In - vitro Sensitivity of Dental Plaque Agents against Synthetic and Natural Antibacterials

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Abstract: *Human oral cavity is inhabited by hundreds of bacterial species that play vital role in maintaining oral health or in shifting to a diseased state such as dental caries. The present study was aimed to assess the effects of commercially available toothpastes on bacteria causing dental plaques and compared with the twig's extract Azadirachta indica L. (Neem), a well-known plant used by common people since ancient period in lieu of brush and paste, as used in modern practices. The dental plaque's samples of forty students were examined and five bacteria were isolated by streak plate method on nutrient agar media and characterized using culture-dependent biochemical methods. The isolates were subjected to in vitro susceptibility tests by measuring zone of inhibition against twelve commercially available tooth pastes and Methanol extract of Neem twig by well diffusion method that was also compared with the sensitivity of Gentamycin as a standard antibiotic. Zone of inhibition in case of neem extract was found more than toothpastes and slightly less than Gentamycin. Out of these ten toothpastes, herbal preparation Dantkanti, a product of Patanjali Ayurved Ltd. showed the maximum sensitivity against all isolates followed by Sensodyne and Thermocyl. Visible white toothpaste was found to be least effective. The affectivity of an antibacterial agent would be help in clinical prophylaxis and thus in the treatment of the infection.*

Keywords: Antibacterial, Dental plaque, Toothpastes, Neem extract, Dantkanti.

1. Introduction

Dental plaque is a biofilm, usually a pale yellow that develops naturally on the teeth, as the microbial community that develops on the tooth surface, embedded in a matrix of polymers of bacterial and salivary origin [1]. Like any biofilm, dental plaque is formed by colonizing bacteria trying to attach themselves to the tooth's smooth surface. Dental biofilms are exceedingly complex and multispecies ecosystems, where oral bacteria interact cooperatively or competitively with other members [2]. With respect to microorganism distribution, the genus *Streptococcus* is present in a high proportion in the soft tissue, saliva, tongue, and supragingival area. Dental biofilms produce acids from carbohydrates that result in caries. A mutans *streptococcus is* the most important bacteria in the pathogenesis of dental caries [3]. This is due to their ability of rapid lactic acid formation from dietary carbohydrates, mainly sucrose and glucose [4].

Microbial acid adaptation and subsequent acid selection of 'low-pH' non-mutans bacteria play a critical role for destabilizing the homeostasis of the plaque by facilitating a shift of the demineralization balance from 'net mineral gain' to 'net mineral loss' (acidogenic stage). Bacteria involved in dental caries may be found naturally in dental plaque but being present in a very small proportion they are only weekly competitive at neutral pH. Low pH favors the survival of acid producing bacteria and secondly it shifts the balance towards demineralization [5]. Once the acidic environment has been established, mutans streptococci and other aciduric bacteria may increase and promote lesion development by sustaining an environment characterized by 'net mineral loss' (aciduric stage). Hence, high proportions of mutans streptococci and/or other aciduric bacteria may be considered biomarkers of sites of particularly rapid caries progression [6]. Many of these

bacteria are usual saprophytes of the oral environment that, in particular situations can overcome and express their virulence factors [7]. Most of the bacteria are harmful and cause plaque and bad breathe [8]. The microorganisms residing in the oral cavity, and their inevitable inter-relationships, are essential components in changing the balance between health and disease [9]. When good oral hygiene practices fail to prevent the development of biofilms, toothpastes and mouthwashes with chemotherapeutic agents can be used. These agents can kill microorganisms in the biofilm. Chlorhexidine, triclosan, essential oils and minerals–agents proven to kill the harmful bacteria and can reduce the degree of plaque and gingivitis, while not allowing disease causing microorganisms to colonize.

Many different products are currently marketed that promised to provide consumers with fresh breath. The active agents that are incorporated into treatment forms include surfactants, antibacterial agents, baking soda, peroxide; metal sacks, herbal and natural extracts and chlorine dioxide [8]. Bacteria growing in biofilms such as dental plaque display an increased tolerance to antimicrobial agents, including those used in dentifrices and mouth rinses [10] [11] [12]. The persistence of antimicrobial action of compounds and formulations in the mouth can be demonstrated by recording the magnitude and duration of the reduction of salivary bacterial counts following a single application [13]. Antimicrobial agents have been used as a chemotherapeutic agent to improve oral health. This in vitro study was carried out to determine the antimicrobial activity of sixteen toothpastes against bacteria isolated from the oral cavity. Tooth brushing with toothpaste is the most widely practiced form of oral hygiene in most countries [14]. A wide range of chemicals, mainly antimicrobial agents, have been added to toothpastes in order to produce a direct inhibitory effect on plaque formation [15]. Antimicrobial mechanisms of toothpastes containing fluoride are through interfering the

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2789

glucose transport, carbohydrate storage, extracellular polysaccharide formation and acid formation by oral streptococci [16].

2. Material and Methods

2.1 Collection of clinical specimens

Saliva and oral swabs were collected from collegiate students of 18 to 20 years age group. The individuals at the time of collection were believed to have healthy teeth. Sterile swab sticks were provided to them and were instructed to rub their tooth surfaces, the tongue and teeth crevices without swallowing saliva as soon as they wake [17] up in the morning, in sterilized tubes containing 2ml normal saline. Samples were stored in a cool place then transported to the laboratory [18].

2.2 Isolation of Bacteria

The samples were labeled and streaks were made on nutrient agar plates. The plates were inverted and incubated at 37^0 C for 24 h. After growth, the isolated colonies were sub cultured into nutrient agar slants and stock cultures were obtained for further study.

2.3 Biochemical Characterization

Isolates were first identified depending on their gram-staining, microscopic examination, catalase test, sugar fermentation study and IMViC test. Growth on nutrient agar media, Mac-conkey agar and Blood agar were studied for cultural characteristics.

2.4 Plant (Neem) Extract

The plant of Neem (*Azadirachta indica* L.) was selected for study. Its twig were collected from college campus and identified with the help of taxonomic key available in departmental library and confirmed with departmental herbaria. The completely dried material was powdered and then allowed for successive extraction in methanol (W/v). The obtained liquid extracts were stored at 4°C in air tight bottle [19] [20].

2.5 Sensitivity Test

Twelve tooth pastes were used in the present study. Tooth pastes stock was prepared in sterile distilled water (0.5 g/ml). Isolates were seeded to minimal media and made 8 mm well with the help of sterile cup borer. Zones were compared with Methanol extract of Neem twig along with Gentamycin as a standard antibiotic. The samples were loaded (25μl) in a well and plates were kept in incubator at 35˚C ± 2˚C for 24 hrs. [21].

3. Result and Discussion

The clinical isolates were subjected to in vitro susceptibility tests against twelve different toothpastes by well diffusion method and zone of inhibition in centimeter was measured. Out of forty samples five bacterial strains were isolated and characterized as mentioned in Table -1, whereas *Streptococcus* species was the predominant bacteria flora. The results of ZOI as the effect of the plant extract and various toothpastes on all five isolates along with standard antibiotic Gentamycin has been recorded in Table -2.The reaction of the plant extract and toothpastes showed different zones of inhibition, whereas maximum measurement was observed in Neem extract, just lesser than Gentamycin. Among tooth pastes it was observed that Patanjali Dantkanti and Patanjali medicate were found to be most effective. Similar type of sensitivity picture is shown by them and that can be attributed to the herbal ingredients in their compositions. It can be stated that fluoride which is the common constituent of nearly all the toothpastes considered in the study has an effect of reducing the oral bacterial flora significantly and that the level of the effectiveness depends on the concentration and time of the exposure of the toothpaste. The reasons for the difference in affectivity of different toothpastes can be due to the uneven or unstable level of concentration of the tooth pastes and the ingredient used. The diffusion method can be used as a preliminary test for detecting antimicrobial activity in substances or products. Since the diffusion phenomenon depends on each substance's physico-chemical properties, as for example its diffusion coefficient, as well as the medium where the diffusion occurs. It is possible to obtain a qualitative indication of antimicrobial activity [22].Therefore, the toothpastes that having the largest microbial inhibition zone and thus, probably the strongest antimicrobial properties may not be necessarily superior to those found to have smaller diameter inhibition zones [16]. The affectivity of an antibacterial agent would help in clinical prophylaxis and thus in the treatment of the infection. Such antibacterial in the form of toothpastes can be used to minimize the load of caries causing organisms of the oral cavity.

Table 1: Biochemical characteristics of Bacterial isolates from the oral cavity-

Isolates	Gram staining	Motility	Sugar fermentation			IMVic test				Catalase	Hemolysis on blood agar
			Glucose	Lactose	Sucrose	Indole	MR	VP	Citrate		
Staphylococcus sp.	+ ve	Non-motile	A +	A +	A +	-	+	+	-	+	-
Streptococcus sp.	+ve	Non-motile	A +	A +	A +	-	+	+	-	-	+
Klebsiella sp.	-ve	Non-motile	A+G+	A+	A+	-	-	+	+	+	-
Gram [-ve] Rod bacteria -A	- ve	Motile	A+G	+	-	+	+	-	+	+	-
Gram [-ve] Rod bacteria -B	-ve	Motile	+	-	-	-	+	-	+	+	-

Table 2: Measurement of the Zones of Inhibition (cm) with different toothpastes, Neem extract and Gentamycin

Toothpaste/ Plant extract/ Antibiotic	Well diameter (cm)	Staphylococcus sp.	Streptococcus sp.	Klebsiella sp.	Gram(-)ve rod bacteria -A	Gram(-)ve rod bacteria -B
Close-up	0.8	1.8	0.9	0.9	1.0	1.2
Colgate max fresh	0.8	1.4	1.6	1.0	1.4	0.9
Colgate salt	0.8	1.1	1.3	1.4	1.1	1.0
Dabur red	0.8	1.3	0.9	1.1	1.5	1.3
Gilmus	0.8	1.2	1.4	1.9	1.2	0.9
Miswak	0.8	1.1	1.5	8.13	1.8	1.0
Patanjali dantkanti	0.8	1.6	1.3	2.3	1.8	1.5
Patanjali medicate	0.8	1.6	1.8	1.1	2.3	1.1
Pepsodent 2 in 1	0.8	1.6	1.2	1.3	1.2	1.8
Sensudine	0.8	1.8	2.3	1.0	1.8	0.9
Thermocyl	0.8	1.5	1.2	1.4	1.4	1.3
Visible white Colgate	0.8	0.9	1.5	1.1	1.0	0.9
Neem Extract	0.8	2.1	2.4	2.6	2.5	1.9
Gentamycin	0.8	2.5	2.5	2.8	2.6	2.0
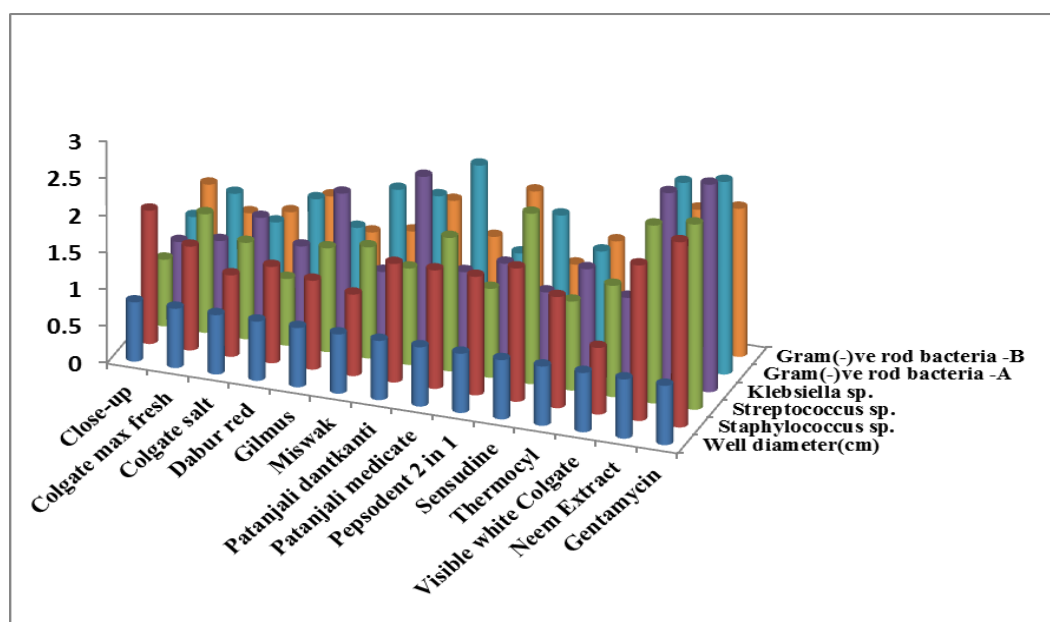
Figure 1: Measurement of the Zones of Inhibition (cm) with different toothpastes, Neem extract and Gentamycin

4. Conclusion

Azadirachta indica L. (Neem) is being used in India since ancient times for treatment of different types of ailments and always showed good results without any side effects. The modern generations having different life styles and dietary habits have gone far away from the traditional practices and they do believe in modern medicines, which may have side effects.

The results of this study are promising enough for the younger generations to turn back to the old Indian herbal collection, which will keep their teeth healthy. Incorporation of *Azadirachta indica* L. (Neem), as an antibacterial agent in combinations with conventional toothpastes will help to reduce the chances of dental associated problems countrywide.

5. Acknowledgment

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