ISSN: 0976-3031

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 10, Issue, 03(G), pp. 31610-31613, March, 2019

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

IN VITRO MICROBIOLOGICAL STUDY ON; AN INDIGENOUS MOUTHWASH (GANDUSHA) FOR PERIODONTAL DISEASE

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DOI: http://dx.doi.org/10.24327/ijrsr.2019.1003.3299

ARTICLE INFO

Article History:
Received 4th December, 2018
Received in revised form 25th January, 2019
Accepted 18th February, 2019
Published online 28th March, 2019

Key Words:

Gandusha, Anti-microbial action, Periodontal disease

ABSTRACT

Gandusha is clinically effective in the management of periodontal disease. This study is an in-vitro analysis on determining the antimicrobial efficacy of the drug using standard Antimicrobial Sensitivity Test against a fungal culture isolated from a patient. The test was performed according to the Well diffusion method having 6 mm diameter wells on Sabouraud Dextrose Agar (SDA). Each well was loaded with the test drug mixed in 20 µl of sterile distilled water and fluconazole 2.5 mg / ml was used as the positive control. According to the results the test drug showed Inhibitory Zone Diameter (IZD) of 15 mm. Therefore it can be stated that the test drug is more effective than the positive control and the isolated culture is sensitive to the test drug as it had shown a IZD of 19 mm while it is intermediate sensitive for the positive control as 15 mm comes under Intermediate Sensitive range (15 – 18 mm). These results support the fact that the indigenous mouth wash (Gandusha) is effective in the management of periodontal disease.

INTRODUCTION

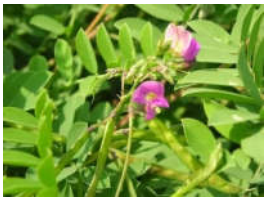
This research presents a microbiological study exploring anti-microbial sensitivity of the indigenous mouth wash on clinical specimen, focusing specially on total zone of growth inhibition. Ayurveda medical system is a one of the great medical system which can cure and prevent many diseases of human in world wide. Ayurveda medicines are becoming popular day-by-day and demand for its usage is increasing not only in the country but also worldwide. The inherent quality of Ayurveda treatment of having negligible side/after effects, has made great potential for its production. Ayurveda medicines are based on plants, animals extract and minerals both in single ingredient drugs and compound formulations.

Ayurveda is an ancient system of medicine and is a rich reservoir of resources even for the dental science. Ayurveda has mentioned various procedures for maintain the oral hygiene. *Gandusha* is the one of the local procedure. *Gandusha* is a method of holding any liquid in the mouth to its full capacity without any movement inside.

This indigenous mouth wash is a decoction preparation which is commonly used in Dental Clinic. This mouth wash was clinically very effective in management of periodontal disease.

This indigenous mouth wash is a decoction preparation by using,

- *Tephrosia purpurea (pila mul)*
- *Pongamia glabra (Magul karanda)*
- *Mimusopus elengi (Munamal)*
- *Embelica ribes (walangasal)*
- *Terminaliya chebula (Aralu)*



Tephrosia purpurea

Pongamia glabra

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Mimusopus elengi

Embelica ribes

Terminaliya chebula

It is a powder form preparation using same parts of each component. Normally *Gandusha* is prepared using 6 parts of water, boiled it reduce to 1 part of water.

Periodontal disease is universal and has been known to affect mankind since the beginning of the recorded history. They also form a major global public problem. Periodontium is the supporting structure of a tooth, helping to attach the tooth to surrounding tissues and to allow sensations of touch and pressure.

The two most common periodontal diseases are Gingivitis and Periodontitis. Periodontal disease is marked by bacterial overgrowth. However, a persistent immune response to chronic infections in the mouth is believed to play a major role in gum destruction. In the healthy mouth, more than 350 species of microorganisms have been found.

Gandusha is usually very effective in the management of periodontal disease. But it is not scientifically evaluated. Microbiological evaluation is a part of scientific method. Therefore, this study is focused on finding, whether there is an anti-microbial action in this indigenous mouth wash.

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 Dental Clinic of National Ayurveda Teaching Hospital in Colombo. It was collected by using cotton buds. Then it was transferred in to Petri plate. (Sabouraud Dextrose Agar)

Cultivation of the isolate in the sample

Drug Preparation

The *Gandusha* was prepared according to the *Gandusha paribasha* of *Sharangadhara Samhitha*.

4.5 g of coarsely powdered drugs are boiled with 6 parts (720 ml) of water, over a mild fire till the liquid is reduced up to one part (120 ml) of the original quantity.

First all the ingredients were identified and authenticated. Thereafter measure the required amounts of each drug. Positive control has been prepared as*Gandusha*. Prepared positive control (+VE) was diluted a 0.5mg of fluconazole into 1ml of distilled water.

Raw material amount	Water amount	Obtain amount
22.5 g (4.5x 5)	720 ml	120 ml

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 dissolved autoclave mixtures, at121^0C for 15-20 minutes.

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 inoculums 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 (Drug) :- 50 µl (prepared drug sample)

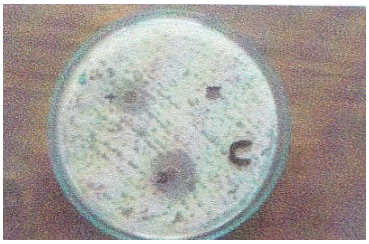
Fluconazole was used as a standard drug and served as positive control, while distilled water was used as negative control. The plates were left at ambient temperature for 15 minutes to allow excess pre-diffusion of extract prior to incubation 24 hours in 37^0C temperature.

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

RESULTS

IZ of sample

IZ of sample AIZ of sample



IZ of sample C

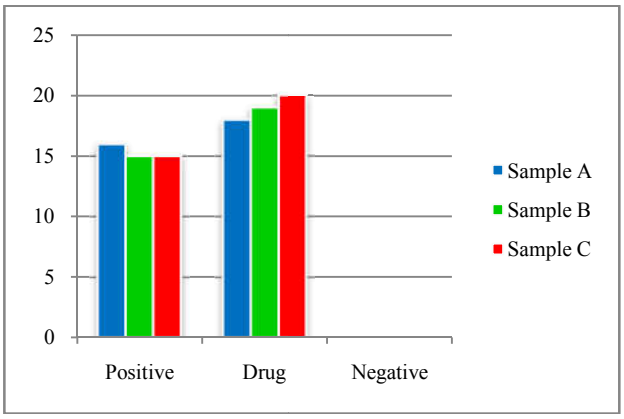
	Zone Diameter (mm)		
Sample	**Positive control**	**Drug**	**Negative control**
A	16 mm	18 mm	00
B	15 mm	19 mm	00
C	15 mm	20 mm	00

IZ of Sample A,B& C

Data Analysis

Frequency

Statistics			
		Inhibition zone of *Gandusha*	**Inhibition zone of Fluconazole**
N	Valid	3	3
	Missing	0	0
Mean		19.0000	15.3333
Std. Deviation		1.00000	.57735



Zone diameters of 3 samples

Results According to T- test

	N (Sample size)	Mean	Std. Deviation	Std. Error Mean
Inhibition zone of *Gandusha*	3	19.0000	1.00000	.57735
Inhibition zone of Fluconazole	3	15.3333	.57735	.33333

According to above table mean of zone size of two samples are not equal. Meanof *Gandusha* is higher than Fluconazole.

DISCUSSION

Anti-microbial properties of medicinal plants are being increasingly reported from different parts of the world. The World Health Organization estimates that plant extract or their active constituents are used as folk medicine in traditional therapies. That was of 80 % of the world population. In this study, the "Indigenous mouthwash" (*Gandusha*) shows significant activity against tested fungus.

Gandusha mean inhibitory zone diameter 15.33 mm. According to this result *Gandusha* have anti-fungal effect against clinical specimen.

According to comparative data analysis the significant anti-fungal activity of the *Gandusha* compared with fluconazole standard anti-fungal drug.

It was concluded that, this indigenous mouthwash has an anti-fungal effect against clinical specimen of oral candidiasis. The result has been confirmed that an anti-fungal effect of indigenous mouthwash is more potent than Fluconazole.

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How to cite this article:

Peiris K.P.P, Samaranayake G.V.P and Dewasurendra D.M., 2019, In Vitro Microbiological Study on; An Indigenous Mouthwash (Gandusha) for Periodontal Disease. *Int J Recent Sci Res.* 10(03), pp. 31610-31613.
DOI: http://dx.doi.org/10.24327/ijrsr.2019.1003.3299

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