Bactericidal Effect of 18 Popular Proprietary and Traditional Tooth-Care Agents, in Rural, Outskirts and Urban West Bengal, on Pure Non-Oral and Undefined Mixed Oro-Dental Culture.

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Abstract

The dental surface and its various crevices are a hotbed for billions of diverse microbial population, necessitating toothpaste to be a unique antibiotic, acting as specific bactericides for the pathogens yet providing broad-spectrum coverage to suppress uncontrolled growth of the normal micro- inhabitants. This pilot study analyzed the antimicrobial efficacy of 9 proprietary, 2 indigenous toothpastes, 4 vintage natural tooth-care products (twig and leaves of Neem, Guava leaves, cow-dung ash), 2 plant- derived traditional pain-relief agents (seora leaves, tobacco), and banana tree skin ash (insecticide, germicide and potential antimicrobial) against an oro-dental non-definite microbial population, and two non-oral pure cultures.

Introduction

Regular oro-dental healthcare is an integral part of our personal hygiene, function and appearance, which includes scrubbing teeth with proprietary tooth pastes and traditional dental care agent (henceforth termed DCA). Most toothpastes ingredients promise comprehensive dental healthcare, particularly protection against cavity, dental plaque and build-up of the pathogenic dental microbial load [1-3]; while others (muktahansi, lava) contain nothing but crushed wood, cow-dung ash (CDA) and a few herbs.

The oro-dental surfaces and crevices form separate subsections within the micro-ecosystem along our dentures, where a plethora of microbes flourish and protect themselves further by forming micro-colonies that grow into plaques [4-6]. The constant warmth, moisture, nutrient- rich

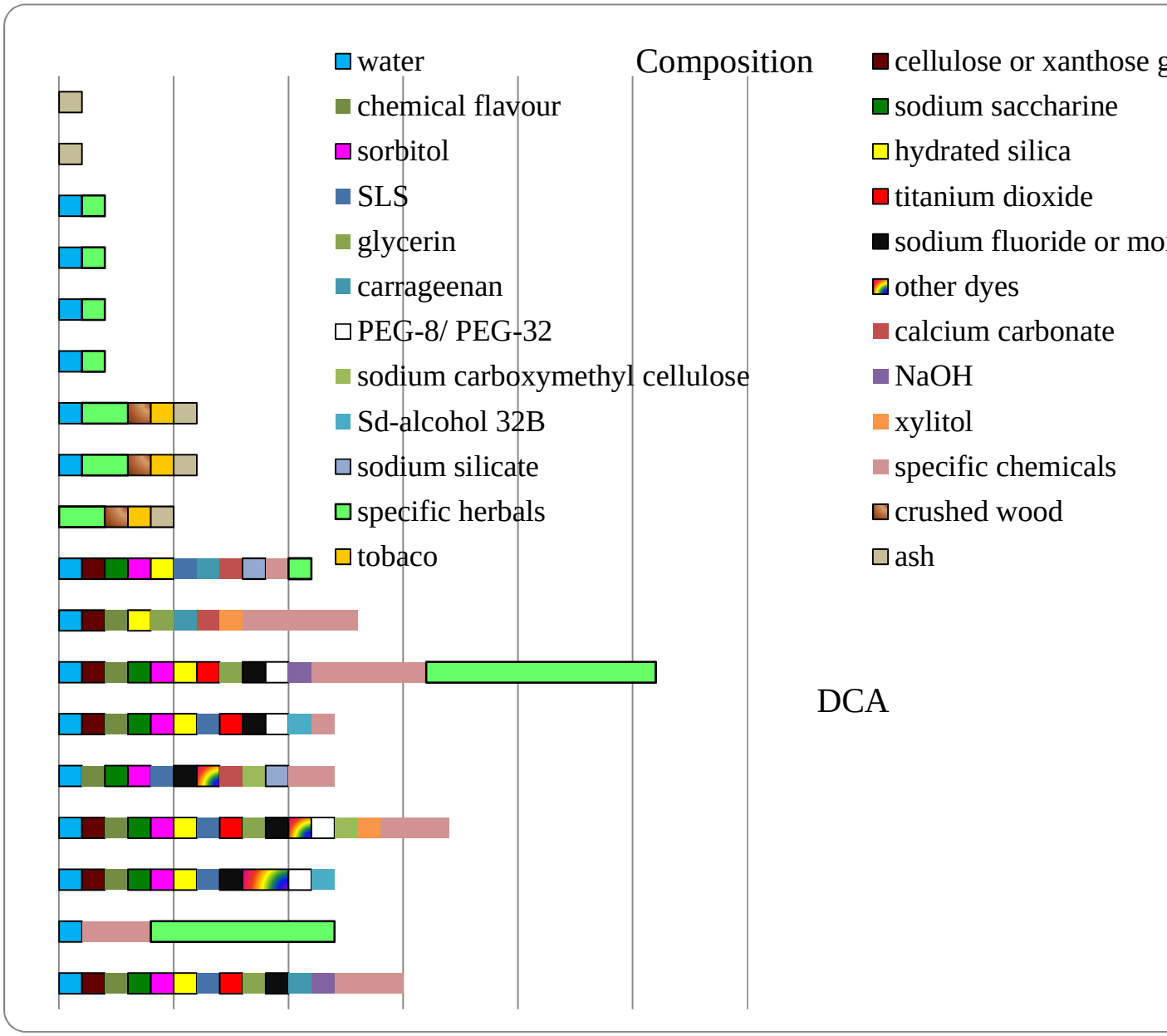
environment with quasi-distinct pockets of facultative- anaerobic, aerobic and anaerobic niches inside the mouth makes it the perfect natural reservoir and growth media for billions of necessary commensals, and opportunistic pathogens [5].This is important, as controlling and sustaining the growth of normal microbiota, while inhibiting the pathogens is the mainstay of maintaining good dental hygiene, which is closely associated with various systemic homeostasis [7]. A good DCA must therefore balance broad spectrum antimicrobial coverage with specific bactericidal properties.

Previous studies of the same genre focused on pure culture of oral commensals or pathogens where the effect of inter-species competitive exclusion, amensalism, commensalism and other mutualisms are excluded [5]; and can't be extrapolated into the practical scenario, where the growth of any one microbe is dependent on or improved upon or inhibited by interaction with other neighboring micro-inhabitants. Such assay thus necessitates a proper test population mimicking at least a fraction of the complex interactions in the diverse microbial ecology present in mouth. We compared the bactericidal efficacy of 18 popular DCA (*vide* Chart-1), against such non-definite mixed oro-dental culture. *E.coli* is chosen as non-oral gram negative control commensal, colonizing the intestine, which may cause some nosocomial oral infection treated based on susceptibility tests [8]. *Bacillus subtilis*, commonly used for testing efficacy for sterilization techniques [6], was our non-oral gram positive control.

Chart-1: Chart-1 cataloged the 18 dental care agents (DCA) chosen for the project and their common ingredients

1. Colgate Total
2. Dabur Red
3. Close-up
4. Amway Glister
5. Cibaca
6. Pepsodent Germicheck
7. Sensodyne
8. Himalaya

9. Babool
10. Tobaco
11. Muktahanshi
12. Lava
13. Guava leaf extract (GLE)
14. Neem leaf extract (NLE)
15. Neem twig extract (TE)
16. Seora Leaf extract (SLE)
17. Cow-dung ash CDA
18. Banana tree skin ash or basna



Methods

Field survey: Random participants (279 male, 286 females) of rural, urbun and outskirts of south eastern West Bengal, were asked close-ended questions on their practiced dental healthcare regime, and related subjects. 20% were aged below 20, 45% were 20-40 years, 25% 41-60 years, <7% 60-70 years, <1% were older than 81. Most (43%) were student, 18% housewife or self-employed, 23% professionals, 5% were educated people of older generation or of lower economic strata, rest 5% were farmers, laborers, hawkers, etc. Product monographs were used as data source for ingredients of the DCA (*vide* Chart-1).

Sample preparation:

Based on survey 12 toothpastes and 6 natural DCA were collected (*vide* Chart-1). Aqueous solutions of the toothpastes (4 mg/ml) were marked C1. The leaves and twig were washed, sundried, ground to powder, autoclaved. After testing solubility in single and double distilled water (ddw), acetone, 50% ethanol, 70% ethanol, methanol, chloroform; 10 ml aqueous (ddw) extracts were prepared from 1g powder, and marked C1. Cleaned, sundried skin of banana tree and cow-dung cake were burned to ashes in closed container, autoclaved; 1g was emulsified in 10ml rice oil, mustered oil, soyabeen oil, peanut oil, olive oil, sunflower oil. Most stable emulsion with least antimicrobial effect, (in mustard oil) was marked C1. Serial dilutions made from C1 were marked C2(1/2-fold), C3(1/4-fold) and C4(1/8-fold).

Oro-dental Microbe collection.

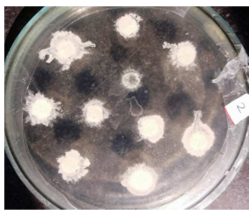
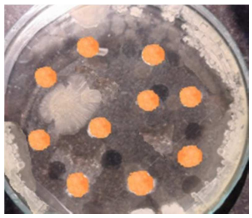
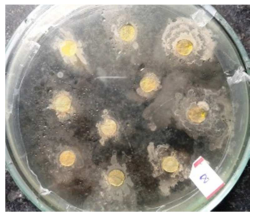
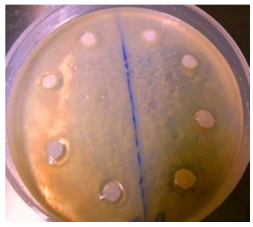
6 young-adult subjects aged 20-23 of both genders having healthy periodontia, without clinical signs of oral mucosal lesions, gum inflammation, caries, severe halitosis and antibiotic protection for the last 6 months, were asked to brush with tap-water, for consecutive two days. On the third day before brushing, microbiological samples from facial and lingular surfaces of peridontia, occlusal surfaces of the molars, crevices between the anterior teeth from both upper and lower dental arch were collected using sterile cotton swabs.

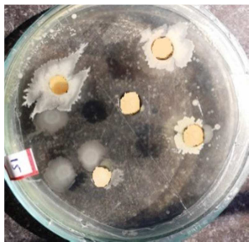
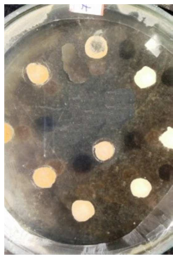
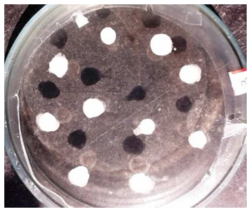
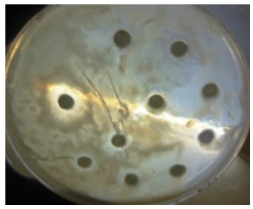
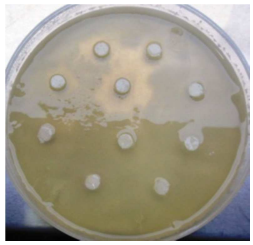
Culture and Susceptibility Test:

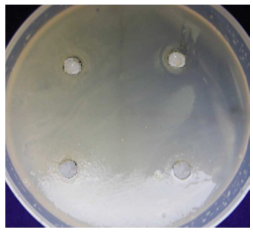
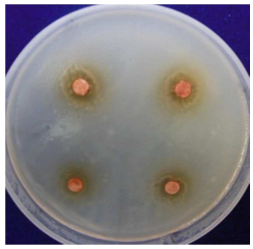
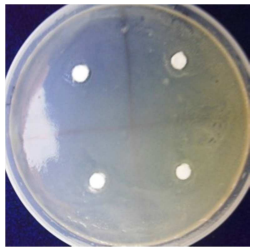
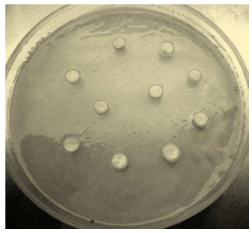
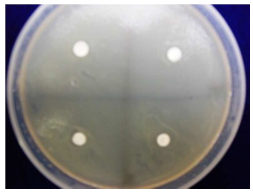
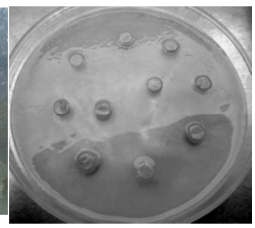
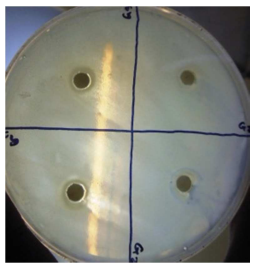
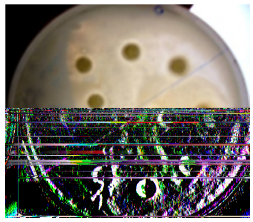
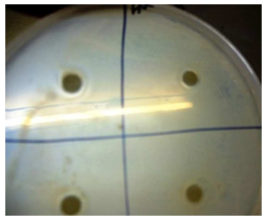
5ml LB media (1%tryptone, 0.5%Yeast extract, 0.5%NaCl), inoculated with

bacterial sample were incubated at 37˚C, pH 7.2 (*E.coli*, *B.subtillis*) and at 36˚C, pH 6.2 (oro-dental sample) for 24 hours after optimization at 570, 590, 600 and 610nm at graded temperature (35˚C, 36˚C, 37˚C, 38˚C), pH (6.2, 6.7, 7.2, 8.2) in both normal and candle jar set-up. Seeding volumes for bacterial lawn were optimized at 300 µl, 500µl and 400µl respectively. Disc diffusion were conducted with autoclaved 6mm Whatmann filter discs soaked with 18 DCA of C1-C4, along with sterile mustard oil and ddw as vehicle controls(*vide* Tables-1, 2). To account for the mixed nature of oro-dental bacterial sample, same sample in multiple discs were placed per plate; zone of clearance was noted in the fraction of clear area per petri-plate using ImageJ 1.46r (Wayne Rasband, National Institute of Health, USA, 2013), and compared with Student's 2-tailed paired t test at $P<0.05$. All tests were done in triplicates. Results were shown in form of representative images of disc diffusion test results.

Table-1 : Susceptibility test results for 18 DCA in 4 graded concentrations (with vehicle control), on dental bacteria. The uneven zones of clearances and profuse resistant colonies are due to mixed nature of the master culture

DCA		C1	C2	C3- left , C4- right
1	Colgate Total			
2	Dabur Red			

3	Close-up			
4	Amway Glister			
5	Cibaca			
6	Pepsodent Germicheck			
7	Sensodyne			
8	Himalaya			

9	Babool			
10	Tobaco			
11	Mukta-hanshi			
12	Lava			
13	Guava leaf extract (GLE)			
14	Neem leaf extract (NLE)			

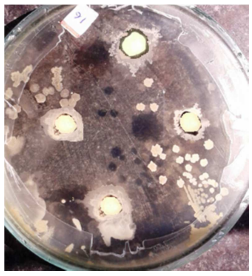
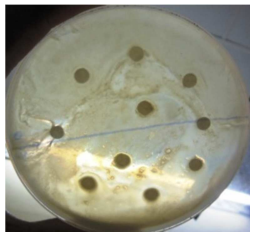
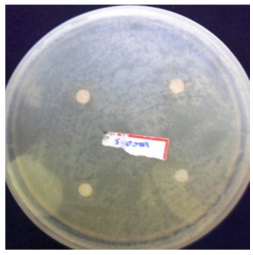
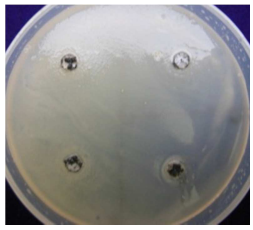
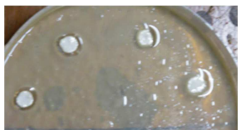
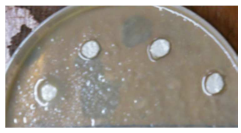
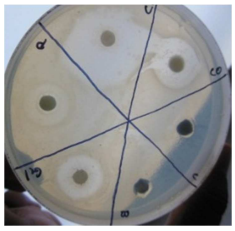
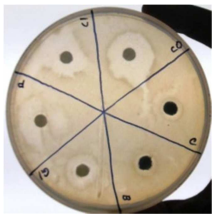
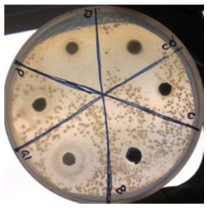
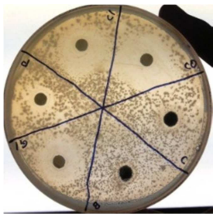
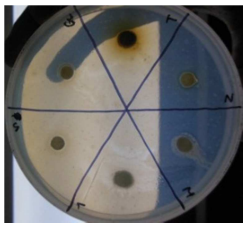
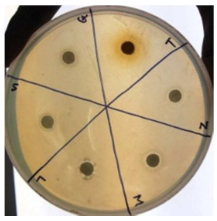
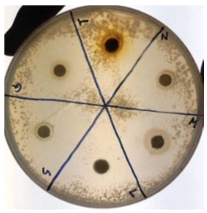
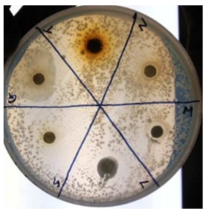
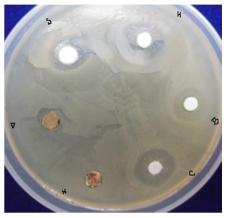
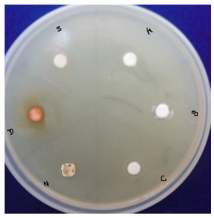
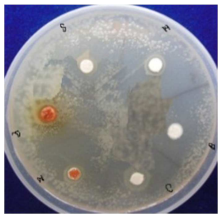
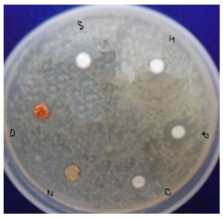
15	Neem twig extract (TE)			
16	Seora Leaf extract (SLE)			
17	CDA			
18	Banana tree skin ash (“basna”)			
Vehicle control		 →mustard oil		 →ddw

Table-2: Antimicrobial activities of 18 DCA in graded dose, on *E.coli* and *B.subtilis*. Only C3, C4 are shown, as at higher concentration the zones of clearances often overlapped

<table>
<tr><td colspan="2">E.coli</td><td colspan="2">B.subtilis</td></tr>
<tr><td colspan="4">Set 1: (counter-clockwise from bottom middle–right): black discs for basna-B (negligible effect) & CDA-C (slightly better); Colgate-Co, Close-up-Cl, Pepsodent-P, and Glister-Gl (good effect).</td></tr>
<tr><td>  </td><td>  </td><td>  </td><td>  </td></tr>
<tr><td>C3</td><td>C4</td><td>C3</td><td>C4</td></tr>
<tr><td colspan="4">Set 2: (counter-clockwise from top-center): vermilion disc with spreading for tobacco-T, followed by GLE-G, SLE-S, Lava-L, Muktahanshi-M, NLE-N suppress B.subtilis more than E.coli.</td></tr>
<tr><td>  </td><td>  </td><td>  </td><td>  </td></tr>
<tr><td>C3</td><td>C4</td><td>C3</td><td>C4</td></tr>
<tr><td colspan="4">Set 3: (counter-clock wise from center-left): orange disc for Dabur-D, followed by slightly brown TE-N show more effect on B.subtilis, but Cibaca- C, Babool- B, Himalaya-H, Sensodyne- S, affect E.coli more at C3.</td></tr>
<tr><td>  </td><td>  </td><td>  </td><td>  </td></tr>
<tr><td>C3</td><td>C4</td><td>C3</td><td>C4</td></tr>
</table>

Results & Discussion

Consumer survey results:

Of 565 participants, 41% used various brands of Colgate as their primary choice. Rest favored other brands (Dabur 13%; Close-up, Glister, Babool and Pepsodent 5-7 % each; Cibaca, Himalaya, Sensodyne 2% each; Vico, Thermosil, Mentodent, Dentasure together made 1%), with no discernable gender or age bias. They mostly belonged to similar demographic and high-end socio-economic strata. About 1% used Tobaco; <1% used tobacco-like agents (Gudakhu Shib, Gul); Muktahanshi, Lava users were 1% each; another 1% used Prasad, Lorden, Dantakathi patajali etc; all of whom belonged to lower socio-economic group, mostly aged, with no gender bias. The rural aged group favored natural products like Neem leaves, Neem Twigs, CDA (3% each), sometimes along with pasted Seora Leaf (1%).

About 30% used >2 brands of toothpaste, around 51% brushed once, 45% brushed twice daily, showing no gender or age bias. Less than 3% brushed more than twice daily and were predominantly female or aged. Toothpastes were chosen according to availability, price, family predilection, brand loyalty, media publicity. Almost all were non-vegetarians, with 17% smokers. Dental problems were not significantly predominant in any particular toothpaste user ($P < 0.05$).

Strength of antimicrobial

Mixed culture:

All DCA, (bar tobacco, lava) were strong antimicrobial at C1 and eradicated most bacterial populations except confluent growth at the edges of plate, with scattered discrete or cluster of resistant colonies. At C1 Muktahansi left interlinked confluent lanes, whereas GLE, TE, CDA, basna formed separate regions of heterogeneous semi-confluent growth interspersed with resistant colonies in clear zones, indicating inherent selective antimicrobial character. The Antimicrobicity of CDA (stronger than basna), could be attributed to increased pH, or cation dis-balance in the media [9].

Colgate, Close-up, Glister, Pepsodent, Cibaca produced clear zones of inhibition at C4; Sensodyne, Babool, Himalaya,

Dabur at C3, Muktahansi at C2 upwords. Antimicrobicity was observed for GLE at C1 (rarely at C2), TE at C4, NLE at C3. SLE displayed strongest activity, even at C4, although at C2-C4, instead of clear zones, heterogeneously decreased intensity of the opaqueness was observed, with discrete resistant colonies, replacing the uniformity of the lawn (*vide* Table-1).

Pure culture: Here also, Colgate, Close-up, Glister, Pepsodent showed strongest activity against both, even at C4, followed by Cibaca, Himalaya, Sensodyene and Babool at C3 for *E.coli* and C4 for *Bacillus*. Such toothpastes should caution against accidental consumption in high amount, which might harm the predominant intestinal commensal. Tobacco, GLE, SLE, NLE, TE, Dabur, Muktahansi, Lava showed almost no effect on *E.coli* at C3, but all of them were strong against *B.subtilis* even at C4, necessitating further study on probable effect on other resident commensal aerobic bacilli. CDA and basna showed negligible effect on *E.coli* at C3, but suppressed *B.subtilis* at C4 (*vide* Table-2).

Dose dependency:

Antimicrobicity of Close-up, Glister, SLE, Sensodyene, CDA, Muktahansi, were significantly ($P<0.05$) dose dependent in all cultures, except for Close-up on bacillus at C3-downwords. The antimicrobial strength of Pepsodent, Colgate, Cibaca, Himalaya, TE, Dabur, did not uniformly increase with concentration.

Nature of antimicrobial:

The only entire circular zone of inhibition observed in mixed culture were for Close-up, Colgate, Glister, Cibaca and Sensodyne at C2, indicating broad spectrum bactericidal properties at high dose, probably attributed to common ingredients like fluorides. Pepsodent, SLE leaving highly irregular overlapping clear zones with stellar projections, interspersed with reticulate rays of confluent growth, could be transcribed as strong but selective bactericidals. Such strong nature can be a double edged sword depending on which particular bacteria they suppressed. Himalaya, Babool produced patches of clearance with different density.

Conclusion:

Previous studies of this nature were limited to pure cultures of selected known predominant oral bacteria and pathogens [1-3,10]. But their veracity and applicability were difficult to extrapolate as in practice, several other factors (eg, interference of network of dynamic metabolic pathways, inter- relationship with other co-inhabitant oral microbes), were involved. We bypassed these limitations in part by exposing random samples of mixed oro-dental microbial load, to 18 DCA; as the toothpastes were purported to act on similar population of unknown oral microbiota. Also we were not restricted to commercial brands only but included indigenous toothpastes and raw natural DCA based on a popularity survey.

We concluded, the natural DCA (SLE being most potent) were more selective antimicrobials though toothpastes with predominantly chemical composition were significantly ($P<0.05$) stronger (Close-up, Pepsodent, Colgate, Glister, Cibaca). The exact potency of the products was hard to grade as out of 18 only 6 showed roughly faithful dose dependency. Also, even keeping aside the huge non-culturable oro-dental bacterial population [5], only a small percentage of the culturable inhabitants were reproduced in the instant project, and as such they do not faithfully represent the microbiology as it is in mouth. Thus the results were not to be considered as a final interpretation of microbicidal properties of the different DCA but a close indication of the same.

References :

1. Olorunfem,P.O., Nyabam,S.K., 2004, "Evaluation of the antimicrobial activities of three commonly available toothpastes on oral microbial flora"' Nig.J.Pharm.Res., 3(1), pp.102-105.
2. De Rossi,A., Ferreira,D.C.A., da Silva,R.A.B., de Queiroz A.M., da Silva,L.A.B., Nelson-Filho,P., 2014, "Antimicrobial Activity of Toothpastes Containing Natural Extracts, Chlorhexidine or Triclosan", Braz.Dent.J.[online], 25(3), pp.186-190.
3. Randall,J.P., Seow,W.K., Walsh,L.J., 2015, "Antibacterial activity of fluoride compounds and herbal toothpastes onStreptococcus mutans: an in vitro study", Aust.Dent.J., 60(3), pp.368-374.
4. Takahashi,N.,2005, "Microbial ecosystem in the oral cavity: Metabolic diversity in an ecological niche and its relationship with oral diseases", Int.Congr.,1284,pp. 103–112.
5. Prescott, Harley and Klein's Microbiology, 5th edition, (2002),ISBN: 0-07-282905-2.
6. Parija, S.C.,Textbook of Microbiology and Immunology, 2nd Edition, ELSEVIER(2012).
7. li,X., Kolltveit,K.M., Tronstad,L., Olsen,I.,2000, " Systemic Diseases Caused by Oral Infection", Clin.Microbiol.Rev.,13(4),pp. 547–558.
8. Coleman Rotstein,C., Evans,G., Born,A., Grossman,R., Light,R.B., Magder,S., McTaggart,B., Weiss,K., Zhanel, G.G., 2008, "Clinical practice guidelines for hospital-acquired pneumonia and ventilator-associated pneumonia in adults",Can.J.Infect.Dis.Med.Microbiol., 19(1),pp.19–53.
9. Ayeni,L.S., Adeleye,E.O., 2012, "Effect of Cattle Dung and Urea Fertilizer on pH and Cations of Soils Selected from Agro-ecological Zones of Nigeria", International Journal of Soil Science,7,pp. 82-90.
10. Roopavathi,K.M., Gopal,S.V., Pushpalatha,G., Bennadi,D., Renushri,B.V., Madhura,A.S., 2015, "Antimicrobial Efficacy of Commercially Available Toothpastes – An In vitro Study", J.Young.Pharm.