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Antimicrobial evaluation of modified herbal and allopathic dental rinse solution against virulent strains.

Mariyah Yacoob Bawa[1], **Afsheen Aqeel**[1], **Saima Asif**[2], **Saeeda Bano**[2] & **Tanveer Abbas**[1]

[1]Department of Microbiology, University of Karachi, Karachi-Pakistan.
[2]Jinnah University for Women, Karachi-Pakistan.

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Corresponding Author Email: mariyah.yacoob@gmail.com
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Abstract

Background: Mouthwashes are antiseptic solutions and produce anti-inflammatory properties against periodontal infections in humans. Several in-vitro studies have been performed against virulent strains, as evidenced by an appropriate knowledge about their quality, effectiveness and safety in this research. This study is designed to test the antimicrobial effects and evaluation of mouthwashes against pathogens involved in dental caries and oral infections.

Methodology: Rinse solution (Mouthwash) has been developed by three different formulations. Anti-caries solutions were applied and checked against virulent bacterial and fungal pathogens. The antimicrobial effectiveness was identified using the agar well diffusion method and minimum inhibitory concentration (MIC).

Results: Chemisol showed potential killing activity and a zone of inhibition against pathogens ranging in 21-30 mm, which comparatively have higher zones than biosol and herbisol. Biosol also indicated efficacy within 11-13 mm zone of inhibition against *Salmonella typhi ATCC-14028, Salmonella enterica ATCC-6017* resistant to herbisol and chemisol. Moreover, biosol exhibited greater MIC against most microorganisms at direct concentration and 1:10 dilution compared to herbisol and chemisol.

Conclusion: The significant difference observed in the antimicrobial activities of herbal, herbal & allopathic combination and allopathic dental washes against pathogenic strains. This study concluded that proper gargling with mouth wash solutions reduces the risk of life-threatening strains from fecal water & food.

Keywords

Oral Infection, Antimicrobial Activity, Mouthwashes, Pathogenic Strains, Chlorhexidine Gluconate.

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Introduction

Mouthwashes are germicide arrangements proposed to diminish the microbial burden in the oral depression, albeit different mouthwashes may be given for different reasons, such as pain-relieving, calming or hostile to parasitic activity. Mouth contaminations are the most genuine danger of causing hazardous diseases[1]. Notwithstanding its extraordinary commonness among youth and senior gatherings, mouthwashes stay the most secure and simplest method of controlling Peri-embed contaminations. Exploration businesses are creating mouthwashes for oral medicines. Periodontitis is an aggravation of gums and teeth supporting constructions, which is mainly connected with an uncontrolled assembling of bacterial plaque biofilms[2]. Gram-negative microorganisms, especially anaerobes, are liable for ongoing periodontal contaminations; they generally dwell on dental surface[3]. These flushes are successful in fortifying the lacquer of your teeth and shielding your teeth from corrosive harm.

The oral cavity biological system addresses a unique example. In general, utilizing a mouthwash with povidone-iodine, fundamental oil or chlorhexidine gluconate altogether diminished plaque and decreases bacterial markers of gum disease. A successful plaque control measure should target plaque arrangement before the developed plaque has occurred. Different sorts of chemotherapeutic specialists have been worked. Subsequently, this investigation has been embraced to know whether these antimicrobial solutions are effective on regular microorganisms present in the oral cavity and, by implication, adds to plaque development. *Porphyromonas gingivalis* and *Veillonella parvula* are significant microbes in damaging periodontal illness in people. The commonness of oral thrush, and different diseases like UTI, vaginitis, contamination of skin, nosocomial diseases, lungs, looseness of the bowels, tooth harms, aggravation, ladies vaginosis and foundational diseases predominantly brought about by *V.parvvula, P.gingivalis, E.coli, S.typhi, S.enterica, P.aeruginosa, B.spizzeni, and S.aureus, C.albicans and A. brazilensis* [4-5].

Transmission of these microorganisms by aberrant contact (fecal-oral course), by direct course (individual to individual by kissing people, particularly babies) and hands are likewise significant repositories of augmentation of ruinous mouth infections[6]. The advancement of an assortment of mouthwashes with the various plan has been finished by the different manufacturers. Studies have shown that oral washes can decrease plaque and more indications of gum disease when utilized, notwithstanding tooth brushing contrasted and tooth brushing alone[7-8]. The current examination work is intended to check the mouthwashes created by new plans (Herbal, natural and allopathic and allopathic dental flush wash) against its tone, taste, breath scent and purifying & increasing of oral holes by their antimicrobial property, microbial virtue, turbidity and murdering action of mouthwashes against eleven ATCC strains[9-10].

Methodology

Study Design

This experimental study has been designed under the aseptic condition in association with the research & development department of Herbion Pakistan (Pvt) Ltd. Laboratory testing have been conducted & performed at the Department of Microbiology, University of Karachi. The study was conducted following declaration of Helsinki and Ethical approval was obtained before the commencement of this study. Three different herbal actives (Essential oils), preservatives and allopathic active have been used in this study.

Development of mouthwashes

a. Herbisol

Herbisol is developed by herbal & herbal compositions, including antimicrobial essential oils includes Clove oil, Tea tree oil, Eucalyptus oil, Peppermint oil, Basil oil, Menthol, and preservatives.

b. Biosol

Biosol is developed by herbal & allopathic combination (herbal/allopathic combo) including essential oils, allopathic active & preservatives.

c. Chemisol

Chemisol is developed by allopathic formulation including allopathic active (chlorhexidine gluconate) and preservatives.

Test microorganisms

Following are the standard test microorganisms that were procured from Oxoid Company of Karachi. All were sub-cultured on specific media, and recommended for different microorganisms such as tryptone soya agar, peptone water and incubation was done at 37°C aerobically and anaerobically. The identification was made by the gram staining method and standard biochemical testing[11-12].

The pathogens include *Veillonella parvula (ATCC-10790), Porhryomonas gingivalis (ATCC-33277), Escherichia coli (ATCC-8739), Escherichia coli (ATCC-14169), Salmonella typhi (ATCC-14028), Salmonella enterica (ATCC-6017), Pseudomona aeruginosa (ATCC-9027), Bacillus spizzeni (ATCC-6633), Staphylococcus aureus (ATCC-6538),* and *Candida albicans (ATCC-10231)*[13].

Antimicrobial Assay

a. Agar Well Diffusion Method

Agar well dispersion strategy utilized indicated by National Committee for Clinical Laboratory Standards (NCCLS)[14].

A sterile swab soaked with tryptone soya stock for bacterial and sabouraud dextrose agar for parasitic suspension (106 cfu/mL) was taken for credit on tryptone soya agar plates. Anaerobic inoculum (106 cfu/ml) taken from trypticase soy stock converged with defibrinated blood or thioglycolate stock of each bacterial culture was spread on tryptone soya agar defibrinated blood. After that, an 8 mm breadth well was loaded up with 100 µl (0.1 ml) of inoculum utilizing 0.5 McFarland standard.

A flame container (5% CO_2) was utilized to set plates and brooded at 37°C for 24–48 hrs to recuperate severe anaerobes *Veillonella parvula (ATCC-10790)* and 48-72 hrs *Porhryomonas gingivalis (ATCC-33277)*. Negative controls were made in wells containing a similar volume of peptone water, methanol, and refined water (without mouth wash).

In contrast, positive controls were made by standard wide range antimicrobial arrangements of imepenem and vancomycin 10 µg and 30 µg separately. Three duplicates were made against tried life form for each mouth wash[15-16].

b. Minimum Inhibitory Concentration (MIC)

The broth dilution technique was used to the determined MIC of these mouthwashes where the direct samples and stocks of 1 ml of the mouth wash were resuspended in 10 ml of Peptone water/ trypticase soya broth/ thioglycolate broth (diluent) to produce 10 fold dilutions 1:10, 1:100, 1:1000, and 1:10,000[17-18]. Each dilution and direct sample were seeded with bacterial suspension 0.1 ml of (1×106 cfu/ml) and incubated for 24-48 hrs at 37°C. Turbidity was observed using a spectrophotometer at 600 nm (nanometer)[17-18].

Statistical analysis

SPSS version 20.0 was used for analysis purpose. The collected data were analyzed using t-test: paired two samples for means that showed actual test results. Moreover, ANOVA-Single factor was checked against MIC results, where p-value < 0.05 was considered statistically significant.

Results

Newly prepared mouthwashes were measured at the different concentrations for the zone of inhibition (mm) as shown in table 1 and MIC by spectrophotometer (Table 2), chemisol showed good killing activity against nine pathogens at direct & 1:10 concentrations except for *Salmonella typhi (ATCC-14028)*, and *Salmonella enterica (ATCC-6017)* showed activity only at direct concentration. Among the examined mouthwashes for the zone of inhibition, it was observed that the difference of direct sample with 1:100 dilutions was significant ($p<0.05$) in herbisol, biosol and chemisol.

Table 1: Antimicrobial Activity of newly developed mouthwashes against microorganisms.

Organisms name (1×106 cfu/mL)	Herbisol			Biosol			Chemisol			p-value
	Zone of Inhibition mm ± SD									
Dilutions	**Direct**	**1:10**	**1:100**	**Direct**	**1:10**	**1:100**	**Direct**	**1:10**	**1:100**	
Gram-Negative, Strict Anaerobic Coccus-shaped bacterium										
Veillonella parvula ATCC-10790	0	0	0	0	0	0	0	0	0	-
Porhryomonas gingivalis ATCC-33277	0	0	0	0	0	0	0	0	0	-
Gram-Negative, Facultative Anaerobic Rod-shaped bacterium										
Pseudomonas aeruginosa ATCC-9027	0	0	0	16±1.1	13±1.1	11±1.1	22±1.3	20±1.3	18±1.3	<0.05
Escherichia coli ATCC-8739	12±1.0	0	0	17±1.4	16±1.4	13±1.4	23±1.5	21±1.5	19±1.5	<0.05
Escherichia coli ATCC-14169	11±0.7	0	0	18±1.7	15±1.7	14±1.7	29±2.2	26±2.2	23±2.2	<0.05
Salmonella enterica ATCC-6017	0	0	0	11±1.0	0	0	0	0	0	<0.05
Salmonella typhimurium ATCC-14028	0	0	0	13±0.7	0	0	0	0	0	<0.05
Gram-Positive , Aerobic Cocci in clusters bacterium										
Staphylococcus aureus ATCC-6538	14±0.4	10±0.4	0	23±1.6	20±1.6	18±1.6	32±2.3	20±2.3	10±2.3	<0.05
Gram-Positive , Facultative Anaerobic Rod-shaped bacterium										
Bacillus spizizenii ATCC-6633	0	0	0	27±1.2	25±1.2	23±1.2	29±2.0	27±2.0	24±2.0	<0.05
Molds										
Aspergillus brazilensis ATCC-16404	15±1.0	13±1.0	0	23±1.2	18±1.2	17±1.2	30±1.6	24±1.6	22±1.6	<0.05
Yeasts										
Candida albicans ATCC-10231	0	0	0	19±1.5	16±1.5	12±1.5	21±2.4	17±2.4	16±2.4	<0.05

Positive Control (K) - 42mm (imepenum) and 37mm (Vancomycin), *Negative Control- 0mm (Distilled Water) and 0 mm (peptone water); mm indicates millimeter and SD (standard deviation). Each value is the mean ± SD of three replications. P-value < 0.05 is considered significant. Average zones of inhibition (±1.0 mm), K= Positive Control

Table 2: Minimal inhibitory concentrations of mouthwashes in percentages (%) against pathogenic bacteria determined by the turbid metric method.

Organisms name (1×106 cfu/ml)	Herbisol MIC-Turbidity O.D				Biosol MIC-Turbidity O.D				Chemisol MIC-Turbidity OD			
Dilutions	**Direct 100%**	**1:10 10%**	**1:100 1%**	**1:1000 0.1%**	**Direct 100%**	**1:10 10%**	**1:100 1%**	**1:1000 0.1%**	**Direct 100%**	**1:10 10%**	**1:100 1%**	**1:1000 0.1%**
Veillonella parvula ATCC-10790	0.359	0.277	0.209	0.205	0.503	0.216	0.171	0.169	0.027	0.040	0.173	0.177
Porhryomonas gingivalis ATCC-33277	0.492	0.374	0.291	0.298	0.614	0.232	0.194	0.181	0.129	0.157	0.169	0.189
Pseudomonas aeruginosa ATCC-9027	0.367	0.211	0.209	0.193	0.222	0.219	0.266	0.272	0.177	0.172	0.177	0.186
Escherichia coli ATCC-8739	0.273	0.229	0.267	0.273	0.267	0.220	0.185	0.174	0.118	0.151	0.202	0.213
Escherichia coli ATCC-14169	0.269	0.210	0.269	0.287	0.251	0.205	0.171	0.159	0.064	0.071	0.207	0.306
Staphylococcus aureus ATCC-6538	0.335	0.219	0.223	0.217	0.272	0.222	0.188	0.172	0.025	0.066	0.212	0.221
Salmonella typhimurium ATCC-14028	0.365	0.190	0.223	0.247	0.310	0.181	0.218	0.235	0.119	0.210	0.296	0.323
Aspergillus brazilensis ATCC-16404	0.223	0.186	0.191	0.122	0.469	0.206	0.171	0.154	0.009	0.110	0.167	0.182
Candidaalbicans ATCC-10231	0.355	0.262	0.314	0.832	0.489	0.222	0.225	0.239	0.117	0.126	0.288	0.349
Bacillus spizizenii ATCC-6633	0.206	0.210	0.231	0.267	0.561	0.213	0.171	0.164	0.110	0.113	0.217	0.228
Salmonella enterica ATCC-6017	0.373	0.257	0.234	0.221	0.358	0.209	0.220	0.262	0.192	0.214	0.305	0.324
Negative controls												
Test samples without microorganisms	0.346	0.167	0.146	0.141	0.574	0.211	0.151	0.162	0.214	0.172	0.160	0.153

OD indicates Optical density

Discussion

Dental illness is very emerging nowadays due to the increased rate of food-borne infection. The majority of organisms disturb oral health, reduce beneficial flora, and increase the growth rate of toxic and opportunistic microbes. Several studies have been conducted on oral toothpaste, ointments, oral wash (gargling) to prevent halitosis, inflammation, painful plaque and gingival infections. In the recent study, it was observed that biosol (combination of herbal & allopathic) mouth rinse is very effective against food-borne microorganisms due to synergistic effect. At the same time, herbisol failed to show significant killing efficacy against the majority of eleven strains[20]. Out of three, chemisol (allopathic dental solution) showed the highest bactericidal and fungicidal

activity and produced a maximum zone of inhibition against seven *Staphylococcus aureus ATCC-6538* (32 mm to 10 mm), *Aspergillus brazilensis ATCC-16404* (30 mm to 22 mm), *Bacillus spizizenii ATCC-6633* (29 mm to 24 mm), *Escherichia coli ATCC-14169* (29 mm to 23 mm), *Escherichia coli ATCC-8739* (23 mm to 19 mm), *Pseudomonas aeruginosa ATCC-9027* (22 mm to 18 mm), *Candida albicans ATCC-10231* (21 mm to 16 mm) *and no zone observed in Veillonella parvula (ATCC- 10790), Porhryomonas gingivalis (ATCC-33277), Salmonella typhi (ATCC-14028), Salmonella enterica (ATCC-6017)*[20].

Biosol also showed good antimicrobial activity against nine microorganisms, the maximum inhibition zone produced against *Bacillus spizizenii ATCC-6633* (27 mm to 23 mm), *Staphylococcus aureus ATCC-6538* (23 mm to 18 mm), *Aspergillus brazilensis ATCC-16404* (23 mm to 17 mm), *Escherichia coli ATCC-14169* (18 mm to 14 mm), *Escherichia coli ATCC-8739* (17 mm to 13 mm), *Pseudomonas aeruginosa ATCC-9027* (13 mm to 9 mm), *Candida albicans ATCC-10231* (14 mm to 8 mm). *Biosol has also shown zone against Salmonella typhi ATCC-14028* (13 mm to 0 mm) *and Salmonella enterica ATCC-6017* (11 mm to 0 mm), *and no zone observed in Veillonella parvula ATCC-10790, Porhryomonas gingivalis ATCC-33277.* The zone of inhibition is very limited in herbisol. Four strains include *Staphylococcus aureus ATCC-6538* (14 mm to 8 mm), *Escherichia coli ATCC-14169* (12 mm to 5 mm), *Escherichia coli ATCC-8739* (10mm to 4 mm) *and Aspergillus brazilensis ATCC-16404* (15 mm to 10 mm). No zone of inhibition was observed against *Veillonella parvula (ATCC- 10790)* & *Porhryomonas gingivalis (ATCC-33277)* due to improper diffusion of oral solution in enriched medium[20].

Newly prepared mouthwashes were measured at the different concentrations for turbidity by spectrophotometer, chemisol showed good killing activity against nine pathogens at direct & 1:10 concentrations except for *Salmonella typhi (ATCC-14028), and Salmonella enterica (ATCC-6017)* showed activity only at direct concentration[13, 17]. Biosol showed effective MIC against *Veillonella parvula (ATCC- 10790), staphylococcus aureus (ATCC-6538), Escherichia coli (ATCC-8739), Escherichia coli (ATCC-14169), Pseudomonas aeruginosa (ATCC-9027), Bacillus spizizenii (ATCC-6633), Aspergillus brazilensis (ATCC-16404) &Candida albicans (ATCC-10231)* at direct concentration. It provides better synergistic/killing effects at direct & 1:10 concentrations and produces better inhibition efficacy against *Salmonella typhi (ATCC-14028) and Salmonella enterica (ATCC-6017)* due to combining herbal formulation and allopathic. Herbisol showed good MIC against *staphylococcus aureus (ATCC-6538), Escherichia coli (ATCC-8739), Escherichia coli (ATCC-14169), Bacillus spizizenii (ATCC-6633) & Aspergillus brazilensis (ATCC-16404).* Tested turbidity (sample, diluent & microorganisms) compared with the negative controls without microorganisms[21].

Further studies should be conducted to determine acute toxicity, hematology & histopathology on mice for clinical studies / in vivo studies. Several chromatography techniques should be applied and developed against herbisol, biosol and chemisol. The quantitative chemical analysis would ensure actual label claim achieved or not in herbal, herbal & allopathic combo and allopathic mouthwashes. The recent discovery would benefit oral health sciences to prevent lethal and incurable illnesses without any significant side effects.

Conclusion

The current research suggests that Biosol (developed by essential oil and chlorhexidine gluconate) showed excellent antimicrobial activity against eleven potential harmful microorganisms. In vitro study has been conducted against formulations. The second most effective dental rinse solution is chemisol, observed comparatively better zones but effective against nine out of eleven strains.

Conflicts of Interest

The authors have declared that no competing interests exist.

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