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Antimicrobial Activity of Herbal Toothpaste against Organisms Causing Dental Caries

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Dental caries occurs as a result of acid produced by bacteria which destroys the enamel and underlying dentin. A study was carried out to isolate microorganisms that cause dental caries in patients belonging to different age groups and find out the antimicrobial activity of various toothpastes against them.

Study Design: Collection of samples for isolation of caries causing organisms, to identify them using biochemical tests and test the activity of herbal toothpastes against them.

Place and duration of study: Samples were collected from Krish Multispeciality Clinic and Prashanth Dental Clinic, Nagole between June'2016 – December'2017 and worked on, at Microbiology Department, St. Francis College for women, Hyderabad.

Methodology: Ten samples of each age group from 10-20 yrs., 20-40 yrs. and 40-60 yrs. were isolated. The organisms were identified by carrying out various biochemical tests according to Bergey's Manual of Systematic Bacteriology and molecular characterization based on 16s rRNA typing. Identified organisms were used to test the antimicrobial activity of herbal toothpastes by employing agar well diffusion method. ANOVA is used for statistical analysis.

Results and Conclusion: Patanjali herbal products like Dantkanti are gaining lot of importance and have become a part of life of common man because of their effectivity. Our studies revealed that out of the various toothpastes tested, Patanjali toothpaste had good antimicrobial activity against all the

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isolates. On comparative analysis of f-ratio and P significant value ($< .05.$) of all pairs, it is observed that Patanjali has good antimicrobial activity against all isolates tested. The efficacy of Meswak is almost similar to that of Patanjali.

Keywords: Dental caries; herbal toothpaste; *Klebsiella pneumoniae*.

1. INTRODUCTION

Dental plaque is a biofilm which is pale yellow in color that develops naturally on the teeth. A dental plaque is formed by colonizing bacteria trying to attach themselves to the tooth surface [1]. This dental plaque gives rise to dental caries. Dental caries denotes the decay or destruction of tooth. It is caused by specific types of bacteria [2]. Four important factors play a role in formation of caries. They are: a tooth surface (enamel or dentin), caries-causing bacteria, fermentable carbohydrates such as sucrose and time [3]. The bacteria produce acid that destroys the tooth enamel and the layer under it, the dentin. Many different types of bacteria normally live in the human mouth [2]. But only a few specific species of bacteria are believed to cause dental caries [4]. The most common bacteria associated with dental cavities are the *Streptococcus mutans*, *Streptococcus sobrinus*, and *Lactobacilli* [5]. They build up on the teeth a sticky film called plaque [2]. Though the plaque is usually associated with the oral diseases which include cavities and other gum diseases, its construction is normal which cannot be prevented. These dental biofilms can be quite serious as their acidic nature causes the demineralization of the teeth resulting in the formation of calculus which is also known as tartar. These are hardened structures that require professional cleaning as they cannot be removed by tooth brush. It is therefore very essential to eradicate these dental plaques to avoid the formation of dental caries and related gum disease [6,7].

To remove plaque and maintain dental hygiene agents like toothpaste and various mouth washes can be used. Toothpaste is a dentifrice which improves the aesthetic appearance and health of the teeth. Toothpaste is commonly used for promotion of oral hygiene, removal of dental plaque and food debris from the mouth [8]. Mechanical plaque removal is one of the most accepted methods of controlling plaque and gingivitis. Several chemical preventive agents have beneficial effects in the control of plaque and to reduce or prevent oral disease. Hence, various chemical formulations were tried in

dentifrices [9]. Chemicals, mainly triclosan and chlorhexidine, have been added in mouth rinses and dentifrices to prevent plaque and gingivitis. But some of these substances show undesirable side effects such as tooth staining and altered taste [10,11]. This led to increased attention on using natural ingredients in herbal dentifrices. Herbal dentifrices like Colgate herbal, Meswak, Dabur red, Neem clove, spearmint herbal are made using sage, chamomile, Echinacea, myrrh, peppermint oil etc. These ingredients have several benefits; chamomile has anti-inflammatory effect, echinacea has immune stimulatory property, sage and rhatany have anti-hemorrhagic properties, myrrh is a natural antiseptic, and peppermint oil has analgesic, antiseptic, and anti-inflammatory properties [12]. Using above herbal ingredients several toothpastes are manufactured and released into the market. Some of them like Colgate herbal, Dabur red, Himalaya complete care, Neem clove paste, Meswak and Dantkanti have gained a lot of importance. Various studies were conducted to assess the antimicrobial activity of these toothpastes. Results of such studies have proved that herbal toothpastes are very efficient in maintaining the oral hygiene. Dabur red containing the following: Pudinasatva, Tomarbeej and laungkatelis is as effective as the conventional non-herbal toothpaste in plaque reduction and reduction of gingivitis [13]. Meswak is a herbal dentifrice containing the pure extract of the Meswak plant *Salvadora persica*, the famous "Toothbrush Tree" which has been used for centuries. The astringent and antibacterial properties of Meswak help reduce tooth decay, fight plaque, and prevent gum diseases [14].

Patanjali dantkanti is a herbal toothpaste made using different components like Akarkara – *Anacyclus Pyrethrum*, Babul (Kikar) – *Acacia Arabica*, Tomar – *Zanthoxylum Alatum*, Neem – *Azadirachta indica*, Pudina (Spearmint) – (Clove) – *Syzygium Aromaticum*, Pippali (Long Pepper) – *Piper Longum*, Vajradanti – *Barleria Prionitis* etc. The above herbal ingredients are known to have antibacterial, anti-inflammatory and anticariogenic properties. It is observed that the herbal toothpaste (Dantkanti) is effective when compared to the conventional toothpaste in

maintenance of oral hygiene and reduction of gum bleeding [15]. It gives protection and long life to teeth and fights against dental problems.

2. MATERIALS AND METHODS

2.1 Sample Collection

The samples were collected from patients suffering with dental caries, from Krish dental clinic and Prashanth dental clinic, Nagole, Hyderabad. Sterile cotton swabs were placed at site of infection for few minutes and then inoculated into sterile nutrient broth. The tubes were properly labeled with patient's age and gender. These samples were stored at 4°C.

2.1.1 Growth media for isolation of organisms

For isolation of dental caries causing bacteria, nutrient agar was used. A loopful of sample was streaked on the nutrient agar plate and the plates were incubated at 37°C for 24h.

2.1.2 Cultural and morphological characters

The colony morphology of different samples was observed. The bacteria were gram stained.

2.1.3 Biochemical properties

Biochemical properties of isolates were used in identification of the organisms. Different biochemical tests like IMViC, glucose fermentation, mannitol fermentation, nitrate reduction, catalase, oxidase tests were carried out based on the Bergey's manual of systematic bacteriology. All the chemicals and reagents used were of analytical grade.

2.1.4 Molecular method of identification (16S rRNA typing)

16S ribosomal RNA (or 16S rRNA) is the component of the 30S small subunit of a prokaryotic ribosome that binds to the Shine-Dalgarno sequence. The genes coding for it are referred to as 16S rRNA gene and are used in reconstructing phylogeny, due to the slow rates of evolution of this region of the gene [16]. The DNA of the sample was isolated and 16s DNA fragment was amplified using PCR. The

consensus sequence of amplicon was generated and analyzed by using BLAST in NCBI.

2.2 Assessing the Antimicrobial Activity of Toothpastes

Agar well diffusion method was used for antimicrobial studies. Nutrient agar plates were prepared. 0.1 ml of culture was seeded and then wells were bored. Into each well 0.3 gm of test toothpastes were added. Antimicrobial activity of each toothpaste was determined by measuring the zones of inhibition.

2.2.1 Statistical tool

ANOVA was used to determine p value, f ratio and the comparative analysis of toothpastes was done using this tool.

3. RESULTS

30 samples were collected from patients belonging to the age groups 10-20, 20-40, 40-60 yrs. The samples were labelled as S1, S5, S6, S8, S9, S10, S12, S13, S14, S16, S17, S18, S20, S22, S25, S26, S28, S29, S30, S31. Biochemical characterization was carried out against the isolates of 10-20 yrs age group. Based on the various biochemical tests, out of the ten samples isolated the organisms belonged to gram negative are identified to be of *Pseudomonas sps*, *Aeromonas sps* and gram-positive nature were identified to be of *Staphylococcal sps*, *Bacillus sps*, as shown in Table 1.

According to various biochemical tests carried out against the isolates of 20-40 yrs the organisms of gram positive nature were identified as *Bacillus sps* and those of gram-negative nature are identified as *Aeromonas sps*, *Pseudomonas sps*, *Klebsiella sps*, *Serratia sps* as shown in Table 2.

Based on the various biochemical tests carried out among the samples isolated from 40-60 yrs age group, the gram-positive organisms are found to be of *Bacillus sps*, *Micrococcus sps* and the gram negative ones are found to be *Pseudomonas sps* and *Citrobacter sps*. The results are shown in Table 3.

Table 1. Biochemical characterization of isolates from patients in the age group of 10-20 yrs

S6	S28	S26	S33	S18, S31	S25, S30
Gram + ve cocci	Gram + ve cocci	Gram + ve rod	Gram + ve rod	Gram –ve rod	Gram – ve rod
Catalase +ve				Oxidase + ve	
Mannitol fermentation +ve	Mannitol fermentation -ve	Starch hydrolysis -ve		Glucose Fermentation + ve	Glucose Fermentation –ve
		Endospore staining +ve			
	Growth on MSA - ve	Nitrate reduction + ve	Nitrate reduction -ve	VP -ve	Nitrate reduction +ve
<i>S. aureus</i>	<i>Staphylococcus sps.</i>	<i>Bacillus pasteuui</i>	<i>Bacillus sphearicus</i>	<i>Aeromonas sps.</i>	<i>Pseudomonas sps.</i>

Key +ve =positive, -ve negative

Table 2. Biochemical characterization of isolates from patients in age group of 20-40 yrs

S8, S13, S16	S10	S12	S14	S20	S22
Gram -ve rods	Gram -ve rods	Gram -ve coccobacilli	Gram -ve coccobacilli	Gram +ve rods	Gram +ve rods
Oxidase +ve		Lactose +ve		Endospore staining +ve	
Glucose fermentation -ve	Glucose +ve	Indole +ve	Indole -ve	Starch hydrolysis -ve	
	VP -ve	VP +ve	VP -ve		
Nitrate reduction +ve		Motility (non motile)		Nitrate reduction +ve	Nitrate reduction -ve
		Citrate test +ve	MR +ve	Catalase +ve	
		H2S test -ve			
<i>Pseudomonas sps.</i>	<i>Aeromonas sps.</i>	<i>Klebsiella sps.</i>	<i>Serratia sps.</i>	<i>Bacillus pasteurii</i>	<i>Bacillus sphaericus</i>

Key, +ve =positive, -ve negative

Table 3. Biochemical characterization of isolates from patients in age group of 40-60 yrs

S1, S5	S17	S29	S9
Gram +ve rods	Gram +ve cocci	Gram -ve rods	Gram -ve coccobacilli
Endospore staining +ve	Mannitol fermentation -ve	Oxidase +ve	Lactose fermentation +ve
Catalase +ve			Indole Test +ve
Starch Hydrolysis -ve	Growth on MSA		Citrate test +ve
Nitrate reduction +ve		Nitrate reduction +ve	VP test -ve
	Glucose fermentation +ve	Glucose fermentation -ve	
<i>Bacillus pasteurii</i>	<i>Micrococcus varians</i>	<i>Pseudomonas sps.</i>	<i>Citrobacter diversus</i>

Key - +ve=positive, -ve negative

3.1 16s rRNA Typing of S12

The gene sequence for 16s rRNA region of S12 sample was isolated, amplified and analyzed using BLAST. It was identified to have 99% identity with *K. pneumoniae* strain DSM 30104.

3.1.1 Sanger sequence chromatogram file data

>Forward Sequence data

TGAGGCGTGAGCTTCCGCGAGCTAACGCAG
TAAATCGACCGCCTGGGGAGTACGGCCGCA
AGGTTAAAACCTCAAATGAATTGACGGGGGCC
CGCACAAGCGGTGGAGCATGTGGTTTAATTC
GATGCAACGCGAAGAACCTTACCTGGTCTTG
ACATCCACAGAACTTTCCAGAGATGCATTGGT
GCCTTCGGGAAGTGTGAGACAGGTGCTGCAT
GGCTGTGCGAGCTCGTGTGTTGTGAAATGTTGG
GTTAAGTCCCGCAACGAGCGCAACCCTTATC
CTTTGTTGCCAGCGGTTCCGGCCGGAAGTCA
AAGGAGACTGCCAGTGATAAACTGGAGGAAG
GTGGGGATGACGTCAAGTCATCATGGCCCTT
ACGACCAGGGCTACACACGTGCTACAATGGC
ATATACAAAGAGAAGCGACCTCGCGAGAGCA
AGCGGACCTCATAAAGTATGTCGTAGTCCGG
ATTGGAGTCTGCAACTCGACTCCATGAAGTC
GGAATCGTAGTAATCGTAGATCAGAATGCTA
CGGTGAATACGTTCCCGGGCCTTGTACACAC
CGGCCGTACACCATGGGAGTGGGTTGCAA
AAGAAGTAGGTAGCTTAACCTTCTG
GAGGGCGCTTACCACTTTGTGATTCATGACT
GGGGTGAAGTCGTA

>S_12 consensus sequence

TGAGGCGTGAGCTTCCGCGAGCTAACGCAG
TAAATCGACCGCCTGGGGAGTACGGCCGCA
AGGTTAAAACCTCAAATGAATTGACGGGGGCC
CGCACAAGCGGTGGAGCATGTGGTTTAATTC
GATGCAACGCGAAGAACCTTACCTGGTCTTG
ACATCCACAGAACTTTCCAGAGATGCATTGGT
GCCTTCGGGAAGTGTGAGACAGGTGCTGCAT
GGCTGTGCGTCAAGTGTGTTGTGAAATGTTG
GGTTAAGTCCCGCAACGAGCGCAACCCTTAT
CCTTTGTTGCCAGCGGTTCCGGCCGGGAAGT
CAAAGGAGACTGCCAGTGATAAACTGGAGGA
AGGTGGGGATGACGTCAAGTCATCATGGCC
TTACGACCAGGGCTACACACGTGCTACAATG
GCATATACAAAGAGAAGCGACCTCGCGAGAG
CAAGCGGACCTCATAAAGTATGTCGTAGTCC
GGATTGGAGTCTGCAACTCGACTCCATGAAG
TCGGAATCGTAGTAATCGTAGATCAGAATGC
TACGGTGAATACGTTCCCGGGCCTTGTACAC
ACCGGCCGTACACCATGGGAGTGGGTTGC
AAAAGAAGTAGGTAGCTTAACCTTCTG
GAGGGCGCTTACCACTTTGTGATTCATGACT
GGGGTGAAGTCGTA

Blast report is shown in Table 4.

3.2 Comparative Analysis of Antimicrobial Activity of Patanjali and other Herbal Toothpastes

Agar well diffusion method was used. Four toothpastes- Patanjali dantkanti, Colgate herbal, Meswak and Dabur red were used against few isolates to determine their antimicrobial activity. The zones of inhibition around each toothpaste are observed as shown in Table 5.

The results from Table 5 indicate that all tested toothpastes demonstrated a significant antimicrobial activity against the tested microorganisms ($P < 0.05$). The antimicrobial activity of patanjali toothpaste against the organisms S1, S3, S6, S12, S25, S20 and *Streptococcus mutans* was very significant. The activity of Meswak was similar to the activity of Patanjali against S6, S12, S20. Meswak though effective against other organisms S1, S3, S25 and *S. mutans* but was not as effective as Patanjali. Meswak and Patanjali were very effective against all the organisms tested than Dabur ($P < 0.05$), and colgate herbal ($P < 0.05$). The data analyzed using ANOVA showed no significant difference among Meswak and Patanjali with respect to their antimicrobial activities ($P > 0.05$).

There is a significant difference in the antimicrobial activities of Patanjali and Dabur. The *f*-ratio value is 10.85303. The *P*-value is .006407. The result is significant at $P < .05$.

Patanjali and Colgate Herbal The *f*-ratio value is 11.53643. The *P*-value is .005304. The result is significant at $P < .05$.

Meswak and Dabur The *f*-ratio value is 7.57293. The *P*-value is .017541. The result is significant at $P < .05$.

Meswak and Colgate Herbal The *f*-ratio value is 8.32574. The *P*-value is .013692. The result is significant at $P < .05$.

The results of antimicrobial activity of 4 test toothpastes against S1, S6 and S12 are shown in Figs. 3, 4, 5 respectively. The antimicrobial activity of the 4 test toothpastes against *Streptococcus mutans* is shown in Fig. 6.

Table 4. Blast report

Description	Max Score	Total Score	Query Cover	E value	Identity	Accession number
<i>Klebsiella pneumoniae</i> strain DSM 30104 16S rRNA gene, partial sequence	1177	1177	100%	0	99%	NR_117686.1
<i>Klebsiella pneumoniae</i> strain DSM 30104 16S rRNA gene, partial sequence	1177	1177	100%	0	99%	NR_117683.1
<i>Klebsiella pneumoniae</i> strain DSM 30104 16S rRNA gene, complete sequence	1177	1177	100%	0	99%	NR_036794.1
<i>Klebsiella pneumoniae</i> strain DSM 30104 16S rRNA gene, partial sequence	1173	1173	100%	0	99%	NR_114715.1
<i>Klebsiella pneumoniae</i> strain DSM 30104 16S rRNA gene, partial sequence	1171	1171	100%	0	99%	NR_117684.1
<i>Klebsiella pneumoniae</i> strain NBRC 14940 16S rRNA gene, partial sequence	1168	1168	99%	0	99%	NR_113702.1
<i>Klebsiella pneumoniae</i> strain DSM 30104 16S rRNA gene, partial sequence	1166	1166	100%	0	99%	NR_117685.1
<i>Klebsiella pneumoniae</i> strain DSM 30104 16S rRNA gene, partial sequence	1166	1166	100%	0	99%	NR_117682.1
<i>Klebsiella pneumoniae</i> strain JCM 1662 16S rRNA gene, partial sequence	1162	1162	98%	0	99%	NR_113240.1
<i>Klebsiella quasi pneumoniae</i> subsp. <i>Simili pneumoniae</i> strain 07A044 16S rRNA gene, partial sequence	1157	1157	98%	0	99%	NR_134063.1

Table 5. Antimicrobial activity of herbal tooth paste against the dental isolates

S.no.	Sample	Patanjali	Dabur red	Colgate herbal	Meswak
1.	S1	60mm	35mm	37mm	50mm
2.	S3	37mm	15mm	0	31mm
3.	S6	30mm	0	0	30mm
4.	S12	43mm	0	0	43mm
5.	S25	41mm	35mm	30mm	34mm
6.	S20	35mm	28mm	28mm	35mm
7.	<i>Streptococcus mutans</i>	45mm	23mm	25mm	34mm

4. DISCUSSIONS

In this study 30 samples were collected from patients suffering with dental caries, belonging to different age groups. The samples were categorized into 3 groups based on the patient's age (10-20 yrs, 20-40yrs and 40-60 yrs). These samples were plated onto nutrient agar and sub cultured in nutrient broth. Various biochemical tests like Indole, glucose fermentation, nitrate reduction etc were carried out to identify the organisms, according to Bergey's manual of

systematic bacteriology. Group of 10-20 yrs - organisms like *Staphylococcus aureus*, *Aeromonas* spp, *Pseudomonas* spp, *Bacillus pasteurii*, *Bacillus sphaericus* were identified. Group of 20-40 yrs- organisms like *Pseudomonas* spp, *Aeromonas* spp, *Bacillus* spp like *B.sphaericus*, *B. alvei*, *B.polymyxa*, *Klebsiella* spp, *Serratia* spp were identified. Group of 40-60 yrs- organisms like *Citrobacter diversus*, *Micrococcus varians*, *Pseudomonas* spp, *Bacillus pasteurii* were identified.

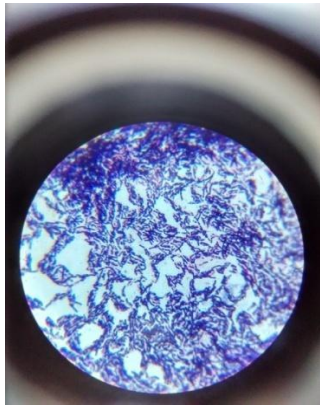


Fig. 1. Gram staining field view of S1

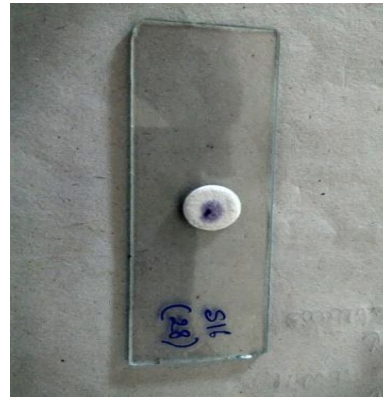


Fig. 2. Oxidase test of S16

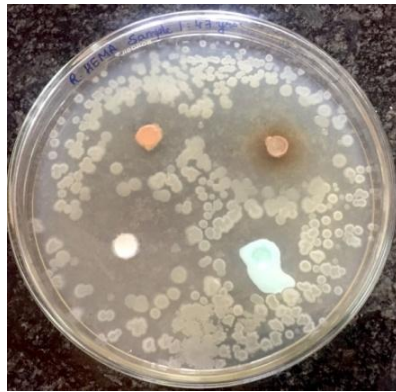


Fig. 3. Antimicrobial activity against S1

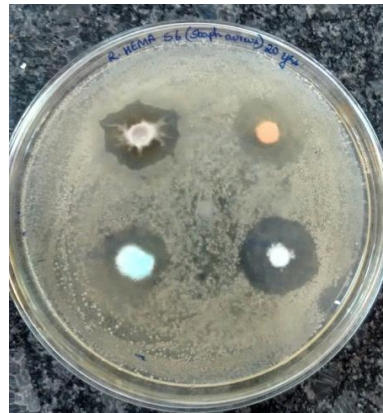


Fig. 4. Antimicrobial activity against S6

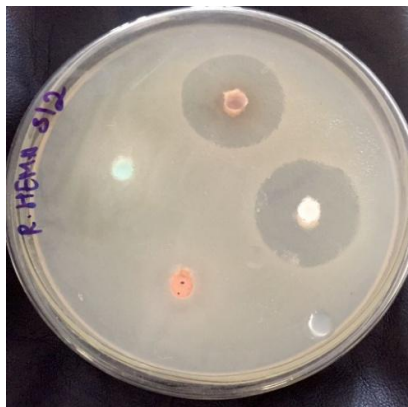


Fig. 5. Antimicrobial activity against S12



Fig. 6. Antimicrobial activity against S. mutans

Studies suggest that *S. aureus* continues to be a frequent isolate in the oral cavity and perioral region and is mostly due to cross-infection [17]. *Aeromonas* sps is known to cause gastroenteritis and also pneumonia in humans [18]. This may be reason behind its presence in

oral cavity. *Pseudomonas* sps cause nosocomial infections. They act as opportunistic pathogens and many strains are isolated from dental unit water lines [19]. This shows that *Pseudomonas* sps are associated with dental infections. *Bacillus* sps are a major part of normal flora of

humans and are also mostly used in different probiotic products. During their consumption the bacteria may colonize the oral cavity and also *Lactobacilli* are known to be associated with dental caries [20]. *Citrobacter* sps are major nosocomial pathogens. In a study conducted to detect quorum sensing among bacteria, *Citrobacter* sps were isolated from dental plaque [21]. *Micrococcus* sps were known to be associated with dental caries and various antimicrobial agents' activity is assessed against them [22]. *Klebsiella* sps are important opportunistic hospital acquired pathogens [23]. *Serratia* sps are nosocomial pathogens and were also found in dental unit water lines [24]. Gram negative cocci form a major role in formation of dental pellicle. After the attachment of gram positive cocci, pathogenic gram negative cocci along with other rods form colonies called corncocks which assist organisms like *Streptococcus mutans* in their pathogenic function [25,26]. The organisms identified can cause dental caries.

Though some of the organisms are a part of normal flora, when there is an imbalance in oral environment, they can cause dental caries. Therefore, removal of plaque to prevent the colonization of caries causing microbes and maintaining oral hygiene is very important. According to studies conducted by Kuldeep *et al.*, 2016 the patients using herbal Dantkanti toothpaste have been protected effectively in terms of reduction of debris, calculus and gingival index factor is statistically significantly low. No adverse reactions of dentifrices products were observed during the trial. Hence antimicrobial studies were done with herbal tooth paste like Patanjali, Dabur, Colgate herbal and Meswak [27]. The zones of inhibition produced around the wells with different toothpastes are as shown in Table 5. Dantkanti has shown good antimicrobial activity against the different isolates, Meswak has also shown good activity like that of Dantkanti. Dabur red and Colgate herbal have shown moderate activity when compared to the other two toothpastes. Several studies have been carried out to test the antimicrobial activity of different herbal toothpastes and few of them like Colgate herbal, Dabur red and Meswak have shown moderate activity against *Streptococcus mutans* and other caries causing organisms [28]. The *S. mutans* culture used in present study belongs to strain MTCC 890 procured from IMTECH, Chandigarh.

The data analyzed using ANOVA shows significant antimicrobial activity of Patanjali toothpaste against all the isolates tested. In one-way ANOVA performed with pairs of toothpaste: Patanjali and Colgate Herbal, Patanjali and Dabur, Meswak and Dabur, Meswak and Colgate Herbal, the f-ratio and p-value derived show the significant antimicrobial activity of Patanjali & Meswak respectively. Though the activity of Meswak is almost similar to that of Patanjali, it is noticed that the antimicrobial activity of Dabur and Colgate Herbal toothpastes vary hugely in comparison. Similar statistical analysis was performed among herbal toothpastes against *S.mutans* and observed that the antimicrobial activity of Patanjali was significant followed by Meswak and Dabur [29].

5. CONCLUSION

The mouth is a favorable habitat for a number of organisms due to the presence of nutrients and secretions. These organisms attach, colonize and accumulate on tooth surface giving rise to dental plaque [30]. Such colonizing bacteria gradually cause dental caries. Several organisms causing dental caries like *Staphylococcus* sps, *Bacillus* sps, *Citrobacter* sps, *Micrococcus* sps, *Pseudomonas* sps, *Aeromonas* sps etc have been isolated and identified in the samples collected from patients of different age groups. Among age group of 10-20 yrs different organisms were identified. *Staphylococcus* was identified only in the samples of this age group. This is in agreement with "A growing evidence suggests that *Staphylococci* can be frequently isolated from the oral cavity of particular patient groups such as children, elderly and some groups with systemic diseases." [31]. Age group of 20-40 yrs: *Pseudomonas* sps was identified in 3 samples falling in the range of 28-34 yrs. The samples of this age group have shown the presence of diverse variety of organisms. Age group of 40-60 yrs: Gram negative cocci predominated in this group; out of the 10 samples five of them were identified to be gram negative cocci.

Among the 4 toothpastes subjected to agar well diffusion method to determine their antimicrobial activity in this assay (Colgate herbal, Meswak, Dantkanti and Dabur red) Patanjali Dantkanti has shown the highest activity against different isolates and also the most common caries causing organism i.e, *Streptococcus mutans*. Meswak has also shown good activity like that of Dantkanti. Dabur red and Colgate herbal have shown moderate activity when compared to the

other two toothpastes. Statistical analysis performed between the toothpaste samples also confirmed the higher efficacy of Patanjali Dantkanti followed by Meswak, Dabur red and Colgate herbal.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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