International Journal Dental and Medical Sciences Research
Volume 4, Issue 3, May-June 2022 pp 261-266 www.ijdmsrjournal.com ISSN: 2582-6018

Anti Microbial Activity of Commercial Lyavailable Five Ayurvedic Dentifrices

[1]SahilVashisht, [2]Dolma Borah
[1]*Post graduate –Subharti Dental Hospital, Meerut*
[2]*Intern,ITS–CollegeofDentalSciencesandResearch,Muradnagar,Dist.Ghaziabad*

Submitted: 15-05-2022 Accepted: 25-05-2022

ABSTRACT
Background: Dental caries is one of the most leading cause of oral diseases among the globe, numerous dentifrices andmouthwashes come to the market for the remedy. Thus, more research arefocussed on natural system of medicine like Ayurvedaand its use for curbing oral health problems. **Aim and Objectives:** To assess the antimicrobial activity of commercially availablefive dentifrices against streptococcus mutans. **Materials and method:** For the present study we used strains of micro-organisms-StreptococcusmutantsStrain-890from MTCC.Torevive thestrainsBrainHeartInfusionAgar(BHIagarmedia) wasused. Double blinding was done and samples were coded with alphabets to avoid examiner bias. The plates were kept inincubation chamber for 24 hours. After 24 hours, 48 hours, 72 hours The zone of inhibition was measured with the help of HiAntibioticZonescalefromHiMediaLaboratoriesLimited,Mumbai.**ObservationsandResults:**Afterassessingtheantimicrobial activity for 24 H, 48 H and 72 H, DantKanti was found to be most effective with the highest zone of inhibition28.16±0.75, followed by Meswak. Whereas, Daburlal and Himalaya had the least effect when compared to their counterparts.**Conclusion:** The result of the current study demonstrated that Dantikanti has the maximum antimicrobial effect against S.mutansfollowed by Meswak and Babol, remaining dentifrices like Himalaya and Daburlal had the least effect against the microbes.Thus, when compared with all other commercially available ayurvedic dentifrices Dantikanti proved to be more effective at 24hours, 48hours and72 hours among allothers.
Keywords: Ayurvedic dentifrices,S.mutans,MTC-890

I. INTRODUCTION

Oralcavityofhumansconsistsoflargenumberofgrampositive and gram negative microorganisms. Several studieshave shown that oral cavity act as aparadise with its warm andmoist environment, growth, proliferation and multiplication. Abiofilm inside the oral cavity is responsible for caries activity,gingivitisandperiodontitis.Theepidemiological studiesevidentlyreflectanoticeableincreaseintheprevalenceofdentalcariesinmanydevelopedanddevelopingcountries.Streptococcusmutantsisoneofthemain opportunisticpathogens of dental caries. The pathogens of dental caries, playacentralroleinfermentingcarbohydrates resulting inacidproduction, and leading to the demineralization of the toothenamel(Ajithkrishnanetal.,2014;Chhina,2016). theproblemofdentalcariescanbepreventedbyvarious documentedmethodsofplaquecontrolwhichmaybechemical,mechanicaloracombinationofboth.Mostcommonly used method is use of dentifrices along with properbrushing techniques. Nowadays a wide range of dentifrices areavailableinthemarketformaintaininggoodoralhygieneusing various mechanisms. Similarly various toothbrushes areavailabletosynergisetheeffectofthesedentifricesforefficientremovalofplaqueanddebris.Alsothereisaparadigm shiftintheuse of herbal and ayurvedic productsrecently, and the oral hygiene products are not an exception toit. The market is flooded with herbal dentifrices even from bigbrands. Many studies have justified the use of antimicrobialchemotherapeutic agents as a means of reducing the levels oforalbacteria,specificallyStreptococcusmutants.Thoughmany herbal dentifrices claim to have antimicrobial propertiessimilartothatofotherchemotherapeuticagentsbutifwesearchtheliteratureverylittleresearchhasbeenconductedtoprove the same. Hence, the present study was undertaken toinvestigateantimicrobialefficacyofdifferentherbaltoothpastesagainstS.mutansbyusingstandardagarwell diffusion method (Bafna, 2015; Latiyanet al., 2017; Bafnaetal., 2015).

Aim and Objectives: To assess the antimicrobial activity

International Journal Dental and Medical Sciences Research
Volume 4, Issue 3, May-June 2022 pp 261-266 www.ijdmsrjournal.com ISSN: 2582-6018

ofcommerciallyavailableayurvedicdentifricesagainst thestreptococcus mutans.

II. MATERIALSANDMETHODS

This in-vitro study was conducted to evaluate and compare theefficacy of herbal toothpastes in reducing the colonization ofstreptococcus mutanscommerciallyavailableherbal toothpasteswere taken and to blind foldthe study, they were labelled as A,B, C, D, E to minimize the observers bias and increase theinternalvalidity of thestudy. Sampleswerecollectedfromthem and their efficacy against S.mutans was evaluated usingmodified agar well disk diffusion method. Standard protocol ofdilution was used for each of them, each dilution was made byadding3mlofdistilledwaterin3gmoftoothpaste.

Bacteriastrains: Forthe presentstudyweusedstrainsofmicro-organisms-StreptococcusmutantsStrain-890fromMTCC. To revive the strains Brain Heart Infusion Agar (BHIagar media) was used. The inoculation of S.mutans in liquidmedium(BHImedia)fromitssinglecolonywasdonetoensure its best growth at 37°C in peptide rich conditions. Allthese steps were performed using sterile technique to avoidcontamination. Using a sterile, disposable inoculating loop orwooden stick, one colony was picked from tube containingviable S.mutanscolonies. The colony was suspended in liquidBHI medium in a sterile 15 ml screw cap conical tube. Cap ontube was tightened to prevent release of CO_2 during growth.Volume was close to maximum of tube to reduce the availableair space. Since the tube was sealed, culture was placed in non-CO_2(ambientair)incubator.Incubationwas donestatically centresweremarked.Byditchmethodusingsterilegelpuncherwasused to cut 3wells equidistant from each otherand was filled with different toothpaste samples (2 ml) withmicropipette.Theplateswerekeptinincubationchamberfor 24hours. After24hours, 48hours, 72hours The zone ofinhibition was measured with the help of Hi Antibiotic Zonescale from Hi Media Laboratories Limited, Mumbai, which iscertifiedtoInternationalStandardsOrganizationand WorldHealthOrganization(WHO)GoodManufacturingPractice.[7-10]

Statisticalanalysis: TheresultswereenteredintotheMicrosoftexcel2010fordataanalysisandinterpretation. Descriptive statistics (Mean and S.D) were calculated and oneway ANNOVA analysis with Bonferroni correction was doneto analyse mean difference among the five dentifrices usingIBMSPSSVersion23.

III. RESULTS

Table2representsthezonesofmicrobialinhibitiondemonstrated by the five commercially available dentifrices atfullstrengthagainsttheS.mutanswithmeanzoneofinhibition. DantKanti and Meswakrepresents the highest meandiameteragainstS.mutansof27.83±1.16and23.58±1.96respectively and Himalaya had the lowest zone of inhibition of7.83±0.75 at24hours.

Table1.Samplewithactiveingredients

Label	Sample	ActiveIngredient
A	Himalaya(TotalSensitive)	Triphala
B	DantKanti(Patanjali)	Neem,Clove,Pudina&Babool
C	Meswak	Calciumcarbonate &Sorbitol
D	Babool	Lavangeoil,Pudina,Camphor
E	DabarLal	Cloveoil, Pudina,Ginger

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Table.2 Descriptivestatisticsformeanzoneofinhibition

		N	Mean±Std.Deviation
24 Hours	Himalaya	6	7.83±0.75
	DantKanti	6	27.83±1.16
	Meswak	6	23.58±1.96
	Babool	6	13.83±0.75
	Dabarlal	6	8.00±0.63
48 Hours	Himalaya	6	8.00±0.63
	DantKanti	6	28.16±0.75
	Meswak	6	23.58±1.96
	Babool	6	13.83±0.75
	Dabarlal	6	8.00±0.63
72 Hours	Himalaya	6	8.00±0.63
	DantKanti	6	28.16±0.75
	Meswak	6	23.58±1.96
	Babool	6	13.83±0.75
	Dabarlal	6	8.00±0.63

overnightat37°C.Culturewascheckedunderalightmicroscopeat40×magnification forpresenceof S.mutans.The method used was by modified agar well disk diffusionmethod. This methodwas used because it is gold standard forin vitro study of anti-microbial properties of specific culture.Thismethodiswidelyusedinotherstudiesandresearchrelated for anti-microbial effects in specific culture (Chhina,2016;Bafna, 2015;Bafnaetal., 2015;Singhetal., 2014).

Antimicrobial Assay: Brain heart infusion agar media wasfixedin the laband then pouredintothesterilestreak platesand was incubated for 1 hour at 37'C until it gets solidifies.TesttubecontainingStreptococcusmutants wasopenedand diluted in 1:1 ratio with distilled water. For the present studyweusedstrainsofmicro-organisms-Streptococcusmutants[MTCC-890].Theorganismswereidentifiedbystandardmicrobiological techniques including colonial characteristics,morphological characteristics and biochemical characteristics.Whole procedure was done under horizontal laminar flow hoodformaking the condition sterile and prevent contamination.Using a sterile cotton swab, Streptococcus mutants was spreadover the media and was cultured in brain heart infusion agarand incubated at 37'c for 48 hours. The toothpaste was dilutedwithpathogenicfreedistilledwaterbymixing3gmoftoothpaste with 3ml of distilledwater. Totally 15 petridishes(3 each for 5 dentifrices) were prepared and Streak plates weredividedinto3equalparts(equalinsurfacearea)and their

Similarly, at 48 hours and 72 hours, DantKanti and meswakhadthehighestzoneofinhibitioncomparedtootherdentifrices. The maximum zone of inhibition was demonstratedat 48 hours by Dantikanti (28.16±0.75) which was unchangedtill 72 hours. Comparison of different dentifrices using one-way analysis of variance (ANOVA) showed that the differenceinmicrobialinhibitionwassignificantforallthefivedentifrices against the S. mutans which can be inferred fromTable3.Tables4indicatestheMultiplecomparisonsusingBonferronicorrectionat24,48and72hoursrepresentsstatistically significant difference in mean zone of inhibitioncomparedtoothers,exceptfortheDaburLal–Himalayagroup (p=0.999,p=1.000).

Table3. One– wayannovaanalysisfor meancomparison

		F	p-value	Sig.
24 Hours	Between Groups Within GroupsTotal	370.111	.000	S
48 Hours	Between Groups Within GroupsTotal	438.608	.000	S

72 Hours	Between Groups	438.608	.000	S
	Within GroupsTotal			

Bonferroni correction used for mean comparison between the different dentifrices. The mean differenceis significantat ≤0.05.S:Significant;NS:Nonsignificant

Table4.Multiplecomparisonusingbonferronicorrectionfor meancomparison

DependentVariable	Dentifrice	Dentifrice	P-value	Sig.
24Hours	Himalaya	DantKanti	.000	S
		Meswak	.000	S
		Babool	.000	S
		Dabarlal	.999	NS
	DantKanti	Himalaya	.000	S
		Meswak	.000	S
		Babool	.000	S
		Dabarlal	.000	S
	Meswak	Himalaya	.000	S
		DantKanti	.000	S
		Babool	.000	S
		Dabarlal	.000	S
	Babool	Himalaya	.000	S
		DantKanti	.000	S
		Meswak	.000	S
		Dabarlal	.000	S
	Dabarlal	Himalaya	.999	NS
		DantKanti	.000	S
		Meswak	.000	S
		Babool	.000	S
48Hours	Himalaya	DantKanti	.000	S
		Meswak	.000	S
		Babool	.000	S
		Dabarlal	1.000	NS
	DantKanti	Himalaya	.000	S
		Meswak	.000	S
		Babool	.000	S
		Dabarlal	.000	S
	Meswak	Himalaya	.000	S
		DantKanti	.000	S
		Babool	.000	S
		Dabarlal	.000	S
	Babool	Himalaya	.000	S
		DantKanti	.000	S
		Meswak	.000	S
		Dabarlal	.000	S
	Dabarlal	Himalaya	1.000	NS
		DantKanti	.000	S
		Meswak	.000	S
		Babool	.000	S
72Hours	Himalaya	DantKanti	.000	S
		Meswak	.000	S
		Babool	.000	S
		Dabarlal	1.000	NS
	DantKanti	Himalaya	.000	S
		Meswak	.000	S
		Babool	.000	S
		Dabarlal	.000	S

Meswak	Himalaya	.000	S
	DantKanti	.000	S
	Babool	.000	S
	Dabarlal	.000	S
Babool	Himalaya	.000	S
	DantKanti	.000	S
	Meswak	.000	S
	Dabarlal	.000	S
Dabarlal	Himalaya	1.000	NS
	DantKanti	.000	S
	Meswak	.000	S
	Babool	.000	S

Bonferroni correction used for mean comparison between the different dentifrices.Themeandifference issignificantat≤0.05. S:Significant;NS:Nonsignificant
Thustherewassignificantdifferenceamongthemeanofallthegroupsandhighestwasreportedfor Dantkanti.

IV. DISCUSSION

The current research was carried out to assess the antimicrobialactivity of commercially available five ayurvedic dentifrices toassess the effectiveness against the S. mutans. According to theresults,DantiKantihasshowedthemaximumzoneofinhibition with the mean diameter of 28.16±0.75 at 72 hours,whereasbabool,daburlalandHimalayashowed minimalchangesinthezone of inhibition.theactivecomponentofDantKanti(neem),havedemonstratedtoexhibitimmunomodulatory,anti-inflammatory,antihyperglycaemic,antiulcer,antimalarial,antifungal,antibacterial,antiviral,antioxidant, antimutagenic and anticarcinogenic properties intheliterature.Menthol($C_{10}H_{20}O$),theactiveconstituentpresentinPudina,perhapsislargelyresponsiblefortheherapeutic potentials of Pudina. It is used to treat liver andspleendiseases,asthmaandjaundice.Theoilisantiseptic,carminative, refrigerant, stimulant and diuretic. Clove oil hasLongley beenuseddirectlytothegumstoeasetoothache.There is evidence that the eugenol in clove oil is effective atfightingseveralknownoralbacteria.Medicinescontaningeugenolarewidelyusedindentistryandsomeresearchsuggests that clove gel may reduce the pain of needle insertionindentistry.Thus,therecanbeasynergisticeffectofthecombinationofclove,neem,baboolandpudinawhichispresentintheDantKanti.Researchcarriedoutbyvariousinvestigators have also demonstrated that they have significantantimicrobial activity and also reported reduction in plaque andimproved periodontal health. In modern India, with the adventof research activities into the naturopathy system of medicinehaselaboratedthebeneficialeffectoftheseherbsinoralhealth. These components are also used in themouthwashesfor the analysis of the antimicrobial property and it has provedto be effective against S.mutans and C.albicans (Singh, 2018;Tomar, 2018;Jyoti,2018;Singh,2014;Shah, 2018).

V. CONCLUSION

The result of the current study demonstrated thatDantikantihasthemaximumantimicrobialeffectagainstS.mutansfollowedbyMeswakandBabol,remainingdentifriceslikeHimalayaandDaburlalhadtheleasteffectagainstthemicrobes. Thus, when compared with all other commerciallyavailable ayurvedic dentifrices Dantikanti proved to be moreeffectiveat24 hours,48 hoursand72 hoursamongallothers.

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