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Comparative evaluation of antibacterial activity of three commercial mouth rinses with different formulations of binary natural mouthrinses

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ABSTRACT: Background
Mouth rinses are the most usual and effective means of preventing oral colonization by micro-organisms. Almost all conventional mouth rinses contain alcohol and fluoride, which are toxic if swallowed in large amounts. An effective substitute to these with all the good qualities and sans its unpleasant effects is highly desirable. Natural ingredients are considered better for our health and the environment.
Objective- The present study aimed to compare the antibacterial activity of different concentrations of neem-cow urine combination (binary product), chlorhexidine and fluoridated mouthrinses against streptococcus mutans using in-vitro method.
Material and Methods
Fresh neem leaves are procured, dried, crushed and mixed with ethanol. Binary products of neem extract prepared in four different concentrations: 1:4(25%), 1:1(50%), 3:4(75%) and full-strength (100%), taking cow urine distillate as the diluent. These samples along with Chlorhexidine, the gold standard among mouthwashes and anti-caries mouthrinses like Fluoritop and kidodent are then tested for their anti-bacterial activity using agar well diffusion method.
Statistical analysis: The obtained data were tabulated and subjected to statistical analysis using one-way ANOVA test and post-hoc-Tukey test.
Results
Among the test groups, Neem showed significantly higher anti-bacterial activity compared to both Fluoritop and kidodent and equivalent to chlorhexidine. Among the binary formulations containing neem, a decline in anti-bacterial activities is observed with the increase in the proportions of cow urine.
Conclusion
Even though certain mouthwashes are available for its anti-cariogenic action, they possess some disadvantages in children such as accidental ingestion and high toxicity. Hence, researchers are focusing on natural alternatives and Neem can be considered as an adjunct to mechanical plaque control, and also promising than fluoridated anti-caries mouthrinses.

KEYWORDS:

I. INTRODUCTION

The tooth surface is unique in that it is the only body part that is not subjected to metabolic turnover. It is, however, subjected to various infections due to factors that favour microbial growth. This microbial growth leads to one of the most widespread diseases of the tooth, i.e., dental caries. Dental caries is an infectious disease influenced by factors such as the relationship between diet and cariogenic microorganisms in the oral cavity and the characteristics of the host. Caries begins in childhood and eventually affects 90% of adults. Even so, dental decay's effect on low-income individuals is disproportionate, leading to earlier onset, more affected teeth, complications, and ultimately teeth lost during adulthood due to caries.

Streptococcus bacteria are mainly responsible for the initial phase of the caries lesion, especially in the enamel (initiation), whereas Lactobacillus is more involved with the progression of caries. Targeting Streptococcus mutans (S mutans) forms the most important measure for the prevention of dental caries[1].

The tremendous backlog of unmet dental needs and ever-increasing demand for care has made it quite obvious that dental diseases cannot be controlled by treatment alone. No matter how sophisticated dental techniques have become, preventive dentistry still remains the foundation on which oral healthcare must be built. Home oral care is an important contributor to oral health and can help lessen the need for extensive dental intervention in the future. Therefore, various chemotherapeutic agents have been developed for home use[2].Mouth rinses are the most usual and easy way and an effective means of preventing colonization by micro-organisms.

Commercial chemical mouthwashes are available in larger number. One of them is chlorhexidine, which is considered as a gold

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standard. It is the most effective anti-plaque agent, but it is not a "magic bullet". However, chlorhexidine has some side effects such as tooth staining on long-term use, taste disturbance, enhanced supragingival calculus formation, and desquamation of the oral mucosa[1]. An effective substitute to chlorhexidine with all the good qualities and sans its unpleasant effects is highly desirable and has been long awaited.

The use of dilute oral fluoride rinse is an important component of preventive dental programs in children. Sodium fluoride mouth rinses are effective in reducing caries and inhibits carbohydrate utilization of oral microorganisms by blocking enzymes involved in the bacterial gylcolytic pathway[3].While mouthwash is not recommended for children younger than 6 years of age, as swallowing reflexes may not be well developed in them and they may swallow large amounts of the mouthwash, which can trigger adverse events—like nausea, vomiting, and intoxication. The upsurge in the prevalence of side effects of many synthetic medicines has encouraged scientists to research for plant based antimicrobial agents and the use of complementary and alternative medicine[2]. A growing number of consumers are embracing this philosophy, that natural ingredients are better for their health and the environment. Hence, there is a need for a mouthrinse which is lot safe and effective in children.

In Veda, cow's urine was compared to the nectar. In substrata, several medicinal properties of cow's urine have been mentioned and are known to cause weight loss, reversal of certain cardiac and kidney problems, indigestion, stomach ache, edema, etc. Cow urine has a unique place in Ayurveda and has been described in 'SushritaSumhita' and Ashtanga Sangraha' to be the most effective substance secretion of animal origin with innumerable therapeutic values. In India, drinking of cow urine has been practiced for thousands of years[3].

Azadirachta indica, commonly known as neem, has attracted worldwide prominence in recent years, owing to its wide range of medicinal properties. Neem has been broadly used in Ayurveda, Unani and Homoeopathic medicine and has become a cynosure of modern medicine. Neem elaborates a vast array of biologically active compounds that are chemically diverse and structurally multifaceted. All parts of the neem tree- leaves, flowers, seeds, fruits, roots and bark have been used conventionally for the treatment of inflammation, infections, fever, skin diseases and dental disorders. The medicinal utilities have been described especially for neem leaf. Neem leaf and its constituents have been verified to exhibit immunomodulatory, anti-nflammatory, anti-hyperglycaemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic and anticarcinogenic properties[4].

Despite the added benefits provided by natural ingredients when compared to chemical mouth rinses such as greater compliance, cost-effectiveness, nontoxic nature and proven antibacterial properties, there has been no study reported in literature that has compared the anti-bacterial effects of fluoride and combinations of natural products for the prevention of dental caries. Hence, this study was taken up to formulate novel and cost-effective mouthwashes that can benefit children thereby reducing the risk of oral diseases in them. The aim of the present study was to compare the antibacterial activity of different concentrations of neem-cow urine combination (binary product), chlorhexidine and fluoridated mouthrinses against streptococcus mutans using agar well diffusion method.

II. MATERIALS AND METHODS

MATERIALS

This in-vitro study used a total of eight solutions as mouthrinses. They are :

Commercially available products;

1. Cow urine distillate(100%)- available in the trade name Go Ark
2. Fluoritopmouthrinse [composition-0.02%Sodium fluoride and phosphate]
3. Kidodentmouthrinse [0.05% Sodium fluoride, Xylitol and Triclosan]
4. Hexidinemouthrinse [Chlorhexidine gluconate 0.2%, propylene glycol, menthol]

Prepared solutions;

5. Neem solution (full strenght) – N100%
6. Neem + Cow urine distillate (1:4)-N25%
7. Neem + Cow urine distillate (1:1)-N50%
8. Neem + Cow urine distillate (3:4)-N75%

All the commercial products were obtained from the local market. The Antimicrobial activity of the above mentioned solutions were evaluated against Streptococcus mutans by Agar well diffusion technique in the laboratory [figure 1]

METHODS

Procurement of neem leaves

Twenty-five grams of mature neem leaves were collected and washed with sterilized distilled water [figure 2]. Fresh leaves were thoroughly cleaned twice using distilled water. They were cut into pieces with the help of scissors/knife and were dried at room temperature and thereafter pulverized

to fine powder using a Kenstar® high-speed electric grinder for 15 min[5].

Preparation of neem solution

Then 20.0 g of dry powder was mixed with 100 ml of 70% (w/v) ethanol for a week in a round bottom flask with occasional shaking [figure 3]. The flask was kept in dark to avoid effect of light on the active ingredients. The extract was then filtered for coarse through Whatman No. 1 filter paper, measured and kept in an airtight container. The alcohol portion has to be removed from the extract by placing it in a petri dish. Aqueous plant extracts were prepared by dissolving the obtained solid residue form in sterile distilled water using glass stirrer, respectively, in the ratio of 1:5 i.e., 20 gm of plant material in 100 ml of water in a sterile 250 ml glass flask. Flasks were then plugged with cotton and kept in refrigerator at 4°C for further use[6].

Preparation of neem-cow urine combination

Binary products of neem extract were prepared in four different concentrations: 1:4(25%), 1:1(50%), 3:4(75%) and full strength (100%), taking cow urine distillate as the diluent. These samples are then tested for its anti-bacterial activity using agar well diffusion method.

'Determination of Antibacterial activity [Agar well diffusion technique]

Culture preparation

30mL Luria Bertani (LB) broth was prepared by adding 0.3g Tryptone, 0.3g Sodium chloride, 0.18g Yeast extract, 30mL Distilled water and autoclaved at 121°C for 15 mins.

Test microorganism

Streptococcus mutans strain (MTCC 497) was inoculated in 30mL of sterilized Luria Bertani (LB) broth and incubated at 37˚ C for 24h.

Media preparation

600mL Luria Bertani (LB) agar media was prepared by adding 6g Tryptone, 6g Sodium chloride, 3.6g Yeast extract, 12g Agar, 600mL Distilled water and autoclaved at 121°C for 15 mins.

Plate preparation

Approximately 40mL of LB agar media was poured into the sterilized petriplates and allowed it to solidify, later 24hrs cultured 200µL inoculum of Streptococcus mutanswas added on the agar plates respectively and spread throughout the plate using spreader.

Sample preparation

50µL and 100µL of the sample was directly used to check the inhibitory concentration of Streptococcus mutans.

Two wells measuring 5mm was made using the well borer in respective plates, 50µL and 100µL of sample were loaded into the respective wells and incubated at 37°C for 24hrs. All the test were done in triplicate to minimize the test error. The inhibition zone for different mouthwashes are measured and noted [figure 4].

III. RESULTS

In the present study the antibacterial activity of the eight mouthwashes were evaluated against Streptococcus mutans. It was determined by agar well diffusion method at 50µl and 100µl loading dose and growth of inhibition zone was measured in millimeters (mm) including the diameter of well (5mm). The results are given in the Table 1.

The obtained data was subjected to statistical analysis using one-way ANOVA test and post hoc-Tukey test.

Table 2 and 3 illustrates the comparison of mean zone of inhibition (ZOI) at 50µl and 100µl respectively between different study groups. This difference in the mean ZOI between different study groups was statistically significant at P<0.001.

Chlorhexidine group showed highest zone of inhibition (19.67 ± 0.58) followed by Neem 100% (17.33 ± 0.58), Neem 75% (14.00 ± 1.00), Kidodent (12.67 ± 0.58), Fluoritop (9.33 ± 0.58) and Neem 50%(8.67 ± 0.58) at 50µl loading dose. However no zone of inhibition was showed by Gow-Arka and Neem 25% (0.00).

Table no. 4 illustrates the comparison of mean ZOI between 50µl and 100µl for different study groups. The test results showed that all the study groups showed higher mean ZOI at 100µl as compared to 50µl and a significant increase in the mean ZOI was noted in the Fluoritop group in 100µl as compared to 50µl at P=0.04. However, in other groups the relative increase in the mean ZOI at 100µl as compared to 50µl did not show any significant differences.

Table 5 & 6 illustrates Multiple comparison of mean ZOI at 50µl between different groups using Tukey's Post Hoc test, revealed that chlorhexidine group showed significantly highest ZOI compared to other groups at P<0.001, which was then followed by Neem 100% showing significantly higher mean ZOI compared to remaining groups at P<0.001 & with CHX at P=0.003, this was followed by Neem 75% showing significantly higher ZOI compared to remaining study groups at P<0.001, then Kidodent group showed significantly higher ZOI compared to remaining study groups at P<0.001, this was then followed by Fluritop and Neem 50% showing

significantly higher mean ZOI compared to Go Arka and Neem 25% at P<0.001. However, no significant differences in the mean ZOI was noted between Neem 75% and Kidodent [P=0.14], Neem 50% and Fluritop [P=0.79] and Go Arka and Neem 25% [P=1.00].

IV. DISCUSSION

Dental caries is a multifactorial disease with several well-known components collectively acting for the disease to manifest. It is recognized to require a host (tooth), a dietary substrate and acidogenic bacteria. Our study objective was to develop a natural anti-caries mouthrinse which is safer and cost-effective. Among pathogenic flora, Streptococcus mutans are considered the main causative microorganism associated with dental caries[7] and hence Streptococcus mutans strain (MTCC 497) was obtained using the culture method for this study. Though mechanical oral hygiene practice aids in the removal of accumulated plaque, the chemical agents like mouthwash can be chosen as adjuncts to reduce Streptococcus mutans[8]. This could be the main mode of oral cleansing in medically compromised patients and elderly, where adequate oral hygiene maintenance could be a major concern[9].

Chlorhexidine is considered to be the "gold standard" antiplaque mouthwash due to its prolonged broad spectrum antimicrobial property. It consists of positively charged cations that bind to negatively charged (anions) bacteria and surface structures in the oral cavity. It exhibits its antimicrobial effect by binding to microbial cell walls, damaging the surface structure, in the process eventually leading to an osmotic imbalance with consequent precipitation of cytoplasm causing cell death[10]. Gram positive cocci especially Streptococcus mutans seems to be sensitive to chlorhexidine which acts by binding to bacterial cell wall and affects its function[8]. In the present study, chlorhexidine has shown the highest anti-bacterial activity against S mutans in comparison to all other samples. This result collaborates with those of George D.E et al[11], Nagappan N et al[12], Parkar SM et al[13] and Nair RA et al[14] wherein chlorhexidine showed similar ZOI against S mutans. However, CHX has some side effects like bitter taste and the formation of extrinsic stains on the teeth and tongue, increased risk of caries due to fermentation and alcohol content, discoloration, altered taste perception, metallic taste and cytotoxic effects on cells[15].

Other commercial mouthwashes used in this study are anti caries mouthwashes with the active ingredient Sodium fluoride which is most commonly used in children for the prevention of dental caries. Fluoride alters the physiochemical properties of teeth by making them more resistant to acid dissolution due to the formation of fluorapatite or fluorhydroxyapatite. It increases the post eruptive maturation, enhances remineralization and inhibits demineralization. Fluoride affects the potential cariogenicity of plaque in many ways. It reduces acid production and eliminates sensitive bacterial population. It also interferes with the formation of cellular polysaccharide that is required for adhesion. It has been known for a long time that fluoride inhibits glycolytic enzyme enolase which causes depletion of phosphenolpyruvate that would reduce sugar transport which, in turn, reduce acid production and glycogen synthesis. Reduced glycogen synthesis would adversely affect the ability of bacteria to survive and ultimately reduce bacterial population in the plaque. Because of the risk of swallowing too much fluoride, fluoride mouthrinses are not recommended for children younger than six years of age[16].

Kidodent MR also possess another ingredient called Triclosan other than Sodium fluoride. Triclosan has been associated with a higher risk of food allergy. A Toxicological Sciences study found that triclosan affected estrogen-mediated responses, and many chemicals that imitate estrogen are known to increase breast cancer risk. Triclosan also suppressed thyroid hormone in rats in a study by Stoker et al[17], showing this chemical to be a potent endocrine disrupter[18]. Considering these drawbacks of mouthwash we require alternative antiplaque agents and have led current research towards natural and biocompatible agents.

Natural products are secondary metabolites synthesised by an organism which behave as defense mechanisms against a vast majority of competing flora and fauna[10]. The major advantages of these natural alternatives are easily availability, affordable, lesser side-effects and lack of microbial resistance[19]. Jacob et. al from his systematic review stated that mouthwash made of natural products can be used as an alternative to chlorhexidine as both showed similar antibacterial efficacy against Streptococcus mutans[10]. Also, literature shows that many herbal extracts in cow urine distillates have been found to be effective as antimicrobials[20]. Several natural extracts with proven antimicrobial efficacy against S mutanshas been tested as mouthwash with conflicting results.

In the present study, neem was used in four concentrations (25%, 50%,75% and 100%) to be tested as a mouthrinse against S mutans by agar-diffusion method in 50µL and 100µL loading

doses. The antimicrobial efficacy of any natural or synthetic agent can be evaluated using broth dilution method, agar dilution method, disc diffusion method, agar well-diffusion method and ditch-plate method. However, agar well-diffusion method was used in the present study as it depends on the diffusion of the tested material to such an extent that growth of the added microorganism is prevented entirely in a zone around the hole containing the test material[21]. The antibacterial efficacy of all the mouthrinses were assessed in 50µL and 100µL loading doses and the current study revealed that the mean ZOI was greater in 100µL loading dose among the two loading doses with a significant increase in the mean ZOI was noted in Fluoritop group. This is in agreement to the findings by Nasreen Banu and V. Gayathri wherein the ZOI increased with the increase in loading dose when antibacterial activity of herbal mouthwashes were tested against oral pathogens[22].

Azadirachta indica (Neem) is perhaps the most useful traditional medicinal plant. The tree is still regarded as "Village dispensary" in India[23]. Different parts of neem have been used for their various pharmacological activities such as antioxidant, antimutagenic, anti-inflammatory, anticarcinogenic, antidiabetic properties. Neem is known to inhibit the bacterial adhesion to saliva-conditioned hydroxyapatite, a composite of bone and enamel. Neem extract also inhibited insoluble glucan synthesis, thereby reducing the adherence of streptococci to tooth surfaces. Azadirachtin, Terpenoid chief constituent of neem, is mainly responsible for the antibacterial properties of neem[24]. In our study, the mean zone of inhibition of Neem 100% (19.33 mm) was almost equivalent to that of chlorhexidine(20.33mm) and the study result is similar to that of Kankariya et al[25] wherein the comparative study exhibited significant result of neem extract against S mutans and the zone of inhibition is also similar to CHX. Also studies by KathariyaM et al[25] and Sharma R et al[26] are in agreement that properties of neem are comparable to that of chlorhexidine whereas, studies by Sajankumar RP et al[27] and Bansal V et al[28] revealed neem as a potent anti-bacterial agent with highest zone of inhibition than chlorhexidine.

Studies by Gupta M et al[29] and Boomathi N et al[30] indicated the combined effect of cow urine and neem whereas Rajapandiyan et al[31] showed the ability of neem-cow urine combination extract to control multiple drug resistance. So, our research team tested anti-bacterial activity of neem-cow urine combination in different proportions (1:4, 1:1, 3:4) against S mutans and study results showed decline in ZOI as the proportion of cow urine is increased in the binary formulations. This is due to the zero ZOI exhibited by cow urine alone and is in disagreement to study done by Dave P et al[32] who showed antibacterial efficacy of cow urine against S mutans and various other pathogens[33,34,35].

Kulakarni and Damle assessed the effectiveness of sodium fluoride, chlorhexidine and triclosan mouth rinses for their antibacterial activity against S. mutans and found that chlorhexidine to be the best[36]. Sundas and Rao[8] carried out a study to evaluate the efficacy of sodium fluoride (0.05%) and chlorhexidine (0.2%) mouth rinses on plaque reduction and found the effectiveness of both to be statistically equal. In the present study N100% showed significantly higher anti-bacterial activity compared to both Fluoritop and kidodent and equivalent to chlorhexidine, thus concludes neem as a potential alternative to fluoridated mouthrinses that can be used as adjunct to mechanical plaque control. This was the only study that compares neem extract against fluoridated mouthwashes. Karale S et al compared the effectiveness of commercially available Sodium fluoride mouthwash with and without Herbal additives in children and reported that Sodium fluoride with herbal additives is found to be the most promising[37]. Mouthwashes should also contain an appropriate amount of free fluorides to provide bioavailability. The lower concentration of free fluoride in comparison with total one is due to the ability of the fluoride source itself to form its complexes and could lead to a decrease in the caries-preventive effect. Thus, we assume that the properties of the neem solution, when used as mouthrinse remain the same and its effectiveness will not be altered due to its constituents.

V. CONCLUSION

Many conditions within the oral cavity require the use of a mouthwash. Almost all conventional mouth rinses contain alcohol and fluoride, which are toxic (even lethal) if swallowed in large amounts. This is not the case with natural herbal mouth rinses. The present in-vitro study hence concludes that Neem at 100 % concentration is equivalent to chlorhexidine and more promising than fluoridated anti caries mouthrinses. However, future in-vivo research on these extracts should be aimed in the direction of finding its safety and efficacy in children.

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TABLES

TABLE 1 Zone of inhibition of Streptococcus mutansin mm

Samples in µL	Plate 1		Plate 2		Plate 3	
	50 µL	100 µL	50 µL	100 µL	50 µL	100 µL
N 25%	-	-	-	-	-	-
N 50%	9	10	9	10	8	9
N 75%	15	19	13	15	14	16
N 100%	17	20	17	19	18	19
Go. Arka	-	-	-	-	-	-
Fluritop	9	13	10	12	9	13
Kidodent	12	14	13	15	13	15
Chlorohexidine	20	20	19	21	20	20

TABLE 2 - Comparison of mean Zone of Inhibition (in mm) between different groups at 50µl using One-way ANOVA test

Groups	N	Mean	SD	Min	Max	P-Value
N 25%	3	0.00	0.00	0	0	<0.001*
N 50%	3	8.67	0.58	8	9	
N 75%	3	14.00	1.00	13	15	
N 100%	3	17.33	0.58	17	18	
Go. Arka	3	0.00	0.00	0	0	
Fluritop	3	9.33	0.58	9	10	
Kidodent	3	12.67	0.58	12	13	
CHX	3	19.67	0.58	19	20	

TABLE 3- Comparison of mean Zone of Inhibition (in mm) between different groups at 100µl using One-way ANOVA test

Groups	N	Mean	SD	Min	Max	P-Value
N 25%	3	0.00	0.00	0	0	<0.001*
N 50%	3	9.67	0.58	9	10	
N 75%	3	16.00	3.00	13	19	
N 100%	3	19.33	0.58	19	20	
Go. Arka	3	0.00	0.00	0	0	
Fluritop	3	12.67	0.58	12	13	
Kidodent	3	14.67	0.58	14	15	
CHX	3	20.33	0.58	20	21	

TABLE 4- Comparison of mean ZOI (in mm) between 50 and 100µl in each group using Student Paired t Test

Groups	Category	N	Mean	SD	Mean Diff	P-Value
N 25%	50µl	3	0.00	0.00	0.00	1.00

	100µl	3	0.00	0.00		
N 50%	50µl	3	8.67	0.58	-1.00	0.68
	100µl	3	9.67	0.58		
N 75%	50µl	3	14.00	1.00	-2.00	0.23
	100µl	3	16.00	3.00		
N 100%	50µl	3	17.33	0.58	-2.00	0.07
	100µl	3	19.33	0.58		
Go. Arka	50µl	3	0.00	0.00	0.00	1.00
	100µl	3	0.00	0.00		
Fluritop	50µl	3	9.33	0.58	-3.34	0.04*
	100µl	3	12.67	0.58		
Kidodent	50µl	3	12.67	0.58	-2.00	0.23
	100µl	3	14.67	0.58		
CHX	50µl	3	19.67	0.58	-0.66	0.74
	100µl	3	20.33	0.58		

TABLE 5- Multiple comparison of mean diff. in ZOI b/w groups at 50µl using Tukey's post hoc Test

(I) Groups	(J) Groups	Mean Diff. (I-J)	95% CI for the Diff.		P-Value
			Lower	Upper	
N 25%	N 50%	-8.67	-10.28	-7.06	<0.001*
	N 75%	-14.00	-15.61	-12.39	<0.001*
	N 100%	-17.33	-18.72	-15.70	<0.001*
	Go. Arka	0.00	-1.61	1.61	1.00
	Fluritop	-9.33	-10.94	-7.72	<0.001*
	Kidodent	-12.67	-14.28	-11.06	<0.001*
	CHX	-19.67	-21.28	-18.06	<0.001*
N 50%	N 75%	-5.33	-6.94	-3.72	<0.001*
	N 100%	-8.67	-10.30	-7.03	<0.001*
	Go. Arka	8.67	7.06	10.28	<0.001*
	Fluritop	-0.67	-2.28	0.94	0.79
	Kidodent	-4.00	-5.61	-2.39	<0.001*
	CHX	-11.00	-12.61	-9.39	<0.001*
N 75%	N 100%	-3.33	-4.97	-1.70	<0.001*
	Go. Arka	14.00	12.39	15.61	<0.001*
	Fluritop	4.67	3.06	6.28	<0.001*
	Kidodent	1.33	-0.28	2.94	0.14
	CHX	-5.67	-7.28	-4.06	<0.001*
N 100%	Go. Arka	17.33	15.70	18.97	<0.001*
	Fluritop	8.00	6.37	9.63	<0.001*
	Kidodent	4.67	3.03	6.30	<0.001*

	CHX	-2.33	-3.97	-0.70	0.003*
Go. Arka	Fluritop	-9.33	-10.94	-7.72	<0.001*
	Kidodent	-12.67	-14.28	-11.06	<0.001*
	CHX	-19.67	-21.28	-18.06	<0.001*
Fluritop	Kidodent	-3.33	-4.94	-1.72	<0.001*
	CHX	-10.33	-11.94	-8.72	<0.001*
Kidodent	CHX	-7.00	-8.61	-5.39	<0.001*

TABLE 6- Multiple comparison of mean diff. in ZOI b/w groups at 100µl using Tukey's post hoc Test

(I) Groups	(J) Groups	Mean Diff. (I-J)	95% CI for the Diff.		P-Value
			Lower	Upper	
N 25%	N 50%	-9.67	-13.05	-6.28	<0.001*
	N 75%	-16.00	-19.39	-12.61	<0.001*
	N 100%	-19.33	-22.60	-16.07	<0.001*
	Go. Arka	0.00	-3.39	3.39	1.00
	Fluritop	-12.67	-16.05	-9.28	<0.001*
	Kidodent	-14.67	-18.05	-11.28	<0.001*
	CHX	-20.33	-23.72	-16.95	<0.001*
N 50%	N 75%	-6.33	-9.72	-2.95	<0.001*
	N 100%	-9.67	-12.93	-6.40	<0.001*
	Go. Arka	9.67	6.28	13.05	<0.001*
	Fluritop	-3.00	-6.39	0.39	0.10
	Kidodent	-5.00	-8.39	-1.61	0.003*
	CHX	-10.67	-14.05	-7.28	<0.001*
N 75%	N 100%	-3.33	-6.60	-0.07	0.04*
	Go. Arka	16.00	12.61	19.39	<0.001*
	Fluritop	3.33	-0.05	6.72	0.04*
	Kidodent	1.33	-2.05	4.72	0.82
	CHX	-4.33	-7.72	-0.95	0.009*
N 100%	Go. Arka	19.33	16.07	22.60	<0.001*
	Fluritop	6.67	3.40	9.93	<0.001*
	Kidodent	4.67	1.40	7.93	0.003*
	CHX	-1.00	-4.26	2.26	0.96
Go. Arka	Fluritop	-12.67	-16.05	-9.28	<0.001*
	Kidodent	-14.67	-18.05	-11.28	<0.001*
	CHX	-20.33	-23.72	-16.95	<0.001*
Fluritop	Kidodent	-2.00	-5.39	1.39	0.45
	CHX	-7.67	-11.05	-4.28	<0.001*
Kidodent	CHX	-5.67	-9.05	-2.28	0.001*

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FIGURES

Figure 1

Figure 2

Figure 3

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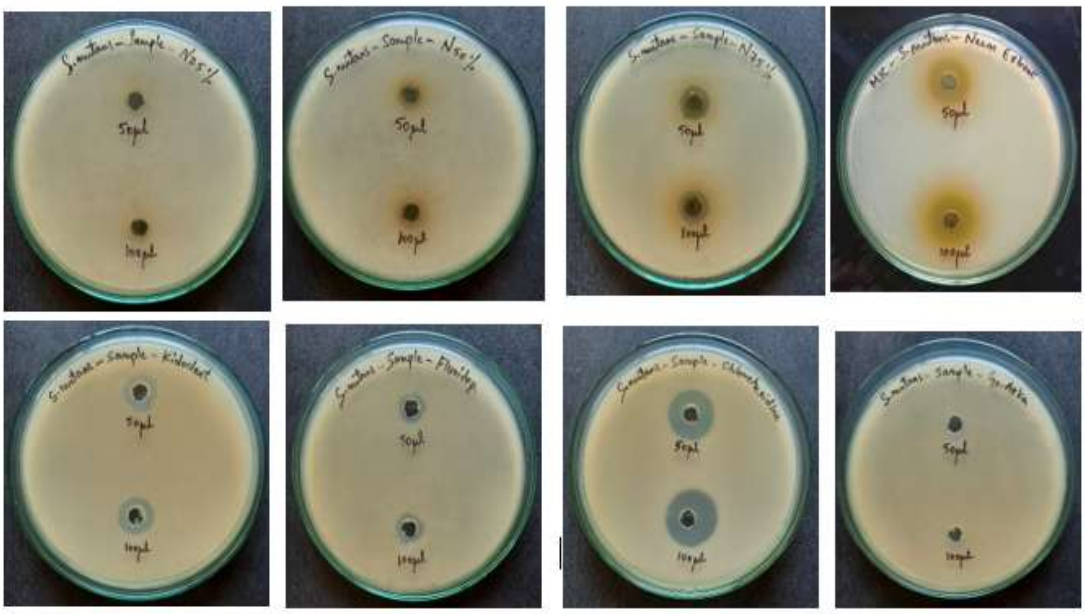

Figure 4