Determination of antimicrobial efficacy of four Ayurveda Local Applications (Pratisarana Yoga) in the management of Periodontal Disease

Peiris K.P.P.1, Wanigasekara D.N.2, Sudesh A.D.H.1, Karunarathne E.D.C.1
1Gampaha Wickramarachchi Ayurveda Institute, University of Kelaniya, Sri Lanka
2Department of Microbiology, Faculty of Science, University of Kelaniya, Sri Lanka

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Abstract – This study evaluates the antimicrobial activity of four Ayurvedic formulations namely Kushtadi Churna, Swethamanjana Churna, Karanjadi Churna and Dasana Sanskara Churna that are used to treat periodontal diseases as local applications. The objectives are to find out the presence or absence of in-vitro antimicrobial efficacy of these four drugs and to evaluate the antimicrobial efficacy of them using agar well diffusion method. The organisms used for the study are Staphylococcus aureus (ATCC 29213), Methicillin resistant Staphylococcus aureus (ATCC 25923) and clinically isolated Candida albicans. The results of the in-vitro antimicrobial efficacy study reveals that Swethamanjana Churna possesses antimicrobial action against all three tested microorganisms. Kushtadi Churna is effective against S. aureus (ATCC 29213) and MRSA (ATCC 25923) and it is unable to affect the growth of C. albicans. Karanjadi Churna and Dasana Sanskara Churna are effective against S. aureus (ATCC 29213) and C. albicans and not effective against MRSA(ATCC 25923). Fluconazole and Amoxicillin are used as positive controls for the yeast and two bacteria respectively.

Keywords: Periodontal disease, antimicrobial activity, Kushtadi Churna, Pratisarana yoga, Dasana Sanskara Churna

1. INTRODUCTION

Any disorder of periodontium; surrounding and supporting tissues of the tooth can be described as a periodontal disease. (Pihlstrom, et al 2005). Although the name "periodontal disease" usually implies inflammatory disorders of periodontitis and gingivitis caused by the pathogenic microorganisms associated with the biofilm formation and dental plaques that form on the teeth (Albandar, et al, 2002), these diseases can also be developmental, traumatic, neoplastic, genetically transmitted or metabolic.(Armitage,2004)

Periodontal diseases are usually caused by bacteria associated with dental plaques. (Socransky and Haffajee, 1990). When the number of virulent bacteria of the plaque is increasing, the resulting illness is classified as gingivitis which is limited to the gums and the pathogenic, bacteria spread to underlying tissues of oral cavity and destroying the connective tissues of teeth, the condition is called as periodontitis. (Marsh, 2003)

The complications associated with gingivitis and periodontitis can be either bacterial, viral or fungal origin. They can cause painful ulcers and abscesses. (Martínez and Ruiz, 2005)

In Ayurvedic Medicine, certain formulations are used to treat periodontal diseases. *Kushtadi Churna, Swethamanjana Churna, Karanjadi Churna* and *Dasana Sanskara Churna* are such formulations that are mostly applied on the affected areas of oral cavity.(Bhai.Rat.Mukha Roga)

Although these formulations are time tested, there are no studies have been carried out to evaluate the efficacy of these formulations scientifically. In this study in-vitro determination of antimicrobial efficacy of these four Ayurvedic formulations is conducted using three microorganisms that are commonly associated with periodontal disease complications. The study focuses on the microbial growth inhibition by the four formulations measuring the inhibitory zone diameter.

2. Materials and Methods

2.1 Sample preparation

2.1.1 Kushtadi Churna test sample preparation

Kushtadi Churna (1g) was mixed with sterile distilled water (10 mL)

2.1.2 Swethamanjana Churna test sample preparation

Swethamanjana Churna (1g) was mixed with sterile distilled water (10 mL)

2.1.3 Karanjadi Churna test sample preparation
Karanjadi Churna (1g) was mixed with sterile distilled water (10 mL)

2.1.4 Dasana Sanskara Churna test sample preparation
Dasana Sanskara Churna (1g) was mixed with sterile distilled water (10 mL)

2.2 Microbial culture preparation
Methicillin Resistant *Staphylococcus aureus* (MRSA) (ATCC 25923), *Staphylococcus aureus*(ATCC 29213), and clinically isolated *Candida albicans* were inoculated in test tubes containing Nutrient Broth separately and incubated overnight at 37^{o}C. After incubation, cultures were adjusted by adding sterilized peptone water until the turbidity matched that of a McFarland 0.5 standard.

2.3 Antibiotic solution preparation
2.3.1 Preparation of Amoxicillin
Amoxicillin (10 mg) was dissolved in sterile distilled water (1 mL) aseptically to obtain the concentration of 10 mgmL^{-1}

2.3.2 Preparation of Fluconazole
Fluconazole (2.5 mg) was dissolved in sterile distilled water (1 mL) aseptically to obtain the concentration of 2.5 mgmL^{-1}

2.4 Antimicrobial susceptibility testing
2.4.1 Antibacterial susceptibility testing of bacteria
The agar well diffusion method was performed to assess the susceptibility of two bacterial cultures against four drugs. Each turbidity adjusted bacterial culture (100 µL) was pipetted out onto Mueller- Hinton Agar plates. Then the inoculum was spread over the agar surface evenly using a sterile cotton swab. Three wells were made on each inoculated agar plate using sterilized cork borer. Four drug preparations (each 50 µL) were added to a well of each labeled plate separately. Amoxicillin (50 µL) was added to the well labelled as "positive control" of all plates. Sterilized distilled water (50 µL) was added to the remaining well of each agar plate as the negative control. The plates were incubated at 37^{0}C for 24 hours. Zone diameter of each inhibition was measured to the nearest millimeter.

2.4.2 Antifungal susceptibility testing of *Candida albicans*
Each turbidity adjusted clinically isolated *C. albicans* culture (100 µL) was pipetted out onto Sabouraud Dextrose Agar plates. Then the inoculum was spread over the agar surface evenly using a sterile cotton swab. Three wells were made on each inoculated agar plate using sterilized cork borer. Four drug preparations (each 50 µL) were added to a well of each labeled plate separately. Fluconazole (50 µL) was added to the well labelled as "positive control" of all plates. Sterilized distilled water (50 µL) was added to the remaining well of each agar plate as the negative control. The plates were incubated at 37^{0}C for 24 hours. Zone diameter of each inhibition was measured to the nearest millimeter.

3. RESULTS AND DISCUSSION
Antimicrobial activity assessed in terms of inhibition zone diameter of four Ayurvedic formulations namely *Kushtadi Churna, Swethamanjana Churna, Karanjadi Churna* and *Dasana Sanskara Churna* that are used to treat secondary infections associated with periodontal diseases.

As these all four Ayurvedic formulations are in powder form, they were dissolved separately in sterilized distilled water to obtain solutions to be used in agar well diffusion method.

Amoxicillin was used as the positive control for bacteria and Fluconazole was the positive control used for the yeast. Both positive controls produced significantly large inhibitory zones on agar plates.

According to the obtained results, *Kushtadi Churna* showed inhibitory action against *Staphylococcus aureus* (ATCC 29213) and Methicillin resistant *Staphylococcus aureus* (MRSA) (ATCC 25923). It was unable to affect the growth of

clinically isolated *Candida albicans*. The inhibitory zone diameter shown by *Kushtadi Churna* with *S. aureus* (ATCC 29213) is lower than its positive control amoxicillin.

When considering about MRSA (ATCC 25923), the inhibitory zone diameter shown by *Kushtadi Churna* is greater than the clear zone of Amoxicillin. However the growth of *C. albicans* was not affected by *Kushtadi Churna.*

Swethamanjana Churna was very effective against MRSA (ATCC 25923) producing a larger inhibitory zone compared to the positive control. *S. aureus* (ATCC 29213) and *C. albicans* were also susceptible to *Swethamanjana Churna* with significantly large inhibitory zones.

Among the used formulations, *Karanjadi Churna* and *Dasana Sanskara Churna* were unable to inhibit the growth of MRSA (ATCC 25923). Although they were not effective against MRSA (ATCC 25923), both of them produced considerably large clear zones with *S. aureus* (ATCC 29213). These two *churna* affected to the growth of *C. albicans* to a certain extent.

Of all the tested Ayurvedic formulations, *Swethamanjana Churna* was found most active against MRSA (ATCC 25923) and against all tested pathogens. As the zone diameter of *Kushtadi Churna* against MRSA (ATCC 25923) is higher than its positive control, it can also be suggested as a good antimicrobial formulation for MRSA(ATCC 25923) associated infections in oral cavity.

4. CONCLUSION

In this study antimicrobial property of four Ayurvedic formulations that have been used for centuries for secondary infections associated with periodontal diseases were evaluated. *Swethamanjana Churna* is concluded as the most effective formulation against tested microorganisms as it can adversely affect the growth of all tested organisms giving inhibitory zone diameters of 23.70±0.30 mm, 26.67±1.20 mm and 26.35±0.40 mm respectively to *S. aureus* (ATCC 29213), MRSA (ATCC 25923) and clinically isolated *C. albicans*.

Kushtadi Churna is effective against *S. aureus* (ATCC 29213) and MRSA (ATCC 25923) producing clear zones of 17.30±0.70 mm and 19.00±0.60 mm of diameter accordingly. It is not effective against clinically isolated *C. albicans*.

Karanjadi Churna can affects the growth of *S. aureus* (ATCC 29213) and *C. albicans* with the inhibitory zone diameter of 21.30±0.89 mm and 18.00±0.21 mm respectively. It is not effective against MRSA (ATCC 25923).

Dasana Sanskara Churna possesses antimicrobial properties against *S. aureus* (ATCC 29213) and clinically isolated *C. albicans* giving the clear zone diameters of 19.33±0.67 mm and 15.45±0.48 mm accordingly while it does not possess any inhibitory property towards MRSA (ATCC 25923).

Table 1. Antibacterial activity and antiyeast activity of ayurvedic formulations

Formulation	Inhibitory zone diameter (mm)		
	S.aureus(ATCC 29213)	MRSA(ATCC 25923)	Clinically isolated *C. albicans*
Kushtadi Churna*	17.30±0.70	19.00±0.60	0.00
Swethamanjana Churna *	23.70±0.30	26.67±1.20	26.35±0.40
Karanjadi Churna*	21.30±0.89	0.00	18.00±0.21
Dasana Sanskara Churna*	19.33±0.67	0.00	15,45±0.48
Positive Control*	29.00±3.40	18.67±0.30	29.67±0.80

* The values are sample mean ± Standard Deviation of three replicates

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