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A Comparative Study on the Anti-Microbial Efficacy of Two Ayurveda Powders (*Prathisarana*) Used In Periodontal Disease

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Abstract

Kushtadi (KU) and *Karanjadi* (KR) are prominently used Ayurvedic powders (*Prathisarana*) in the management of periodontal diseases. This study was carried out for the determination of antimicrobial efficacy of these two drugs using standard Antimicrobial Sensitivity Test against *Candida albicans* and a fungal culture isolated from a patient. The Antimicrobial Sensitivity Test was performed according to the Well diffusion method having 6 mm diameter wells on Sabouraud Dextrose Agar (SDA). Each well was loaded with test drugs mixed in 20 µl of sterile distilled water and fluconazole 2.5 mg/ml as the positive control. According to the results, KR showed an average Inhibition Zone Diameter (IZD) of 20 mm for the isolated culture while KU did not show any inhibition. The results were similar for *Candida albicans* and KR showing IZD 18 mm, KU IZD 0 mm while positive control giving an inhibition of 25 mm. Therefore, it can be concluded that both *Candida albicans* and the isolated culture were sensitive only for KR making it the only effective drug out of the two drugs tested in this study.

Keywords: *Kushtadi*, *Karanjadi*, *Prathisarana*, Anti-microbial, periodontal diseases

Introduction

Ayurveda is an ancient system of medicine and is a rich reservoir of resources even for the dental science. The periodontium is composed of alveolar bone, periodontal ligament, cementum and gingiva. The two most common periodontal diseases are Gingivitis and Periodontitis. Common cause for periodontal disease is infection of micro-organisms. Bacteria are the major organisms for periodontal infection.

The goals of periodontal disease treatment are to promote reattachment of healthy gums to teeth, reduce swelling, the depth of pockets and risk of infection and inhibit disease progression. But ayurvedic treatment is special, it's action is not only reduce symptoms, but also maintained the oral hygiene too.

To overcome these problems, in Ayurvedic classics several treatment modalities such as *Prathisarana*, *Gandoosha* and *Kavala* have been mentioned for management of periodontal diseases.

These *Karanjadi Pratisarana*and *Kushtadi Pratisarana* were clinically very effective in management of

periodontal diseases. But these are not significantly evaluated.

Microbial evaluation is a part of scientific method. In this study it is focused on finding whether there is an anti-microbial action in these *Prathisarana*.

Methodology

The study was based on anti-microbial susceptibility test by using clinical specimen in Agar well diffusion method.

Collection of clinical sample

Clinical sample was collected from the patient at Denal Clinic of National Ayurveda Teaching Hospital in Colombo. Then it was transferred in to petri plate.

Figure 1: Cultivation of the isolate in the sample

Drug Preparation

The *Prathisarana* was prepared according to the *Choorna Paribhasha* of *Sharangadhara Samhitha*. First all the ingredients were identified and authenticated, then measured the required amounts of each drugs. After cleaned the ingredients prepared the *prathisarana*. Prepared fine powders were mixed well and stored in the air tight container.

Disc preparation for AMST

All the glass wears were sterilized with aluminum foil wrapping by using hot air oven at least 2 hours for 160^0C. All the aqueous solutions were prepared with sterile distilled water. Medias and distill water were sterilized by autoclaving at pressure about 15 psi (per square inch); temperature 121^0C for 15 minutes before use in experimental procedures.

Preparation of SDA plates

As for the suppliers instruction 65g of SDA mixed in a conical flask with 1000 ml of sterile distilled water and boiled the mixture up to dissolve the medium completely. Then the media was sterilized non absorbable cotton pad as mentioned in above.

The sterile media allowed to cool up to 45^0C in water bath for 15 minutes and poured in to sterile petri dishes up to 4mm in a height in sterile environment without bubbling and transferred to the refrigerator for ambient temperature at 4^0C for 18 hours.

Preparation of Nutrient media

13 g of Nutrient Broth was mixed in a conical flask with 1000ml of sterile distilled water the mixture was dissolved completely and boiled under the medium temperature. 13 g of powder was mixed with peptone water in a conical flask with 1000ml of sterile distilled water the mixture was dissolved completely and boiled under the medium temperature. The mixtures were auto claved for 15-20 minutes in 121^0C.

Preparation of the Inoculums

The inoculum was taken from the clinical sample by using the loop and it was transferred to nutrient broth. It was incubated for 18 hours in 37^0C temperature. The prepared dilution was used for the broth which are the maximum growth of the fungus in the clinical sample.

- 10^{-1} dilution series – Put 1 ml broth in to 9ml of peptone water
- 10^{-2} dilution series – Put 1 ml -1 dilution series in to 9ml of peptone water
- 10^{-3} dilution series – Put 1 ml – 2 dilution series into 9 ml of peptone water

According to the 0.5 McFarland standards selected the 10^{-2}dilution series for the ABST test.

Seeding the plates

All the SDA plates were allowed to warm up to room temperature before seeding from the refrigerator. Hence any excess moisture will be absorbed in to the medium.

From nutrient broth, 18 hours old fungal culture was taken and the inoculum were seeded over the solidified SDA plates using a sterile cotton swab in order to get a uniform microbial lawn. Then the plates left on a sterile area for excess fluid to be absorbed.

Anti-fungal sensitivity test

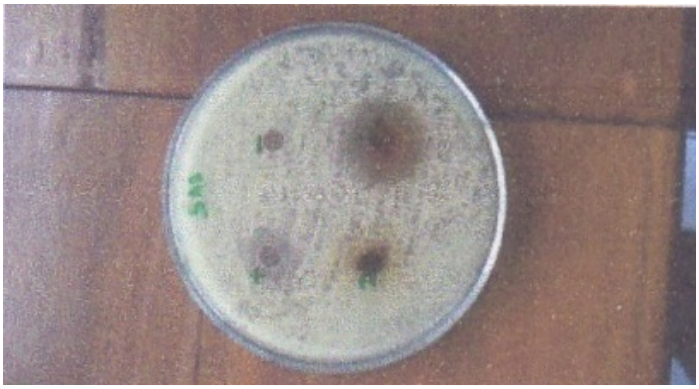
The kieby-Bauer well diffusion method was used for testing the sensitivity of fungus to mouth washes. The wells were bored using sterile Cork broker of 5 mm in diameter and about 3 cm apart from each well. There were 4 wells in one plate. Three plates were prepared for Anti-fungal sensitivity test and it was labeled.

- + (positive controller) :- Anti-fungal 50 µl (Fluconazole)
- - (negative controller) :- 50 µl (Sterilized distilled water)
- D_1 (Drug) :- (*Kushtadiya*)
- D_2 (Drug) :- (*Karanjadiya*)

Fluconazole was used as a standard drug and served as positive control, while distil water was used as negative control. The plates were left at ambient temperature for 15 minutes to allow excess pre-diffusion of extract prior to incubation 24 hours in 37^0C temperature.

Anti-fungal sensitivity was obtained by determining the zone of inhibition around the well and it was compared with standard drug.

Results



Figue 2 : IZ of sample 1

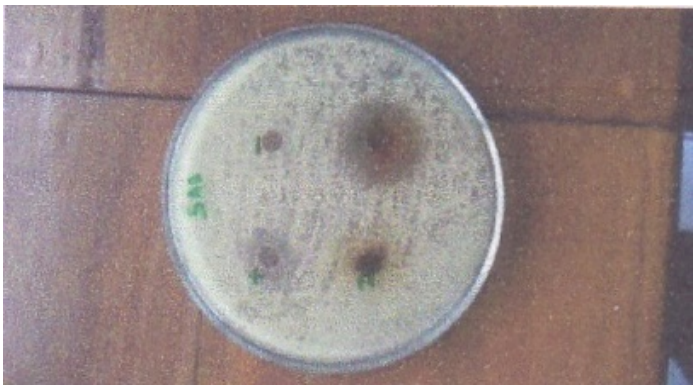


Figure 3 IZ of sample 2

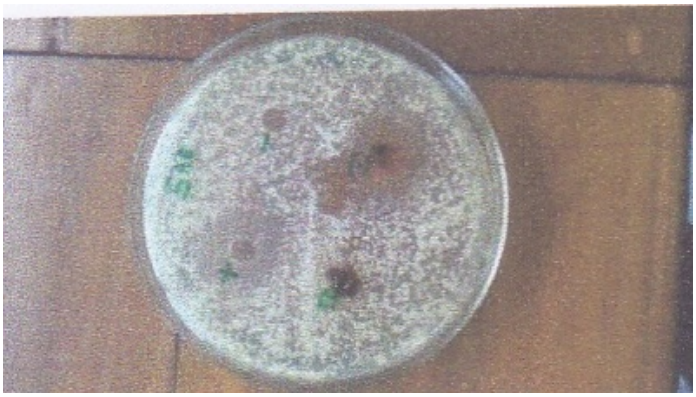


Figure 4: IZ of sample 3

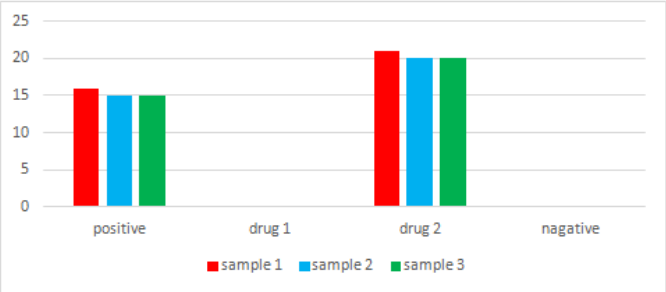
		Zone Diameter (mm)		
Sample	Positive control	Drug $_1$	Drug $_2$	Negative control
1	16 mm	00	21 mm	00
2	15 mm	00	20 mm	00
3	15 mm	00	20 mm	00

IZ of Sample 1,2 & 3

Data Analysis

Frequency

		Karanjadiya	*Kushtadiya*	Fluconazole
N	Valid	3	3	3
	Missing	0	0	0
Mean		20.3333	.0000	18.0000
Std. Deviation		.57735	.00000	2.64575
Variance		.333	.000	7.000
Range		1.00	.00	5.00



Zone diameter of 3 samples

Results According to T- test

	N	Mean	Std. Deviation	Std. Error Mean
Karanjadiya	3	20.3333	.57735	.33333
Kushtadiya	3	.0000	.00000[a]	.00000
Fluconazole	3	18.0000	2.64575	1.52753

According to above table mean of zone size of two samples are not equal. Mean of *Karanjadi Prathisarana* is higher than *Kushtadi Prathisarana*. Therefore, it was concluded that the antifungal effect of *Karanjadi Prathisarana* is higher than the antifungal effect of *Kushtadi Prathisarana.*

According to above table mean of zone diameter of two samples are not equal. Mean of *Karnajadi Prathisarana* higher than Fluconazole. Therefore, it can be concluded that the antifungal effect of *Karanjadi Prathisarana* is higher than the antifungal effect of Fluconazole.

Discussion

In this study, the "*Karanjadi Prathisarana*" shows significant activity against tested fungus. *Karanjadi Prathisarana* mean inhibitory zone diameter is 20.33mm. According to this result *Karnajadiya* has an antifungal effect against clinical specimen. Therefore, this result further confirmed that *Kushtadiya* has not an antifungal effect against clinical specimen. The comparative data analysis of the significant antifungal activity of the *Gandusha* compared with standard antifungal drug, fluconazole.

Finally it can be concluded that *Karanjadi Prathisarana* has an antifungal effect against the clinical specimen of oral candidiasis than *Kushtadi Prathisarana* and also it has strong antifungal effect than fluconazole.

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