LETTER TO THE EDITOR

ANTIMICROBIAL EFFICACY OF A NEW *MIMUSOPS ELENGI (LINN)* INCORPORATED HERBAL PRODUCT ON SELECTED ORAL AND PERIODONTAL PATHOGENS: AN *IN-VITRO* MICROBIOLOGICAL STUDY

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To the Editor,

Dental biofilm eventually leads to the development of caries, gingivitis, and periodontal diseases. Bacterial dental plaque is crucial in the initiation and progression of gingival and periodontal diseases (1). Plaque control can be achieved by mechanical and/or chemical methods. Dentifrices and mouthwashes incorporate compounds, either synthetic and/or natural and are effective mainly by the action on microorganisms by inhibiting their growth and blocking some of their enzymatic reactions (2). Among the mouthwashes, chlorhexidine (CHX) is considered to be the gold standard. However it is known to have side effects such as alteration in patient taste sensation, staining of teeth and less commonly, mucosal erosion or parotid swelling (3). Phytomedicine involving use of traditional medicines has proven safe by the national health care systems. World Health Organization (WHO) guidelines define herbal medicines as finished labeled medicinal products containing an active ingredient, i.e., obtained from the aerial or underground parts of botanicals or other plant materials or their combination (4). Hence, a polyherbal product incorporating *Mimusops elengi (Linn)* (5), known to possess various medicinal properties was used in our study.

A panel of standard strains of microorganisms used in the present study are implicated in oral and periodontal diseases. These include gram-positive organisms including *Streptococcus mutans*, *Lactobacillus acidophilus, Staphylococcus aureus, Enterococcus fecalis*, gram negative bacteria including *Escherchia coli,Pseudomonas aeruginosa, Porphyromonas gingivalis,Fusobacterium nucleatum,* and *Aggregatibacter actinomycetemcomitans* and the pathogenic yeast *Candida albicans* (6,7). To the best of our knowledge, this study is the first of its kind assessing antimicrobial potential with the essential herbal combination. Each component in the formulation was selected after meticulous understanding of their pharmacological actions so that the combination is a product of paramount benefit. This study aimed to assess the antimicrobial efficacy of a polyherbal product (Bakul dant powder^{TM}) (PH) in comparison with CHX against standard oral and periodontal microbial strains.

MATERIALS AND METHODS

The study was conducted in full accordance with the declared ethical principles (World Medical Association Declaration of Helsinki, version VII, 2013. Ethical

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clearance was obtained from the institution's independent ethics committee (Approval No. SRC/ETH/2018-19/116).

Preparation of the polyherbal plant product

The freshly prepared polyherbal product was acquired from the senior doctor of Ayurveda, India who is the proprietor of the product. The parts used and proportions of each plant material are listed in Table I. As reported in previous studies, there can be limited antibacterial potency due to the extraction methods employing various chemicals and crude extracts to get the plant extracts (8). It is therefore suggested that pure plant compounds isolated from plants might exhibit better antibacterial activity. Owing to this concept, we used the native pure form of the plants as a mixture to retain their vital properties in natural form.

Microbial strains used

The antimicrobial effects were determined against standard strains of bacteria that are involved in different stages of oral and periodontal diseases. The strains of microorganisms used were gram positive organisms facultative anaerobic bacteria including *Streptococcus mutans (ATCC 25175)* and *Lactobacillus acidophilus (ATCC 4356), Staphylococcus aureus (ATCC 12598)*, gram-negative bacteria including *E. coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 25619), Porphyromonas gingivalis (ATCC 33277), Fusobacterium nucleatum (ATCC 25586), Aggregatibacter actinomycetemcomitans (ATCC 29523), Enterococcus fecalis (ATCC 35550)* and *Candida albicans (ATCC 2091)*. The strains were procured from Himedia laboratories Pvt. Ltd., Mumbai, India.

Preparation of the stock solution

The stock solution was prepared by dissolving 100 mg of PH in 1 ml of 0.25% Dimethyl sulfoxide (DMSO). 0.25% DMSO is not known to exhibit antibacterial activity and it is further diluted as different concentrations of the compound are prepared, ruling out any possible antibacterial activity of its own (9). 50 µl of CHX (0.2% Rexidin™ mouthwash) was used for the assay. A total of 8 concentrations of the PH were prepared as follows: i) 100% - Neat, ii) 75% - 75 µl compound in 25 µl of DMSO, iii) 50% -50 µl compound in 50 µl of DMSO, iv) 25% - 25 µl compound in 75 µl of DMSO, v) 10% - 10 µl compound in 90 µl of DMSO, vi) 5% - 5 µl compound in 95 µl of DMSO, vii) 2.5% - 2.5 µl compound in 97.5 µl of DMSO and viii) 1.25% - 1.25 µl compound in 98.75 µl of DMSO.

Antimicrobial susceptibility assay

Agar disc diffusion method was used for assessing the antimicrobial activity of the PH in comparison with standard CHX. The media used was Brain Heart Infusion agar. The agar plates were brought to room temperature before use. In anti-fungal disc diffusion method, Sabouraud agar medium was used. Using a swab, the colonies were then transferred to the plates. The turbidity was then adjusted with broth to equal that of 0.5 McFarland turbidity standard. Within 15 min of adjusting the inoculum to a McFarland 0.5 turbidity standard, a sterile cotton swab was dipped into the inoculum and rotated against the wall of the tube above the liquid to remove excess inoculum. The entire surface of agar plate was swabbed three times, rotating the plates approximately 60° between streaking to ensure even distribution. The inoculated plate was allowed to stand for at least 3 min but no longer than 15 min before making wells.

Followed by the inoculation of the agar plate, a hollow tube of 5 mm diameter was taken and heated. It was pressed on above the inoculated agar plate and removed immediately by making a well in the plate. Likewise, five wells were made on each plate. 50 µl of dilutions of the compound were added to each of these into the respective wells on each plate. Following this, the plates were incubated for 18-24 h at 37ºC. Bacterial strains were maintained on blood agar media, mitis salivarius agar, and brain-heart infusion agar as appropriate for that species. These media were obtained and prepared in accordance with the manufacturer's instructions (HiMedia Laboratories Pvt Ltd., Mumbai, India). Once the lawn of growth was confluent or nearly confluent, the diameter of inhibition zone was measured in millimeters using a vernier caliper (Fig. 1).

Determination of MIC (Minimum Inhibitory Concentration)

For the broth dilution assays, 9 serial dilutions of PH and CHX ((i.e., 100, 50, 25, 12.5, 6.25, 3.12, 1.6, 0.8, 0.4, and 0.2 mg/ml) were done with Brain–Heart Infusion broth (BHI). The concentration as prepared for disc diffusion for 100% neat was taken and was serially diluted. In the initial tube 20 µl of PH and CHX was added

into the 380 µl of BHI broth. For dilutions 200 µl of BHI broth was added into the next 9 tubes separately. Then from the initial tube 200 µl was transferred to the first tube containing 200 µl of BHI broth. This was considered as 10^{-1} dilution. From 10^{-1} diluted tube 200 µl was transferred to second tube to make 10^{-2} dilution. The serial dilution was repeated up to 10^{-9} dilution for each drug. In each serially diluted tube, 200 µl of bacterial suspension was added. The tubes were then incubated at 35°C for 48-72 h and observed for turbidity. The MIC was read, which was defined as the highest dilution of the agent that inhibited bacterial growth, as determined by lack of turbidity. The experiments were repeated in triplicates for each strain.

RESULTS

In the present study, an initial screening was carried out to assess the antimicrobial efficacy of a new herbal product against standard strains of oral and periodontal pathogens. A zone of inhibition of more than 8 mm signifies that the micro-organisms are susceptible to the tested products. Table II summarizes the result of susceptibility of experimented organisms against different concentrations of the PH and CHX. Zones of inhibition assessed by disc diffusion were 25.00±1.00 mm for *C. albicans*, 23.33±0.57 mm for *S. mutans*, 20.66±0.57 mm for *P. gingivalis* for the PH . In comparison, zones of inhibition for CHX were 28.00±2.00 mm for *C. albicans,* 30.00±1.00 mm for *S. mutans* and 28.00±1.73 mm for *P. gingivalis.* Though CHX had better results, PH also exhibited inhibition against the pathogens (Fig. 2)

Sensitivity of specific organisms to different dilutions of PH and CHX was analysed by treating the selected strains of micro-organisms. Table III presents the MIC exhibited by the PH and CHX against tested micro-organisms. It is noteworthy that, all of the selected bacterial strains were sensitive to both PH and CHX at 100 mg/ml and 50mg/ml. Similar inhibitory effects against *P. aeruginosa* (0.76±0.15), *E. Coli* were observed with both PH and CHX (1.6±0.25), *C. albicans* (0.8±0.16) and *P. gingivalis* (3.10±0.018). However, for the other strains of microorganisms, PH was found to be

Table I. *Plant materials used and their proportions*

Botanical name	Local name	Parts used	Proportions used (%)
Mimusops elengi(Linn)	Bakul saal	Bark	22
Azadirachta indica A	Neem saal	Bark	11
Acacia nilotica	Babul saal	Stem	11
Acacia catechu	Khair saal	Bark	11
Terminalia chebula	Hirda	Fruit	11
Terminalia bellirica	Bahera	Fruit	11
Emblica officinalis	Amalaki	Fruit pulp	11
Alum	Turti	Hydrated double sulfate salt of aluminium	2
Syzygium aromaticum	Lavang	Dried flower buds	5
Rock salt	Saindhav	Mineral	5

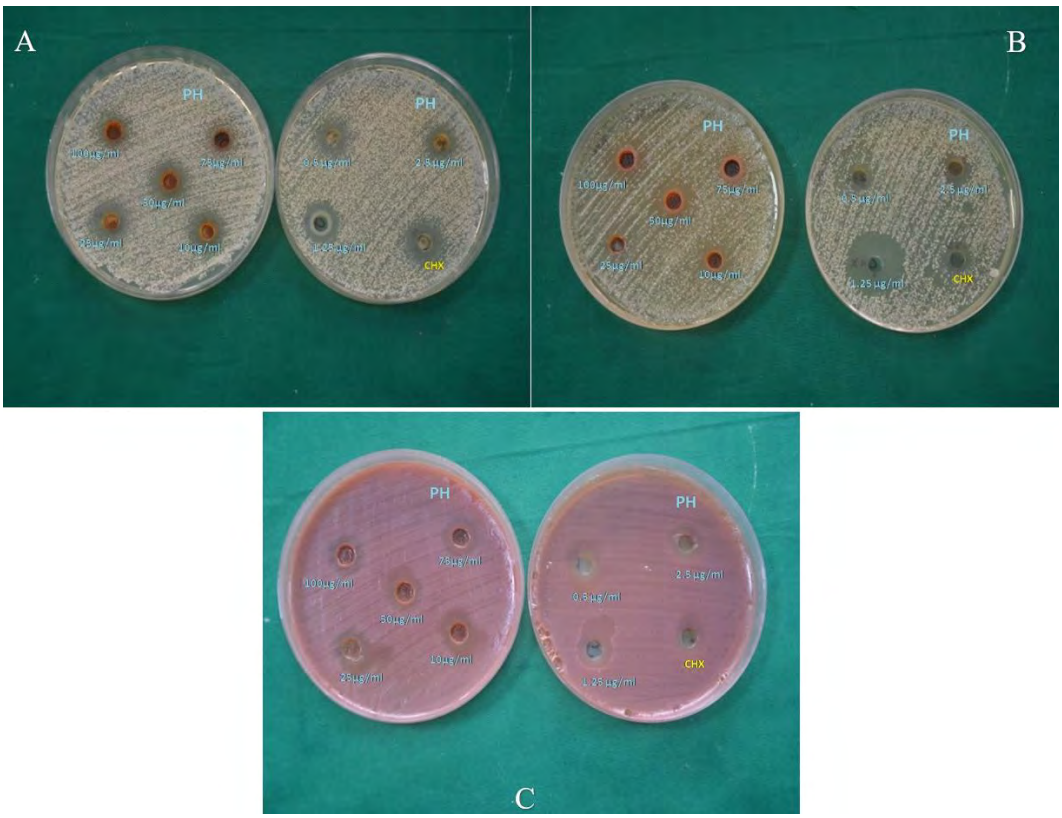

Fig. 1. *Antimicrobial activity of the polyherbal product (PH) and chlorhexidine. (CHX) against* C.albicans ***(A)***, S. mutans ***(B)***, P. gingivalis ***(C)***.

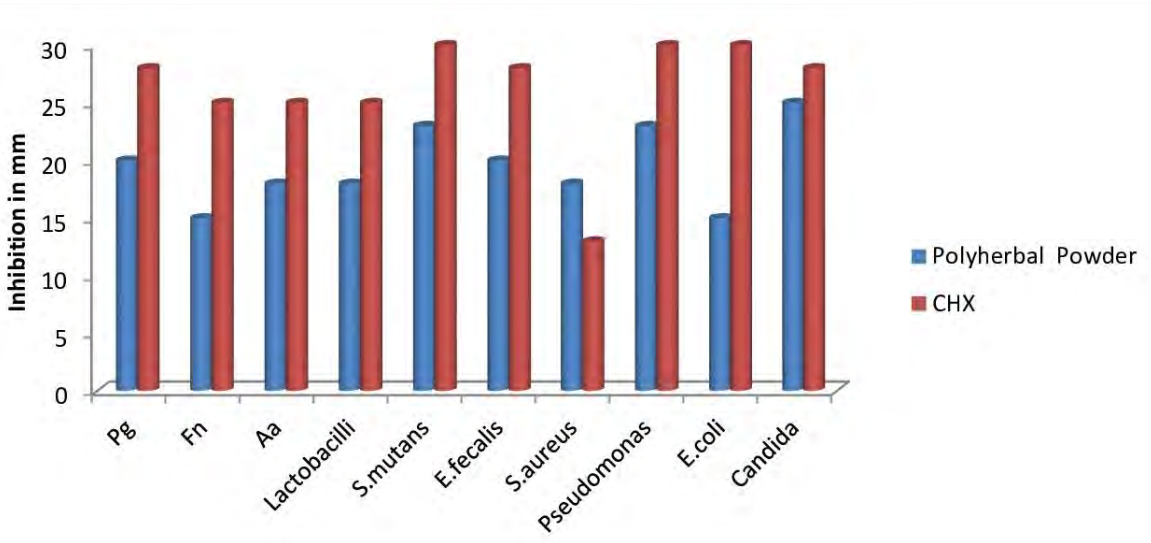

Fig. 2. *Effects of the polyherbal product and chlorhexidine against standard oral and periodontal microorganisms at 100µl/ml.*

Table II. *Comparison of the effects of PH and CHX on standard oral and periodontal microbial strains using Disc Diffusion method at 100 µl/ml*

Microorganisms	Polyherbal product (PH) (100 µl/ml)	CHX (100 µl/ml)
	ZONE OF INHIBITION in mm	
P. gingivalis	20.66 ±0.57	28.00±1.73
F. nucleatum	14.66±1.52	24.66±1.52
A. actinomycetemcomitans	19.33±1.52	26.66±1.52
L. acidophilus	18.66±0.57	25.00±1.00
S. mutans	23.33±0.57	30.00±1.00
E. fecalis	20.00±1.00	27.66±1.52
S. aureus	18.66±2.08	11.66±1.52
P. aeruginosa	22.66±1.52	30.66±1.52
E. coli	15.66±1.15	30.66±1.52
C. albicans	25.00±1.00	28.00±2.00

Table III. *Minimum Inhibitory Concentration (MIC) (mg/ml) of polyherbal product and CHX against the tested micro organisms*

Microbial strains	*P. gingivalis*	*F. nucleatum*	*A. actinomycetm-comitans*	*L. acidophilus*	*S. mutans*	*E. fecalis*	*S. aureus*	*P. aeruginosa*	*E. coli*	*C. albicans*
Poly-herbal Powder	3.10± 0.018	6.17± 0.11	50.66± 3.05	25.66± 2.08	6.2± 0.012	12.26± 0.87	3.12± 0.019	0.76± 0.15	1.6± 0.25	0.8± 0.16
CHX	3.12± 0.019	0.8± 0.016	6.25± 0.012	0. ± 0.16	0.8± 0.016	0.4± 2.00	0.4± 2.00	0.8± 0.016	1.6± 0.25	0.8± 0.16

effective at higher concentrations. Though CHX had better inhibitory effect even at low concentrations against the microorganisms, PH exhibited the inhibitory effects over the micro-organisms at variable concentrations (Fig. 3).

DISCUSSION

Considering the adverse effects of CHX, herbal mouthwashes have gained attention but none has been able to match that of CHX (10). This is the first study of its kind incorporating vital plant products such as *Mimusops elengi (Linn)* and other crucial plant materials in adequate proportions. *Mimusops elengi (Linn)* is well documented in literature to possess antibacterial, antifungal and antiinflammatory potential due to the inherent chemical composition (5). The other herbs incorporated in the product include *Azadirachta indica, Acacia nilotica, Senegalia catechu, Terminalia bellirica, Emblica officinalis, Terminalia chebula, Alum* and *Syzygium aromaticum* are wellknown for their medicinal potential. In our recently published work by Bhavikatt SK and co-workers, we reported good anti-inflammatory potential of the polyherbal product as assessed by gelatin zymography (8).

In the present study, though PH had inhibitory effects at higher concentrations, MIC by broth dilution technique showed highest sensitivity with CHX for all the selected bacterial strains, followed

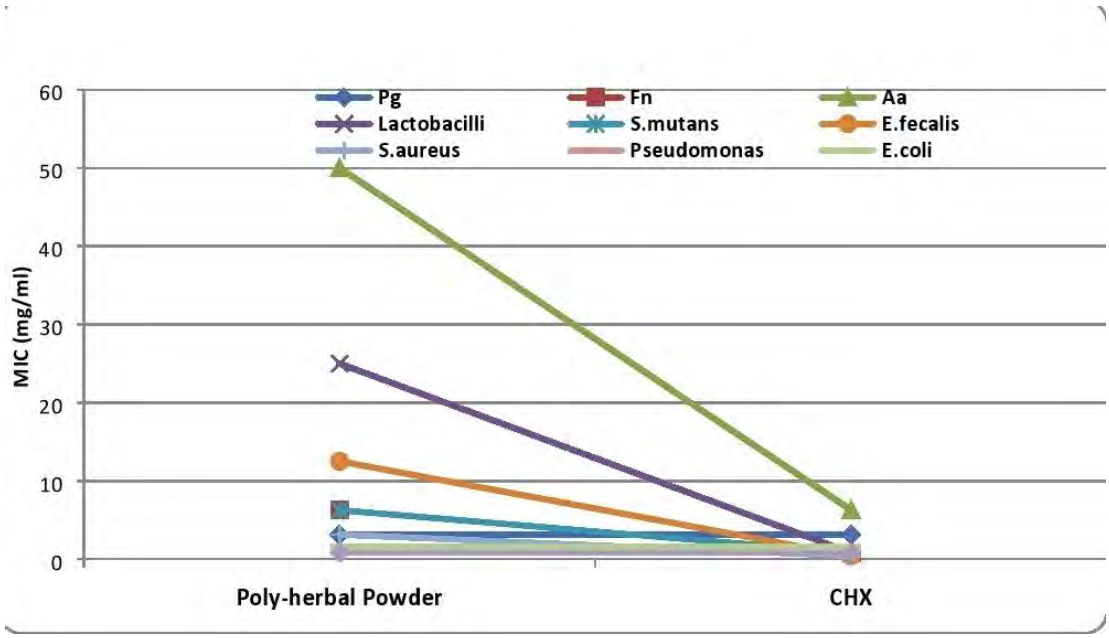

Fig. 3. *Antibacterial sensitivity of the oral and periodontal microorganisms as evaluated by broth dilution.*

by the PH. However, when MIC was assessed, PH had a significantly lesser effect as compared to CHX against *P. gingivalis*, *F. nucleatum*, *A. actinomycetemcomitans*, *L. acidophilus, S. mutans, E. fecalis, S. aureus, P. aeruginosa, E. coli and C. albicans*. CHX was more effective than PH. These findings reinforce the earlier findings that, variation in the media can affect the MIC values of a compound and that MIC values are method dependent. It may be that the constituents of the agar media could have influenced some of the antimicrobial properties of the poly-herbal mouthwash. Similar results were reported by Pathan et al (11) in their study.

PH showed maximum zones of inhibition of against *C. albicans* followed by *S. mutans*, but less as compared to CHX. This inhibition of poly-herbal powder against both Candida and *S. mutans* can be due to both, antifungal and anti-microbial property of the solution. The results are in agreement with Prabhakar et al. (12) in relation to antimicrobial activity. PH showed a comparable inhibitory effects over *S. mutans, S. aureus and C. albicans* to the standard CHX.

In conclusion, although CHX showed superior results, PH proved to have antimicrobial activity against oral and periodontal pathogens. It can therefore be used as a herbal alternative and as an adjunct to the conventional oral hygiene methods. However further studies with clinical efficacy on large samples of population are anticipated to validate the findings of the present study.

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REFERENCES

1. Colombo APV, do Souto RM, da Silva-Boghossian CM. et al. Microbiology of oral biofilm-dependent diseases: have we made significant progress to understand and treat these diseases? Curr Oral Health Rep 2015; 2:37-47.
2. Teles RP, Teles FRF. Antimicrobial agents used in the control of periodontal biofilms: effective adjuncts to mechanical plaque control? Braz Oral Res 2009; 23(1):39-48.
3. Ernst CP, Canbek K, Dillenburger A, Willershausen B. Clinical study on the effectiveness and side effects of hexetidine and chlorhexidine mouthrinses versus a negative control. Quintess Int 2005; 36(8):641-52.
4. World Health Organization, WHO Traditional Medicine Strategy 2002–2005, World Health Organization, Geneva, Switzerland, 2002.
5. Gami B, Pathak S, Parabia M. Ethnobotanical, phytochemical and pharmacological review of Mimusops elengi Linn. Asian Pac J Trop Biomed 2012; 2(9):743-48.
6. Chenicheri SRU, Ramachandran R, Thomas V, Wood A. Insight into oral biofilm: primary, secondary and residual caries and phyto-challenged solutions. Open Dent J 2017; 11:312-33.b
7. Khatoon Z, McTiernan CD, Suuronen EJ, Mah TF, Alarcon EI. Bacterial biofilm formation on implantable devices and approaches to its treatment and prevention. Heliyon 2018; 4(12):e01067.
8. Bhavikatti SK, Alqahtani NA, Bhat KG, Aggarwal VP, Karobari MI. Evaluation of anti-inflammatory activity of a Mimusops elengi (linn)- incorporated herbal product : a zymographic analysis. J Biol Regul Homeost Agents 2020; 34(1).
9. Valgas C, Souza SM, Smânia Elza FA, Smânia Jr A. Screening methods to determine antibacterial activity of natural products. Braz J Microbiol 2007; 38(2):369-80.
10. Gupta D, Nayan S, Tippanawar HK, et al. Are herbal mouthwash efficacious over chlorhexidine on the dental plaque? Pharmacognosy Res 2015; 7(3):277-81.
11. Pathan MM, Bhat KG, Joshi VM. Comparative evaluation of the efficacy of a herbal mouthwash and chlorhexidine mouthwash on select periodontal pathogens: An in vitro and ex vivo study. J Indian Soc Periodontol 2017; 21(4):270-5.
12. Prabhakar K, Kumar LS, Rajendran S, Chandrasekaran M, Bhaskar K, Sajit Khan AK. Antifungal activity of plant extracts against candida species from oral lesions. Indian J Pharm Sci 2008; 70(6):801-3.