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Comparative Evaluation of the Efficacy of Various Herbal Dentifrices Alone and In Combination against Streptococcus Mutans and Lactobacillus Acidophilus– An In Vitro Study

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Abstract

Background: The good oral hygiene practices are the key to the prevention of dental diseases. The proper technique and right oral hygiene products keep check on dental plaque which is one of the primary etiological factors for dental diseases. Several oral hygiene products are made antimicrobial by adding antimicrobial agents such as metal salts, phenols, herbal extracts, enzymes, essential oils, and bisbiguanides to combat the invading and potentially harmful. This study was conducted to evaluate and compare the antimicrobial efficacy of dentifrices containing herbal extracts as their therapeutic agent against dental caries causing microbes, Streptococcus mutans and Lactobacillus acidophilus.

Methodology: The potential antimicrobial herbal materials considered for the study were Terminalia chebula, Acacia catechu, Aloe vera, Azadirachta indica, Green tea and combination of all. The dentifrices were prepared using aqueous extract of each herbal material as the therapeutic agents. Then antimicrobial properties of these prepared dentifrices were evaluated by measuring the zone of inhibition at 24 hours and 48 hours using well diffusion method against Streptococcus mutans and Lactobacillus acidophilus. The data obtained was subjected to statistical analysis using Student's t-test for intragroup comparison and ANOVA test for multiple group comparisons followed by Tukey's post hoc test for group wise comparisons.

Results: Among the test dentifrices used, maximum zone of inhibition was observed with respect to group VI (Combination dentifrice) at 50% concentration and group II (Acacia catechu dentifrice) and group VI (Combination dentifrice) at 100% concentration after at 24 and 48 hours against Streptococcus mutans. Similarly maximum zone

of inhibition was observed with respect to group I (Terminalia chebula dentifrice) followed by the group VII at 50% concentration and group III (Aloe vera dentifrice) at 100% concentration after at 24 and 48 hours against Lactobacillus acidophilus.

Conclusion: Among the herbal dentifrices tested, Combination dentifrice and Acacia catechu dentifrice were better in inhibiting Streptococcus mutans at different concentration followed by other test dentifrices. The Terminalia chebula dentifrice was efficient against Lactobacillus acidophilus in different concentration followed by other test dentifrices.

In the present In-vitro study, dentifrices prepared with herbal material showed promising antibacterial activity against cariogenic bacteria i.e. Streptococcus mutans & Lactobacillus acidophilus and in future these tested herbal products can be potential alternatives to conventional dentifrices as therapeutic dentifrices.

Keywords: Herbal; dentifrice; Streptococcus mutans; Lactobacillus acidophilus

Introduction

The moist and tempered environment and metabolic substrate make oral cavity a unique and ideal ecosystem in the human body for bacterial growth[1]. The dental plaque, a structurally and functionally-organized biofilm coated on tooth structure harbors numerous species of microorganisms predisposing to poor oral hygiene [2,3]. Undisturbed dental plaque matures and increases its pathogenicity with time. The matured and pathogenic plaque is main predisposing factor for dental caries[4]. Whereas dental caries without any predilection to any sexes, races, age groups, socio-economic strata prevalent globally[5].

Apart from physical plaque control, there are chemicals used as antiplaque agents in the dentifrices and mouth rinses such as triclosan, essential oils and chlorhexidine as therapeutic agents to control bacterial plaque, aiming at improving the efficacy of daily oral hygiene control measures. [6,7]

In recent years the public interest grown in alternative health care, especially application of natural or herbal health care products after the scientific re-introduction of traditional Indian system of medicine recognized by laws and acts, authorized by the councils. [8,9,10] Because of public inclination, commercial scope and competition among the manufacturers, the interest in plants with antibacterial and anti-inflammatory activity has increased many fold. [11]

Even though traditionally used, scientifically proven certain medicinal herbs are far away to reach field of dentistry as means of antibacterial oral hygiene products especially dentifrices. The modern day dentifrices formulations include abrasive agents, tensoactives, humectants, thickening agents, flavoring, coloring agents and antimicrobial agents such as metal salts, phenols, herbal extracts, enzymes, essential oils, and bisbiguanides. [12,13,14] However, little is known about the behavior of the above discussed herbs when incorporated in the dentifrices.

Hence, with this background, the study was conducted using, the herbal extracts of Terminalia chebula, Acacia catechu, Aloe vera, Green tea, Azadirachta indica as antimicrobial/therapeutic agent in the dentifrices and were evaluated for their antimicrobial efficacy against Streptococcus mutans and Lactobacillus acidophilus.

The objectives of the study were to evaluate and compare the antimicrobial efficacy of dentifrices containing Terminalia chebula, Acacia catechu, Aloe vera, Green tea, Azadirachta Indica and in combination against Streptococcus mutans and Lactobacillus acidophilus.

Methodology

The in-vitro study was conducted to evaluate the antibacterial efficacy of herbal dentifrices against Streptococcus mutans & Lactobacillus acidophilus.

The ethical clearance was obtained from Bapuji Dental College & Hospital, Davangere and necessary permission were obtained from Bapuji College of Pharmacy, Davangere, and Maratha Mandal N. G. H. Institute of Dental science & Research centre, Belgaum.

1. Preparation of the tooth pastes.

a) Preparation of aqueous extracts: After procurement and authentication by the faculty of Department of Pharmacognacy, Bapuji College of Pharmacy, Davangere, the dried forms of dried ripe fruit of Terminalia chebula, dried bark of Acacia catechu and green tea leaves ground to coarse powder whereas Aloe vera leaf gel was scraped and accurately weighed. The aqueous extract of each herbal material and also a combination of all were obtained through cold maceration technique.

Later filtered aqueous herbal extracts were collected in conical flasks and transferred to a tared flat bottomed dish, dried by evaporation on the water bath at 105^{o} C for 6 hours, cooled in the dessicator for 30 mins and weighed immediately. [15]

b) Formulation of dentifrices: Dentifrices were formulated as per the Table A, given below. The ingredients were weighed individually in digital weighing machine; water and glycerine were measured with the help of pipette and glass beaker. The measured constituents were then transferred to the porcelain mortar and triturated with the pestle till pricking sound was heard. Later dentifrice was transferred to the tube. [16] Colgate total served as the positive control and sterile distilled water as the negative control. [10]

Table 1: Ingredients used in preparation of dentifrices along with test herbal material.

Ingredient	Percentage
Abrasive (calcium carbonate)	30%
Water	30%
Humectants (glycerine)	30%
Foaming agent (sodium lauryl sulphate)	2%
Binding agent (carboxy methyl cellulose)	2%
Therapeutic agent (aqueous extract of the test materials)	5%
Preservative	1%

Table 2: The grouping of different test materials.

Test groups	
Group I	Terminalia chebula dentifrice
Group II	Acacia catechu dentifrice
Group III	Aloe vera dentifrice
Group IV	Green tea dentifrice
Group V	Azadirachta indica dentifrice
Group VI	Combination of the above
Control groups	
Group VII	Colgate total (positive control)
Group VIII	Sterile distilled water (negative control)

2. Evaluation of the antibacterial efficacy of the dentifrices against Streptococcus mutans and Lactobacillus acidophilus.

The antimicrobial activities of the dentifrices were tested on two common dental caries causing pathogens, namely: Streptococcus mutans (ATCC 25175) and Lactobacillus acidophilus (ATCC 4356) obtained from the American Type Culture Collection (ATCC), USA. The nutrient broth was used to get the viable growth of microbes from freeze dried form. When compared with McFarland 0.5

turbidity standards, turbidity in test tube confirmed the growth of microbes. Agar well diffusion assay was used to evaluate the antimicrobial potential of the dentifrices. The Brain Heart Infusion (BHI) agar and Blood agar used as growth media to Streptococcus mutans and Lactobacillus acidophilus respectively. Within 15 min of adjusting the inoculum to a McFarland 0.5 turbidity standard, with a help of sterile cotton swab the agar plates were inoculated with the swab technique. On solidified agar media six wells of 5 mm diameter were made using a sterilized hollow tube device. 50 mg of test dentifrice were placed into the respective wells on each plate. Plates were incubated within 15 min of dentifrice placement at 37º C in the CO_2 incubator. Each dentifrice was tested at 100% concentration (full strength) and in 50% concentration {1:1 dilution (50% w/w) using sterile distilled water (half strength)}. To ensure the consistency of all findings, the experiment was performed and repeated six times under strict aseptic conditions.

Measurement of zone of inhibition

The zone of microbial inhibition was measured after 24 hours and 48 hours on the underside of the petri dishes using Vernier calipers. Diameter of inhibition zone to nearest whole millimeter was measured and recorded. All the measurements of zone of inhibition were carried out by a single examiner [17]

Statistical Analysis

All the results were subjected for appropriate statistical analysis.. Intragroup comparisons were made using paired t-test, one way ANOVA was used for multiple group comparisons followed by Tukey's post - hoc test for group wise differences. [18]

Results

The study was conducted with an objective to evaluate the antimicrobial efficacy of herbal dentifrices against Streptococcus mutans & Lactobacillus acidophilus by standard well diffusion method. All the dentifrices were tested for 50% concentration {1:1 dilution (50% w/w) using sterile distilled water (half strength)} and 100% concentration (full strength). The zone of inhibition observed at two time interval i.e. at 24 hours and 48 hours were measured using Vernier calipers in millimeters.

The zone of inhibition was observed on all the agar plates inoculated with the strains. Group VII, the positive control, Colgate total produced complete zone of inhibition for Streptococcus mutans whereas for Lactobacillus acidophilus it was comparable with the test dentifrices. The negative control produced no observable inhibitory effect.

Observation from Table 1

All the test dentifrices at 50 % concentration showed zone of inhibition with slight or no increase in the zone of inhibition at 48 hours against Streptococcus mutans. Highest mean zone of inhibition was shown by group VI (combination dentifrice) followed by group II (Acacia catechu dentifrice), group I (Terminalia chebula dentifrice), group IV (Green tea dentifrice), group III (Aloe vera dentifrice), group V (Azadirachta indica dentifrice).

At 100 % concentration, maximum zone of inhibition was shown by group II (Acacia catechu dentifrice) and group VI (Combination dentifrices) followed by group I (Terminalia chebula dentifrice), group IV (Green tea dentifrice), group V (Azadirachta indica dentifrice), least by group III (aloe vera dentifrice) both at 24 hours and 48 hours against Streptococcus mutans. Group I (Terminalia chebula dentifrice) and group V (Azadirachta indica dentifrice) showed slight increase in the zone of inhibition at 48 hours.

Observation from Table 2

In the intergroup comparison, group I (Terminalia chebula dentifrice) significantly inhibited Streptococcus mutans at

50 % concentration than group III (Aloe vera dentifrice) and group V (Azadirachta indica dentifrice) both at 24 hours and 48 hours. Similarly, group II (Acacia catechu dentifrice), group IV (Green tea dentifrice) and group VI (Combination dentifrices) significantly inhibited Streptococcus mutans at 50% concentration than group III (aloe vera dentifrice) and group V (Azadirachta indica dentifrice) at 24 hours and 48 hours.

In the intergroup comparison, against Streptococcus mutans, at 100 % concentration, group I (Terminalia chebula dentifrice) showed significant zone of inhibition than group III (Aloe vera dentifrice) and group V (Azadirachta indica dentifrice). Group II (Acacia catechu dentifrice) showed significant zone of inhibition than group III (Aloe vera dentifrice), group IV (Green tea dentifrice) and group V (Azadirachta indica dentifrice). Group VI (Combination dentifrice) showed better zone of inhibition than group III (Aloe vera dentifrice), group IV (Green tea dentifrice) and group V (Azadirachta indica dentifrice).

Observation from Table 3

At 50 % concentration, at 24 hours, highest mean zone of inhibition was shown by group I (Terminalia chebula dentifrice), followed by the group VII (Colgate total), group II (Acacia catechu dentifrice), group VI (Combination dentifrice), group IV (Green tea dentifrice), least by group III & V (aloe vera and Azadirachta indica dentifrices), against Lactobacillus acidophilus. At 48 hours, highest mean zone of inhibition was displayed by group I (Terminalia chebula dentifrice), followed by group V (Azadirachta indica dentifrice), group IV (Green tea dentifrice), group II (Acacia catechu dentifrice), group III (Aloe vera dentifrice), group VI (Combination dentifrice) and least by group VII, Colgate total. There was significant increase in the zone of inhibition at 48 hours as compared to 24 hours among all the test dentifrices excluding the positive control. Group I, (Terminalia chebula dentifrice) and group V (Azadirachta indica dentifrice) were highly significant in inhibiting Lactobacillus acidophilus at 48 hours with p value < 0.001.

At 100% concentration, at 24 hours, maximum zone of inhibition was shown by positive control, group VII(Colgate total) followed by, group VI (Combination dentifrice), group IV (Green tea dentifrice), group II (Acacia catechu dentifrice), group III (Aloe vera dentifrice), group I (Terminalia chebula dentifrice) and group V (Azadirachta indica dentifrice) against Lactobacillus acidophilus. At 48 hours, in 100% concentration, maximum zone of inhibition was shown by group III (Aloe vera dentifrice), group II (Acacia catechu dentifrice), group I (Terminalia chebula dentifrice), group VII (Colgate total), group IV (Green tea dentifrice), group VI (Combination dentifrice) and group V (Azadirachta indica dentifrice). All the test dentifrices showed significant increase in the zone of inhibition at 48 hours.

Observation from Table 4

In the intergroup comparison, at 50% concentration, against Lactobacillus acidophilus, there was no significant difference between the groups.

In the intergroup comparison, at 48 hours, group III (Aloe vera dentifrice) showed significant zone of inhibition against Lactobacillus acidophilus when compared to group V (Azadirachta indica dentifrice).

Discussion

The oral cavity harbors numerous microbes with a perpetual warm and moist environment serves as a hot bed for the residence and development of numerous microbial populations.[19] To combat these pathogens, ingredients possessing antimicrobial activity, such as chlorhexidine, triclosan, essential oils, fluorides, and many other herbal

extracts are added in several oral hygiene products including dentifrices. [6,7,19]

Dental plaque formation is a progressive and dynamic process in which, the bacteria are an essential component and the association between the levels of Streptococcus mutans, as well as of Lactobacillus and the presence of caries in humans is well established.[20,21,22] The use of herbal medicines/extracts in dental hygiene products are gaining importance in recent times as many of them are already in use by people in various forms and formulations in their routine lives. They are traditionally accepted, culturally amiable and some of them are easily available with minimal or no adverse effects/side effects. [19]

In the present study, the antimicrobial properties of individual dentifrices were measured by testing the zone of inhibition on the selected bacteria. [19]

The individual studies by Binney A et al (1996) [23] and Xu T et al (2005) [24] uphold the antibacterial and antiplaque efficacy of Colgate Total dentifrice. Hence used as the positive control and sterile distilled water as the negative control in the present study. On in-vitro assay Colgate total dentifrice completely inhibited Streptococcus mutans, however the zone of inhibition for Lactobacillus acidophilus was comparable with the test dentifrices.

Azadirachta indica dentifrice

Neem (Azadirachta indica) plant contains components which can inhibit oral pathogens and possess broad range antibacterial activity. [25,26]

In the present study, Azadirachta indica dentifrice showed zone of inhibition against Streptococcus mutans and Lactobacillus acidophilus which was consistent with the study by Stoeken JE (2007) who demonstrated antimicrobial effects of Neem extract against Streptococcus mutans, Lactobacillus, S. faecalis and other pathogens.[27] The present study result also supports an in vivo study conducted by Sudha Patil and in vitro study by Prashant GM et al in 2007 against Streptococcus mutans.[28] However when compared to the other test dentifrices, Azadirachta indica dentifrice displayed lesser zone of inhibition against Streptococcus mutans which may be because, in our study, we used the extract of Neem leaves which contain cyclic trisulphide and cyclic tetrasulphide, which are potent antifungal.

Terminalia chebula dentifrice

Antimicrobial effects of Terminalia chebula extract have been demonstrated against many pathogens. The plant extract of Terminalia chebula contains tannin as its active compound, which is well recognized for its anti-microbial activity. [29, 30] According to Aneja et al.(2009) in their study, all the tested extracts of Terminalia chebula were highly effective against two of the tested dental caries causing bacteria. [31]

In the present study, Terminalia chebula dentifrice showed antimicrobial activity against Streptococcus mutans and Lactobacillus acidophilus which is in accordance with the study conducted by Jagtap and Carounanidy et al (2007). [32, 33] however, in the current study the aqueous extract of Terminalia chebula was used as an ingredient in the dentifrice, where as in the other studies the extracts were directly tested for their antimicrobial efficacy.[33]

Acacia catechu dentifrice

The main chemical constituents of Acacia catechu are catechin, quarcetin, Taxifolin etc., possesses antifungal, antiviral, antibacterial, anti-inflammatory and anti-oxidant activity.[34] Lakshmi.T et al (2012), in their study revealed that the acacia catechu plant extract were rich in Quercetin (0.070%w/w) and the antioxidant, antimicrobial, anti-inflammatory, anticancer activity is due to the presence of Quercetin. [34]

In the present study, Acacia catechu dentifrice showed the antimicrobial potential against Streptococcus mutans and

Lactobacillus acidophilus which is consistent with the study conducted by Geetha et al in an In-vitro evaluation of anti-bacterial activity of heartwood extract of acacia catechu on oral microbes. [35] The study also supported the study conducted by Pawar (2005) who used a dentifrice herbal tooth powder, the composition of which were Acacia catechu, Menthol and camphor in the proportion of 91%, 2.7% and 6.3% respectively. The herbal tooth powder reported to significant reduction in plaque, gingivitis and dental calculus respectively.

Aloe vera dentifrice

The antibacterial activity of Aloe vera gel is attributed to a number of pharmacologically active compounds including anthraquinones, aloin, aloe-emodin. [36,37,38] These active molecules inhibit protein synthesis by bacterial cells, thus explaining their antimicrobial activity. [39,40]

The present study showed the antimicrobial activity of aloe vera dentifrice against Streptococcus mutans and Lactobacillus acidophilus which is consistent with the findings of Lee et al. [10] in 2004. However, Lee et al. did not test the antimicrobial efficacy against Lactobacillus acidophilus. The present study result was also consistent with the study reported by Kambizi L (2008) [41], in which Aloe vera gel exerted strong bactericidal activity against both cariogenic and periodontopathic bacteria, and indicated that S. mutans was highly sensitive to Aloe vera gel. [10,41]

Green tea dentifrice

The main polyphenols in green tea are catechins, which are found to inhibit Streptococcus mutans and Streptococcus sobrinus. [42,43]

Otake et al. studied the anti-caries effect of green tea polyphenols in-vivo and reported that polyphenolic compounds present in green tea possess high inhibitory effect against S. mutans bacteria growth and acid produced from it. [44]

The current study result regarding the green tea dentifrice was consistent with the findings of Rasheed and Haider (1998) [45] and the study by Tsuchiya et al. who reported that green tea extract possess antibacterial activity against cariogenic bacteria, attributed to presence of catechins in saliva after rinsing. [46]

In the present in vitro study, Colgate total completely inhibited Streptococcus mutans in 50% and 100% concentration, Combination dentifrice and Acacia catechu dentifrice showed higher efficiency in inhibiting Streptococcus mutans. The higher efficiency of Combination dentifrice and Acacia catechu dentifrice can be attributed to the amount of catechins present.

In our study, Terminalia chebula dentifrice, Acacia catechu dentifrice, Green tea dentifrice and Combination dentifrices were better than Aloe vera dentifrice and Azadirachta indica dentifrice in inhibiting Streptococcus mutans. The higher efficiency Terminalia chebula dentifrice may be due to the “Tannins”, which is the active compound, a well-recognized anti-microbial.[29,30] The acacia catechu dentifrice and Green tea dentifrice efficiency may be attributed to the catechins which are found to be inhibitory against Streptococcus species.[43]

At 50 % concentration, against Lactobacillus acidophilus all the tested herbal dentifrices showed better efficiency than positive control, Colgate total at 48 hours. Terminalia chebula dentifrice showed maximum inhibition of Lactobacillus acidophilus at 50% concentration at the end of 48 hours. However, at 100% concentration, at 24 hours, maximum zone of inhibition was shown by positive control, Colgate total against Lactobacillus acidophilus. At 48 hours, maximum zone of inhibition was shown by Aloe vera dentifrice in full strength.

Therefore the toothpastes having the largest inhibition zone and thus have the strong antimicrobial properties may not be necessarily superior to those showing small

inhibition zones or no inhibition zone. [47] Because when used in-vivo the saliva dilutes toothpastes, the level to which the antimicrobial activity are buffered or lost in dilution in-vivo is of interest.

Conclusion

Incorporation of aqueous extracts of herbs in the dentifrices, (Terminalia chebula dentifrice, Acacia catechu dentifrice, Aloe vera dentifrice, Green tea dentifrice, Azadirachta indica dentifrice and the Combination dentifrice) showed significant antimicrobial properties against Streptococcus mutans and Lactobacillus acidophilus

Although positive control completely inhibited Streptococcus mutans in 50% and 100% concentration, Combination dentifrice and Acacia catechu dentifrice showed higher efficiency in inhibiting Streptococcus mutans

Terminalia chebula dentifrice, Acacia catechu dentifrice, Green tea dentifrice and Combination dentifrices were better than Aloe vera dentifrice and Azadirachta indica dentifrice in inhibiting Streptococcus mutans

In 50 % concentration, at 48 hours, all the tested herbal dentifrices showed better efficiency than positive control, against Lactobacillus acidophilus. Terminalia chebula dentifrice showed maximum inhibition of Lactobacillus acidophilus at 48 hours.

The maximum amount of antibacterial activity at full strength was shown by positive control at the end of a day and Aloe vera dentifrice at the end of two days against Lactobacillus acidophilus.

Overall, the findings of the present study suggested that the tested herbal dentifrices may be an alternative to conventional dentifrices in the maintenance of oral hygiene and hence in the management of dental caries. Further research efforts are needed to establish guidelines to ensure the efficacy and safety of herbal dentifrices tested.

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Legend Tables

Table 1: The effect of dentifrices at 50% concentration (1:1 dilution) and 100% concentration (full strength) on Streptococcus mutans at 24 hours and 48 hours.

Groups	at 50 % concentration (1:1 dilution)			at 100% concentration (full strength)		
	24 hours Mean ± SD	48 hours Mean ± SD	t-test*	24 hours Mean ± SD	48 hours Mean ± SD	t-test*
I (TC)	14.8 ± 1.9	15.0 ± 1.8	t = 1.00 p = 0.36	16.2 ± 1.2	16.7±1.0	t = 2.24 p = 0.36
II(AC)	15.7 ± 0.5	15.7 ± 0.5	t =0.00 p =1.00	17.2± 1.0	17.2 ±1.0	t =0.00 p =1.00
III(AV)	10.7 ± 2.3	10.8 ± 2.3	t =1.00 p =0.37	12.7 ± 0.8	12.7± 0.8	t =0.00 p =1.00
IV(GT)	13.8 ± 1.2	13.8 ± 1.2	t =0.00 p =1.00	15.3 ± 0.8	15.3 ± 0.3	t =0.00 p =1.00
V(AI)	9.7 ± 1.8	9.8 ± 1.7	t =1.00 p =0.36	12.8 ± 0.4	13.0 ± 0.6	t =1.00 p =0.36
VI (Com)	15.8 ± 1.0	15.8 ± 1.0	t =0.00 p =1.00	17.2 ± 0.4	17.2 =/- 0.4	t =0.00 p =1.00
ANOVA**	F = 16.77, **p < 0.01**	F = 16.84, **p < 0.01**		F = 37.27, **p < 0.01**	F = 38.54, **p < 0.01**	

* Student's paired t test **One way ANOVA test

Table 2: The inter-group comparison for Streptococcus mutans at 50 % concentration (1:1 dilution) and 100% concentration (full strength) at 24 hours and 48 hours

	GROUPS	at 50 % concentration (1:1 dilution)		at 100% concentration (full strength)	
		24 hours	48 hours	24 hours	48 hours
		ANOVA F =16.77, **p < 0.01**	ANOVA F =16.84, **p < 0.01**	ANOVA F = 37.27, **p < 0.01**	ANOVA F = 38.54, **p < 0.01**
*** INTERGROUP COMPARISONS (p – VALUES)	I – II	-	-	-	-
	I – III	< 0.01, S	<0.01,S	< 0.01, S	<0.01,S
	I – IV	-	-	-	-
	I – V	< 0.01, S	< 0.01,S	< 0.01, S	< 0.01,S
	I – VI	-	-	-	-
	II – III	<0.01, S	<0.01,S	<0.01, S	<0.01,S
	II – IV	-	-	<0.05, S	<0.05,S
	II – V	<0.01, S	<0.01,S	<0.01, S	<0.01,S
	II – VI	-	<0.01,S	-	-
	III – IV	< 0.05, S	<0.05,S	< 0.01, S	
	III – V	-	-	-	-
	III – VI	<0.01, S	<0.01,S	<0.01, S	<0.01,S
	IV – V	< 0.01, S	<0.01,S	< 0.01, S	<0.01,S
	IV – VI	-	-	<0.05, S	<0.05,S
	V - VI	<0.01, S	<0.01,S	<0.01, S	<0.01,S

*** Tukey's post hoc test

Table 3: The effect of dentifrices at 50 % concentration (1:1 dilution) and 100% concentration (full strength) on Lactobacillus acidophilus at 24 hours and 48 hours.

Groups	at 50 % concentration (1:1 dilution)			at 100% concentration (full strength)		
	24 hours Mean ± SD	**48 hours Mean ± SD**	**t-test***	**24 hours Mean ± SD**	**48 hours Mean ± SD**	**t-test***
I (TC)	13.2 ± 1.9	21.5 ± 1.8	t =14.94 **p < 0.001**	12.3 ± 1.0	17 ± 1.9	t = 6.87 **p < 0.01**
II(AC)	12.2 ± 0.4	17.7 ± 4.0	t = 3.42 **p < 0.05**	13.3 ± 0.8	18.2 ±2.4	t = 4.14 **p < 0.01**
III(AV)	11.7 ± 0.5	17.3 ± 1.9	t = 7.93 **p < 0.01**	12.5 ± 1.0	19.5 ± 3.8	t = 4.65 **p < 0.01**
IV(GT)	11.8 ± 1.0	18.5 ± 2.9	t = 6.32 **p < 0.01**	13.7 ± 1.8	16.3 ± 1.2	t = 6.32 **p < 0.01**
V(AI)	11.7 ± 2.3	19.0 ± 2.4	t =22.0 **p < 0.001**	12.2 ± 1.5	15.5 ±2.1	t = 3.07 **p < 0.05**
VI (Com)	12.0 ± 1.1	17.0 ± 2.2	t = 5.37 **p < 0.01**	14.2 ±1.6	16.0 ± 0.9	t = 3.05 **p < 0.05**
ANOVA**	F = 1.01, p < 0.43, NS	F = 2.35, p < 0.07, NS		F = 2.24, p < 0.08, NS	F = 2.67, **p < 0.05**, S	

* Student's paired t test **One way ANOVA test

Table 4: The inter-group comparison for Lactobacillus acidophilus at 50 % concentration (1:1 dilution) and 100% concentration (full strength) at 24 hours and 48 hours.

	GROUPS	at 50 % concentration (1:1 dilution)		at 100% concentration (full strength)	
		24 hours	**48 hours**	**24 hours**	**48 hours**
		ANOVA F =1.01, p < 0.43, NS	ANOVA F= 2.35, p < 0.07, NS	ANOVA F = 2.24, p < 0.08, NS	ANOVA F = 2.67, p < 0.05, S
*** **INTERGROUP COMPARISONS (p – VALUES)**	I – II	-	-	-	-
	I – III	-	-	-	-
	I – IV	-	-	-	-
	I – V	-	-	-	-
	I – VI	-	-	-	-
	II – III	-	-	-	-
	II – IV	-	-	-	-
	II – V	-	-	-	-
	II – VI	-	-	-	-
	III – IV	-	-	-	-
	III – V	-	-	-	**< 0.05**, S
	III – VI	-	-	-	-
	IV – V	-	-	-	-
	IV – VI	-	-	-	-
	V - VI	-	-	-	-

*** Tukey's post hoc test