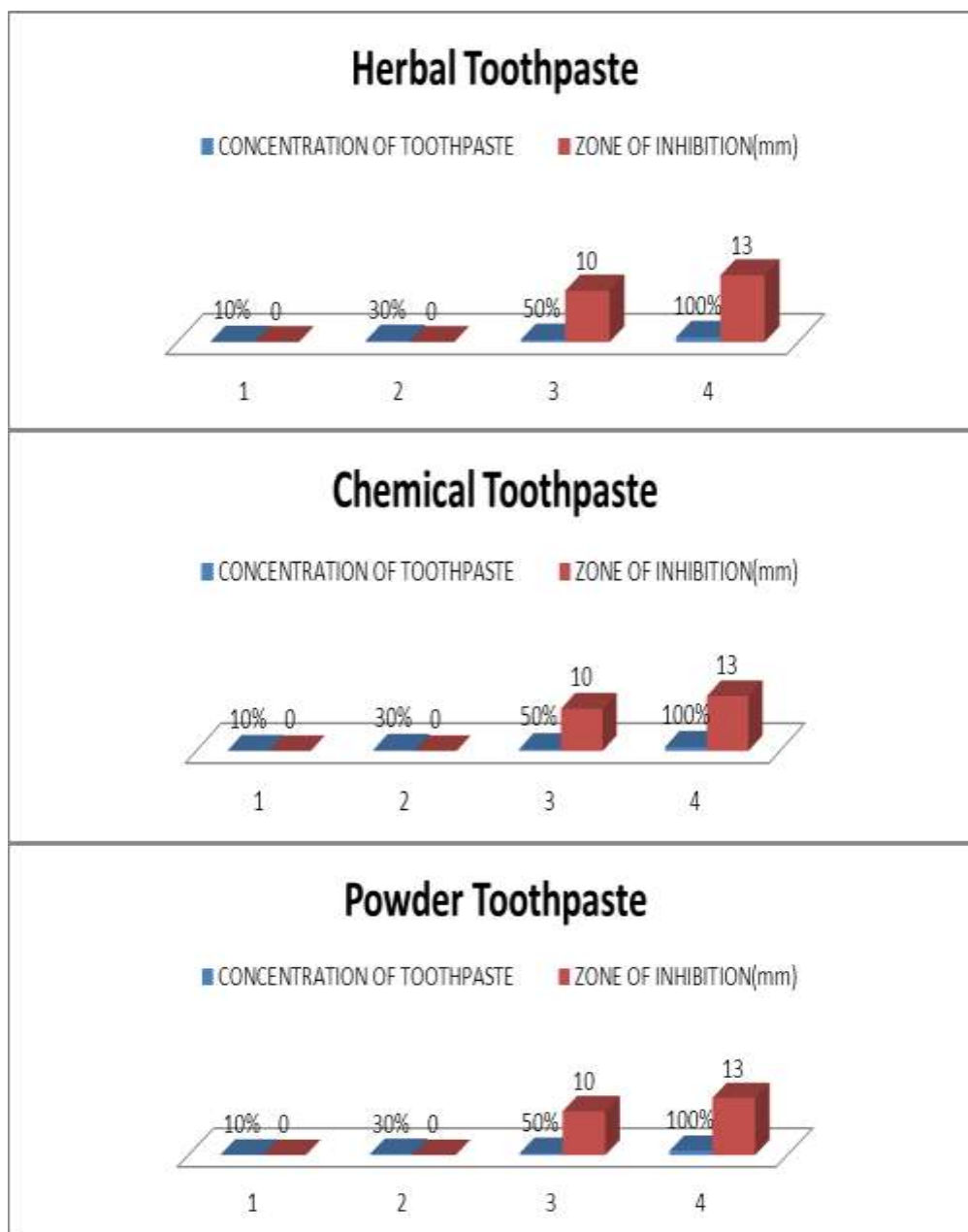


[Table2]: Effect of various concentrations of toothpastes on *Streptococcus*

CONCENTRATION OF TOOTHPASTES %	ZONE OF INHIBITION(mm)		
	HERBAL	CHEMICAL	RED POWDER
10	12	8	00
30	19	11	00
50	22	12	00
100	30	14	00

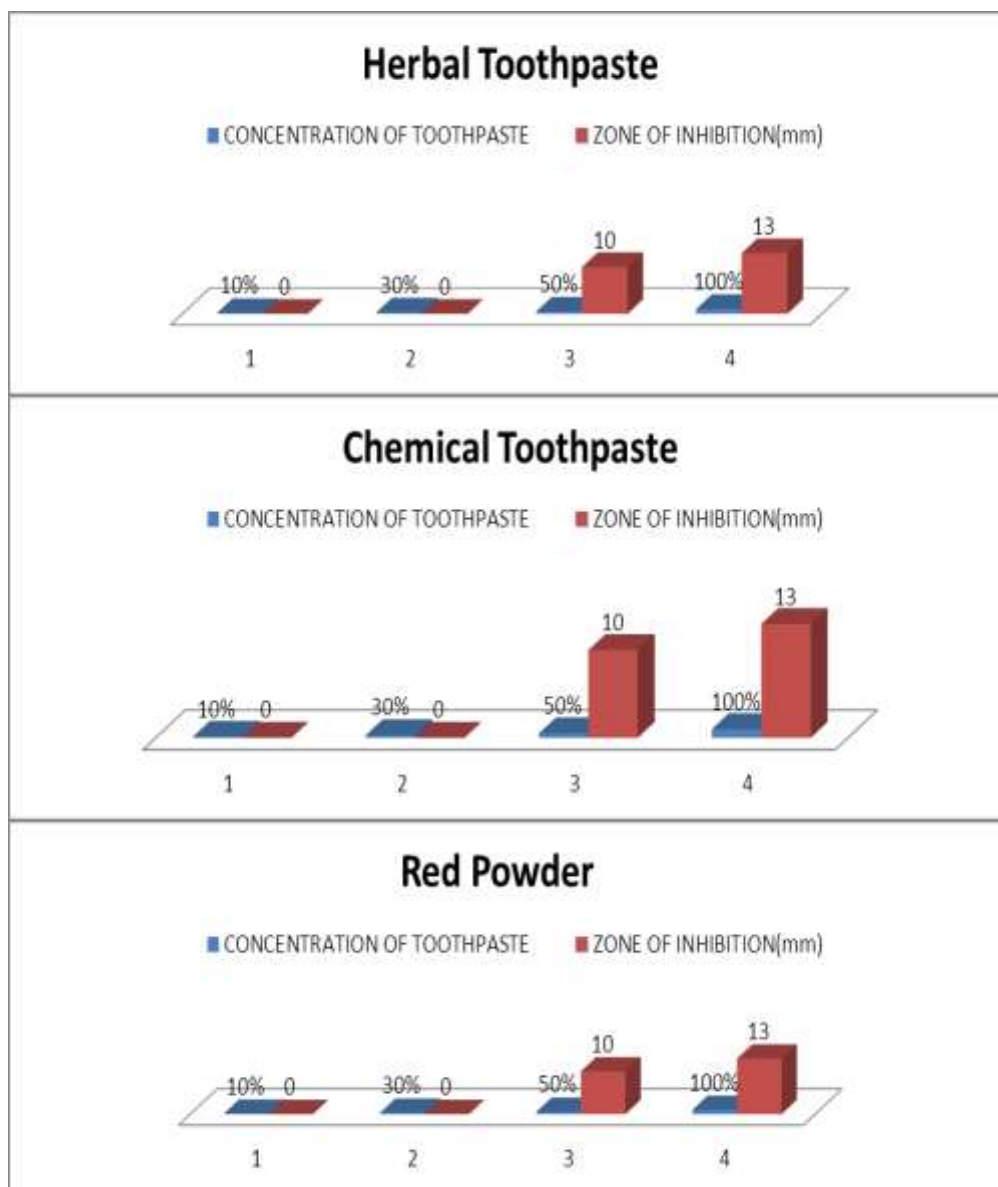
[Table3]: Effect of various concentrations of toothpastes on *Staphylococcus*

CONCENTRATION OF TOOTHPASTES %	ZONE OF INHIBITION(mm)		
	HERBAL	CHEMICAL	RED POWDER
10	11	00	00
30	14	10	00
50	16	13	00
100	19	16	11



[Table 4]: Effect of various concentrations of toothpastes on *Lactobacillus*

CONCENTRATION OF TOOTHPASTES %	ZONE OF INHIBITION(mm)		
	HERBAL	CHEMICAL	RED POWDER
10	12	00	00
30	13	11	00
50	14	14	10
100	17	15	13



DISCUSSION

An attempt has been made to enrich the knowledge of antibacterial activity of 10%, 30%, 50% and 100% concentration of different toothpastes on *Streptococcus*, *staphylococcus*, *Enterococcus* and *Lactobacillus* bacteria.

Red powder toothpaste does not show resistance with *Enterococcus* and *Staphylococcus* At any concentration, but with *staphylococcus* and *lactobacillus* it showed resistance at 50% and 100% concentration.

Chemical toothpastes shows resistance with *Streptococcus*, *staphylococcus*, *Enterococcus* and *Lactobacillus* bacteria at 30% 50% and 100% concentration but does not shows resistance with *staphylococcus* and *lactobacillus* at 10% concentration. When the

concentration is decreases the resistance of the toothpaste is also decreases. The reason of this antimicrobial effect include the presence of fluoride, which is known to exert an anticariogenic action, and silica acting as an abrasive and preventing accumulation of plaque; alkaloids, known to exert an analgesic action, also contribute towards dental well-being. The oils have carminative, antiseptic, and analgesic effects. (Sharma *et al.*, 2014).

Resistance was significantly higher in herbal toothpaste than chemical and red powder toothpaste. It shows significantly highest zone of inhibition with *Enterococcus* in 100% concentration.

The reasons for resistance of herbal toothpaste is presence of herbal ingredients like Babool and neem extracts (Sharma *et al.*, 2014). It may include hydrophilic compounds such as polyphenols, gums (poly-saccharides) and tannins. There is increasing evidence to support that the plants of genus *Acacia* are relatively high in bioactive secondary compound and are thus likely to hold promise for drug discovery. Secondary compounds in *Acacia* are important for a variety of functions, chief among these are Anti-cancer (triterpenoid and saponins), diuretic (glucosides), natriuretic (glucosides), important nutraceutical (poly-saccharide and gum) anti-digestive disorder (saponins, tannins and flavanoids), anti-oxidant (polyphenols), anti-plasmodial (treptamine, tannins, organic acids and saponins(Saini *et al.*, 2008).

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