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Efficacy Evaluation of Commercially Available Toothpaste And Herbal Actives .

Devi shree V[1], Sam Isaac [2] , Preethi Jeyakumar [3]
[1](Department of Biotechnology ,Womens Christisn , College . University of Madras), India)
[2](R&D ,TTK Healthcare ,,India)
[3](Department of Zoology , Stella Maris, College , University of Madras ,India)
Corresponding Author: Preethi Jeyakumar

***Abstract**:The antimicrobial activity of one gel based toothpaste, two normal toothpastes, four herbal toothpastes, two herbal samples was tested against buccal cavity organism. Isolated organism from the selected media has been characterized by using biochemical tests. Pseudomonas aeruginosa, Staphylococcus aureus and Bacillus sp was identified microscopically. The different level of antimicrobial activity was observed by zone of inhibition using disc diffusion and well method.*

***Keywords:** Antimicrobial , Disc diffusion ,Toothpaste , , Zone of inhibition*

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I. Introduction

The most common oral diseases are dental cavities, periodontal (gum) disease, oral cancer, oral infectious diseases, trauma from injuries, and hereditary lesions. In 2015 Dr. Dominic Campopiano suggested that Oral care products containing a natural plant chemical that stops bacteria harming teeth could help prevent decay On April 2012 World Health Organization reported, around 60–90% of children and nearly 100% of adults have dental cavities. The objective of this study is to isolate the microorganism from buccal cavity , characterize the organism and then to evaluate the efficacy of commercially available toothpaste and herbs was test against the isolated oral pathogens.In 2014 Rossi et al., evaluated the antimicrobial effect of toothpastes containing natural extracts, chlorhexidine or triclosan. The effectiveness of toothpastes was evaluated against yeasts, Gram-positive and Gram-negative bacteria using the disk diffusion method. The toothpastes containing chlorhexidine, triclosan or natural extracts exhibited antimicrobial activity. In 2015 Roopavathi et al., compared seven toothpastes which have been tested for their antimicrobial activity against three oral pathogens namely, Streptococcus mutans, Escherichia coli and Candida albicans by well agar diffusion assay. In E.coli, Toothpaste 1 has maximum zone of inhibition in 1:1 ratio and statistically significant (p=0.003).In Streptococcus mutan, Toothpaste 5 has maximum zone of inhibition in 1:1 ratio and statistically significant (p=0.003). In candida albicans, toothpaste 6 has maximum zone of inhibition in 1:1 ratio and statistically significant (p=0.003). Triclosan containing toothpastes formulations are more effective in control of oral micro flora.Seven different brands of toothpastes and two herbs. Acacia nilotica sample were purchased from local market in Chennai. Azadirachta indica sample was collected from the college campus. Close up (gel based), Colgate, Signal (toothpaste), Dabur Meswak, Dabur red, Himalaya herbal, Vicco vajradanti (herbal toothpaste), were collected in pharmacy. In two conical flask 50ml of water was poured and boil. In that boiling water, Acacia nilotica powder was added in one conical flask and in another conical flask Azadirachta indica stick was added and make them to boil for 15-20 mins and incubated for overnight. After incubation, herbs were extracted using filter paper.

The organism was isolated from two people. Age: 23, Sex: female, no disease. The sample was taken before brushing mouth with sterile swab. Then it was streak plated in the nutrient agar in petri dish. The inoculated plates were then incubated at 37°C for 24 hours and colonies were observed.

II. Preparation of Culture Plates

The following culture plates were prepared.

2.1 Mannitol Salt Agar

Mannitol salt agar was prepared by dispersing 111.02g of the mannitol salt agar in 1 liter of Distilled water. Sterilize by auto-caving for 15 minutes at 121°C. Then the agar was poured in plates and after solidification plate was streaked with organism which is isolated in nutrient medium. Incubated the plates at 37°C for 24-48 hrs.

III. Cetrimide Agar

Cetrimide agar was prepared by dissolves 46.7g of cetrimide agar in 1 liter of distilled water. Sterilize the medium and required plates were autoclaved at 121°C for 15 mins. Then the agar was poured in plates and after solidification plate was streaked with organism which was isolated in nutrient medium. Incubated the plates at 37°C for 24-48 hrs.

IV. Lactobacillus Mrs Agar

Lactobacillus MRS Agar was prepared by dispersing 55.15g of Lactobacillus MRS Agar in 1 liter of distilled water. Sterilize the medium in autoclave at 121°C for 15 mins. Then the agar was poured and after solidification plate was streaked with the organism which was isolated from nutrient plate. Incubated the plate at 37°C for 24-48 hrs.

V. Microscopic Examination

5.1 Gram Straning

A glass slide was taken and wiped with 70% ethanol. Made a smear with loop full of organism and heat fixed the slide. Flood the smear with primary stain (Crystal violet) and allowed it to react for 1-2 minutes. Wash the slide in tap water. Further the smear is treated with the mordant (Gram's Iodine) for 1-2 minutes. Excess Gram's Iodine is removed by washing in tap water, and then it is treated with decoloursing agent (95% Ethanol). After Ethanol treatment the smear is water washed and flooded with counter stain (Safranin) for 1-2 minutes. Finally the slide is washed with water, air dried and observed under oil immersion.

VI. Biochemical Characterization

6.1 Indole Test

Indole broth was prepared by dispersing 1% of tryptone and 10gm of peptone in 1L of distilled water. It was sterilized in autoclave at 121°C for 15 mins. One of the tryptone broth was inoculated with organism and another test tube was kept as a control. The tube was incubated 37°C for 48 hrs. After 48hrs of inoculated few drop of kovax reagent was added to the test tubes. The cherry red colour formation indicates positive and no colour change indicates negative result.

VII. Methylred And Vouges Proskauer Test:

Preparation of 17gms of MRVP broth was dissolved in 1L of distilled water. Then the test tubes and broth was autoclaved at 121°C for 15 mins. One of the MRVP broth was inoculated with respected organism and another test tube was kept as a control. The tube was incubated at 37°C for 48 hrs. After incubation, for MR test methyl red is used as indicator if it forms distinct red ring it indicates positive and yellow color ring indicates negative result and For VP test, barritt reagent A and barritt reagent B was add as indicator, for positive result indicate with pink-red ring was formed, if no pink- red ring is formed indicates negative.

VIII. Oxidase Test

A glass slide was taken to which loop full of organism was placed and by using sterile forcepes sterile oxidase disc was placed. If it turn purple color it indicates positive if no color change indicates negative result.

IX. Catalase Test

In a test tube, 1ml of hydrogen peroxide reagent was added and loop full of organism was inoculated. If bubble formation occurs it indicates positive result and if no bubbles or few bubble formation it indicates negative result.

X. Gelatinase Test

Preparation of 5gms of peptone, 1.5gms of beef extract and 120gms of gelatin was dissolved in 1L of distilled water. The broth and test tube were autoclaved at 121°C for 15 mins. Broth was poured in test tubes. One of the test tube loop full of organism was inoculated and another test tube was used as control. Then the test tube was incubated at 37°C for 48 hrs. After incubation, the test tubes were placed in refrigerator 10-15 mins. Frozen state indicates negative result whereas liquid or gel formation indicates positive result.

XI. Antimicrobial Activity

11.1 Nutrient Broth

Nutrient broth does not solidify because it is not an agar. To prepare nutrient broth, 5g of peptone, 5g of sodium chloride, 1.5g of yeast extract and 1.5g of beef extract was dissolved in 1 liter of distilled water. Broth and test tube were sterilized at 121°C for 15 mins. Then the nutrient broth was poured into test tubes. One test tube was used as control and another tube were inoculated with respective organism. Then the tubes were incubated at 37°C for 18-24 hrs.

XII. Sample Dilution

The samples were diluted in 4 different ratios. Dilution at 1:1 ratio, 1g of toothpaste were dissolved 1ml of distilled water, 1:2 ratio 1g of toothpaste were diluted in 2ml of water, 1:4 ratio 1g of toothpaste were diluted in 4ml of water and 1:8 ratio 1g of toothpaste were dissolved in 8ml of water. For herbal extract, 30gm of herbal sample were diluted in 30ml of distilled water and the sample to boil, and then incubate over night at room temperature for 24hrs. Then filter the extract by using filter paper. The filter extract were diluted at 1:1, 1:2, 1:4 and 1:8 ratio by above dilution procedure.

XIII. Antimicrobial Assay

The antimicrobial sensitivity of the test strain of toothpaste and herbal samples was determined by Kirby-Bauer disc diffusion method and wall method. To determine the sensitivity of sample, Prepare Muller Hinton agar by dissolving 38.0g of MH agar in 1000ml of distilled water. The autoclave the media and required petri dishes at 121°C for 15 mins. After autoclaving, pour the agar in petri plates wait till the agar solidifies. Then streak agar plate with respective organisms by using sterile swab. A well was made use of cork borer on the surface of the agar plate. The concentrated extract was added in the wall. Another method, using sterile disc (using filter paper in this procedure). Place the sterile filter in plate by using forceps to which the diluted samples were added. Then place sample loaded filter paper in the agar plate. Then incubate the plates at 37°C for 24 hrs. After incubation zone were identified and zone were measured using scale. No zone indicates absence antimicrobial activity.

XIV. Figures And Tables

Table 1: Represents Biochemical characterization of isolated organisms

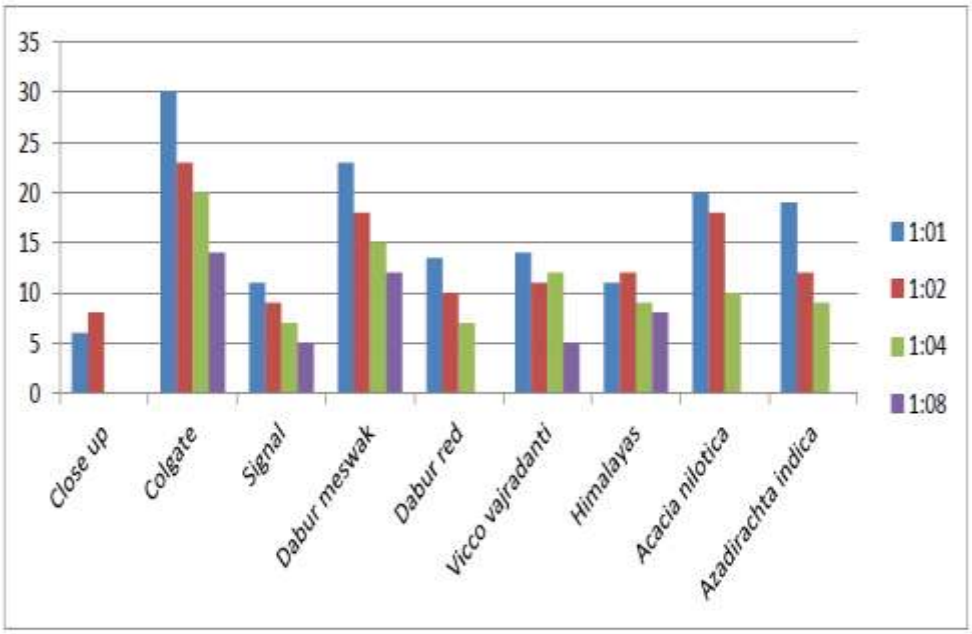
Organism	**Staphylococcus Aureus**	**Pseudomonas Aeruginosa**	**Bacillus Sp**
Indole Test	Negative	Negative	Negative
Methyl Red Test (Mr)	Positive	Negative	Positive
Vouges Proskauer Test (Vp)	Negative	Negative	Negative
Catalase Test	Positive	Negative	Positive
Oxidase Test	Negative	Positive	Positive
Gelatin Test	Positive	Positive	Positive

Table 2: zone of inhibition (in mm) of antimicrobial

Samples	**1:1**	1:2	1:4	1:8
Close Up	6	8	0	0
Colgate	30	23	20	14
Signal	11	9	7	5
Dabur Meswak	23	18	15	12
Dabur Red	13.5	10	7	0
Vicco Vajradanti	14	11	12	5
Himalaya	9	8	7	9
Azadirachta Indica	19	12	9	0
Acacia Nilotica	20	18	10	0

activity by Staphylococcus aureus

The graphical image for zone of inhibition in staphylococcus aureus

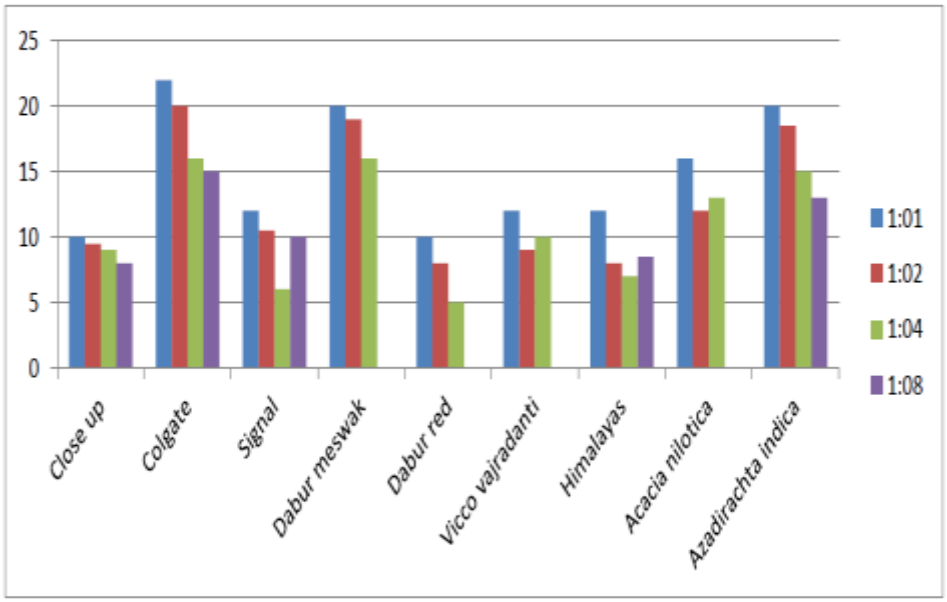


For Pseudomonas aeruginosa, The maximum zone of inhibition 20mm was identified in acacia nilotica at 1:2 dilution. The minimum zone of inhibition 6mm in Azadirachta indica at 1:8 dilution. In 2014 Rossi et al., result shows that the dentifrice containing natural plant extracts (Parodontax(r)) was the only formulation that had activity against Gram-negative bacteria (Psudomonas aeruginosa) P. aeruginosa is related to periodontal diseases, and Candida albicans, the most common fungal pathogen, is involved in candidiasis and most superficial or systemic infections, even dental caries. The toothpaste containing triclosan (Sanogil(r)) showed the highest antimicrobial activity against Gram-positive microorganisms and yeasts; however, it did not demonstrate antimicrobial activity against P. aeruginosa. The plant extract-based dentifrice was the only product able to inhibit the growth of Gram-negative bacteria (P. aeruginosa). Thus the maximum zone is obtained at Acacia nilotica, minimum zone was found in Himalaya.

Table 1 Zone of inhibition (in mm) of antimicrobial activity by Bacillus sp

SAMPLES	**1:1**	1:2	1:4	1:8
Close up	10	9.5	9	8
Colgate	22	20	16	15
Signal	12	10.5	6	10
Dabur Meswak	12	19	16	0
Dabur red	10	8	5	0
Vicco vajradanti	12	9	10	0
Himalaya	12	8	7	8.5
Azadirachta indica	20	18.5	15	13
Acacia nilotica	16	12	13	0

The graphical image for zone of inhibition in Bacillus sp



The maximum zone was observed at Colgate as 22mm in 1:1 dilution and another maximum zone at Azadirachta indica as 20mm in 1:1 dilution ratio. The minimum zone of inhibition was obtained in Dabur red. In 2014 Nwakanma, result shows that Lactobacillus sp has maximum zone at Sample A as 10mm size and minimum zone at Sample E as 2mm size. In 2015 Sugnanam, for Solanum trilobatum average zone was observed. In acetone 20mm, 18mm in petroleum ether and 10mm in chloroform was obtained. In 2011 Sorna et al., result shows that Azadirachta indica and Salvadora persica for Lactobacillus sp. it's of 14 mm and 16 mm respectively. The antibiotic disc streptomycin shows inhibition of about 19mm for Lactobacillus sp. The maximum zone was observed in Colgate and Azadirachta indica, minimum zone was obtained in Dabur red.

XV. Conclusion

The antimicrobial activity of one gel based toothpaste, two normal toothpastes, four herbal toothpastes, two herbal samples was tested against buccal cavity organism. Isolated organism from the selected media has been characterized by using biochemical tests. Pseudomonas aeruginosa, Staphylococcus aureus and Bacillus sp was identified microscopically. The different level of antimicrobial activity was observed by zone of inhibition using disc diffusion and well method. For Staphylococcus aureus, the maximum zone was observed in Colgate was 30mm in size at 1:1 ratio dilution by disc diffusion method. The maximum zone of inhibition in Colgate, minimum at signal and Vicco vajradanti in 1:8 dilution by disc diffusion. The average zone of inhibition was observed in other toothpastes. For Pseudomonas aeruginosa, the maximum zone was observed in 20mm in size at 1:2 dilution by wallmethod.The minimum zone of inhibition in 6mm in size Azadirachta indica at 1:8 dilution. For Bacillus sp, the maximum zone of inhibition was observed in Colgate as 22mm in size and Azadirachta indica as 20mm in size in 1:1 ratio of dilution. The minimum zone of inhibition was Dabur red toothpaste as 10mm in size in 1:1 dilution.

In the oral pathogen, Staphylococcus aureus has maximum zone of inhibition when compare to the other organism isolated from buccal cavity against selected toothpastes

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